Proceedings of the 2012 ANZCCART Conference

“Thinking Outside the Cage: A Different Point of View”

Tuesday 24th to Thursday 26th July, 2012
Rendezvous Hotel Scarborough
Perth, Western Australia

ISBN 978-0-9874657-0-2

These proceedings were edited by Dr Geoff Dandie (CEO, ANZCCART)

Acknowledgements:

ANZCCART would like to thank the following organization for their financial support of this conference:

Table of Contents

2012 ANZCCART Conference

Programme

Tuesday 24th July

8.00 – 9.00am	**Conference Registration Desk Opens**	
Session 1:	**Introduction**	
9.00 – 10.30am **(to include Morning Tea)**	C & D Member introductory event A & B Member gathering Animal Welfare Officers' collective Technicians & Animal Facility Managers Research Ethics Managers & Directors	Chair: David Brockway Chair: Barbara Frey Chair: Deirdre Bourke & Julie Bellamy Chair: Deirdre Bourke & Julie Bellamy Chair: Kim Gifkins & Erich von Dietze
10.30 – 10.45am	Formal welcome (Dr Geoff Dandie; CEO, ANZCCART)	
10.45 – 11.15am	Housekeeping announcements	
11.15 – 11.30am	*Formal Welcome and Opening* Lyn Beazley; Chief Scientist, Western Australia	
11.30 – 12.30pm	*"Human Guinea Pigs and the Nobel Prize"* Barry Marshall; Nobel Laureate	
12.30 – 1.30pm	**Lunch** (Held in the Ocean Room)	
Session 2:	**Papers & Workshops: Research**	**Session Chair:** Chris Prideaux
1.30 – 2.00pm	*"Lessons from rodent models of cerebrovascular dysfunction"* John Mamo; Curtin University	
2.00 – 2.30pm	*"Koalas and People: Ethics and Impacts, Science and Society"* Darryl Jones; Griffith AEC Chair	
2.30 – 3.00pm	*"Salvaging the conscience: The Biomedical Research Dilemma and the Animal's Point of View"* John Schofield; Director of Animal Welfare	
3.00 – 3.30pm	**Afternoon Tea**	
Session 3:	**Challenging our Understanding**	**Session Chair:** Gill Sutherland
3.30 – 4.00pm	*"Behind the Scenes of Animal Production"* Deb Hopwood / Pip Milton; ARC	
4.00 – 4.30pm	*"Fishy Business – The set up and maintenance of a zebra fish colony"* Doreen Mackie; Edith Cowen University	
4.30 – 5.30pm	*"The Unnatural Relations between Artistic Research and Ethics Committees"* Stuart Hodgetts; Symiotica	
6.00 – 8.00pm	**Cocktail function held in Rendezvous Hotel Ocean Room**	

Wednesday 25th July

Session 4:	**New perspectives in medical & scientific research**	**Session Chair:** Dr Jeni Hood
9.00 – 9.45am	*“Burning questions; Can animal research help change patients' lives?”* Fiona Wood; University of Western Australia	
9.45 – 10.30am	*“Improving outcomes at the edge of viability through research in the fetal and preterm lamb"* Jane Pillow; University of Western Australia	
10.30 – 11.00am	**Morning Tea**	
Session 5:	**Animals for human health**	**Session Chair:** Mandy Paterson
11.00 – 11.45am	*"The Importance of Animal Models in Alzheimer's Disease Research"* Giuseppe Verdile; Edith Cowen University	
11.45 – 12.30pm	*“One researcher's personal and scientific experiences of studying cancer using animal models”* Delia Nelson; Curtin University	
12.30 – 1.30pm	**Lunch**	
Session 6:	**Papers & Workshops: Teaching**	**Session Chair:** Sally Bannerman
1.30 – 2.00pm	*“Holistic approaches to animals in teaching: who learns from whom?”* Teresa Collins; Murdoch University	
2.00 – 2.30pm	*“Animal Ethics Committees and Biomedical Research in Singapore”* Mark Vinson Vallarta; National University of Singapore	
2.30 – 3.00pm	*“Testing times – developing awareness while fostering research: improving health, welfare and production in South African Merinos”* Annelie Cloete	
3.00 – 3.30pm	**Afternoon Tea**	
Session 7:	**Papers & Workshops: Animal Care & Maintenance**	**Session Chair:** Melissa Linderman
3.30 – 4.00pm	*“A review of the effects of space allocation and housing density on measures of wellbeing in laboratory mice”* Alexandra Whittaker; University of Adelaide	
4.00 – 4.15pm	*“The Importance of the Experimental Environment of the Stress Response in Sheep”* Maggie Honeyfield-Ross; University of Auckland	
4.15 – 4.30pm	*“Developing Monitoring Guidelines for Fish Welfare”* Miriam Sullivan; University of Western Australia	
4.30 – 4.45pm	*“Education of Undergraduate Animal Science Students in Professional Practice – Can we ensure compliance and educational outcome?”* Jo-Anne Chuck; University of Western Sydney	
4.45 – 5.00pm	Honorary Life Members address to conference	
7.00 – 11.00pm	**Conference Dinner at Frasers Restaurant, Kings Park** **Transportation to be provided from hotel – Buses arriving at 6:00pm**	

Thursday 26th July

Session 8:	**Wildlife: Research & teaching**	**Session Chair:** Kirsty Dixon
9.00 – 9.45am	*“Animal trapping and animal welfare”* Mike Calver; Murdoch University	
9.45 – 10.30am	*“Public perception of pest and wild animal management – changing it for the better”* Peter Mawson; Perth Zoo	
10.30 – 11.00am	**Morning Tea**	
Session 9:	**Panel Discussion**	**Session Chair:** John Schofield
11.00 – 1.00pm	**Euthanasia Panel Discussion** *“Introducing the Controversies”* Leisha Hewitt; University of Western Australia *“Humane euthanasia of pigs”* Barbara Frey *“Lethal sampling of stingrays for research”* Owen O’Shea; Murdoch University *“The management of any beached whales”* Doug Coughran; Department of Environment and Conservation *“Reptile Euthanasia: No Easy Solution?”* Kris Warren; Murdoch University *“Euthanasia of large animals for humane reasons”* Simone Vitali; Perth Zoo	
1.00 – 2.00 pm	**Lunch**	
Session 10:	**Drawing it all together**	**Session Chair:** Sarah Wylie
2.00 – 3.00pm	*“Managing nutritional requirements in a world of change: linking nutritional physiology to animal behavior”* Dean Revell; CSIRO Discussion including: – *“What they eat and where they go”* by Dean Revell; CSIRO – *“Relocation and adaptation”* by Dean Thomas; CSIRO – *“Adapting to a scientific environment to make sense of measurements”* by Samantha Bickell; CSIRO	
3.00 - 3.30pm	*“Positive re-enforcement training in pigs”* Melissa Lindeman, University of Western Australia	
3.20 - 3.50pm	Honorary Life Member address	
3.50 – 4.30pm	Conference summary and close by Geoff Dandie; ANZCCART	
4.30 pm	**FINISH**	

Conference Presentations – Proffered Papers

Editors Note: In accordance with arrangements made by the Local Organizing Committee, some Keynote presenters elected not to submit papers for inclusion in these proceedings. Other Presenters were for a variety of reasons, unable to submit manuscripts as noted.

Koalas, Roads and People: Impacts and Perceptions

Darryl Jones
Environmental Futures Centre,
Griffith University, Brisbane, QLD 4111 Australia

Abstract

The coexistence of koalas and people in many urban areas throughout Australia poses one of our most significant conservation challenges. In locations such as south-east Queensland, Coffs Harbour and Port Macquarie, rapidly expanded suburban development directly threatens the existence of natural populations of this iconic species. Although, *everyone* demands that koalas be protected, our cars, dogs and desire for new houses is leading directly to the on-going extinction of local populations. The number of koalas in southern coastal Queensland has declined by about 60% in less than a decade, yet the region adds about 500 people each week. We tend to think that conservation is about saving wildlife in some distance 'natural' area; confronting the possibility of the extinction of koalas in our own backyards requires major readjustments of perceptions and prescriptions.

We have been investigating the movements and threats to koalas in sites throughout the Koala Coast, a highly fragmented landscape straddling the eastern side of Greater Brisbane. Koalas occur in many small, often isolated bushland tracts which are almost all bounded by major roads and suburban development. Rather than remaining in the 'bush', however, koalas often move widely through the matrix of streets and houses as they make use of key resources and seek social contacts. The long-term survival of such urban koalas will depend ultimately on our perception of the meaning of 'wildlife' and 'conservation'.

Introduction

Possibly the most significant milestone for humanity to have been reached already during the present century, has passed with relatively little acknowledgment. According to a number of authorities including the United Nations, sometime during 2007, for the first time in the history of the human species, most people now live in cities. By far the most rapid change in this direction has occurred in the developing world where the move from the countryside to the exploding megacities is accelerating (McDonnell *et al*. 2009). The long-term implications of the monumental phenomenon can only be guessed but many have raised concerns over the numerous environmental, financial, health, welfare and social challenges facing these massive urban centres.

Somewhat less conspicuous yet potentially of profound importance is the way that the vast numbers of urban people will come to view nature and even other species. One of the most obvious changes associated with the move into the city is the increasing distance – both geographic and perceptual – between natural landscapes and their resident animals and people. Where some level of direct contact with the non-human world was a typical element of daily life for people living in the farms, villages and small towns humans have spent most of their history, such contact is rapidly becoming unusual. This severing of the intimate connection between people and the natural world has been termed the 'extinction of experience' (Miller 2005).

There is currently considerable interest and concern about the practical and political implications of this social dimension of urbanization, with attention being drawn to issues such as the so-called 'nature-deficit disorder' (Louv 2005) and the likely decline of support for conservation from a generation of people raised in urban environments (McDonnell *et al*. 2009).

Many wildlife managers working in urban areas report apparent increases in complaints and demands for action in relation to traditionally tolerated wildlife (D. Jones, unpublished data). For example, non-destructive species are frequently targets for relocation while even the presence of some animals is sometime regarded as unacceptable by some householders. Perhaps the most unexpected example of such intolerance is the increasingly widespread negative attitudes expressed toward koalas by numerous human residents living in areas supporting these animals. Despite the dramatic decline in the populations of koalas in Queensland and New South Wales, the expansion of suburban developments has brought humans and koalas together in an uneasy coexistence.

Koalas and people coexisting

The coexistence of koalas and people in many urban areas throughout eastern Australia poses one of our most significant conservation challenges. In locations such as southern Queensland, Coffs Harbour and Port Macquarie, rapidly expanded suburban development directly threaten the existence of natural populations of this iconic species. Although, the community demands that koalas be protected, the cars, dogs and desire for new houses is leading directly to the on-going extinction of local populations in these areas. The number of koalas in south-east Queensland, for example, has declined by about 60% in less than a decade (State of Queensland 2009), while the region's human populations grows by about 500 new people each week. We tend to think that conservation is about saving wildlife in some distant 'natural' area; confronting the possibility of the extinction of koalas in our own backyards requires major readjustments of perceptions and prescriptions.

Detailed studies by the Queensland Government's Koala Conservation Unit (see Dique *et al*. 2003) and others have documented a catastrophic decline in the numbers of koalas in the 'Koala Coast' region of coastal southern Queensland. The primary causes of this have been attributed to three distinct but intertwined influences: disease, dog attacks and road mortality (State of Queensland 2009). Although each of these issues can be investigated separately, it is becoming increasingly clear that these can also be regarded as unavoidable symptoms of urban development and the resulting fragmentation and isolation of koala populations. Koalas in this region are increasingly being compressed into smaller and more isolated patches, typically surrounded by heavily populated suburbs with the associated roads, cars and dogs. These koalas are being forced into unusually high densities, leading to greater competition for limited feeding resources. Moreover, rather than avoiding the disturbed and potentially dangerous landscapes of the suburbs and staying "in the bush where they belong" (to paraphrase a prominent local sentiment), many koalas venture out into gardens and backyards in search of traditional feeding trees now often marooned in house yards. These foraging adventures – now revealed as highly typical of a majority of these koalas – are clearly high-risk journeys, with a plethora of dangers including the expected dogs and cars, but also the potentially lethal hazards of swimming pools, electrocution and becoming trapped in the maze of fences.

Koalas and roads

We have been investigating the movements and threats to koalas in sites throughout the Greater Brisbane area of southern Queensland. This is a highly fragmented landscape currently undergoing a spectacular growth in suburban development with both the expansion of housing and an intensification of the road network. Recognising the threat posed by roads, in 2009 the Queensland Government initiated an innovative project which attempts to reduce the key impact of road mortality (separate projects have been aimed at addressing the two other major threats to koalas: dogs and disease). The relevant department, Queensland Transport and Main Roads (QTMR), using the detailed information available on the locations of the largest populations of koalas, selected a series of sites where koala habitat existed on both sides of major roads. These sites were the focus of two main activities by QTMR: the construction of specially-designed fencing aimed at preventing koalas from gaining access to the road; and the construction of ‘safe passages’ under the road as well as the installation of the first koala overpass.

The involvement of my research group from Griffith University (Applied Road Ecology) in this landmark project has been to monitor the movements of the koalas in the six nominated study sites and to assess the extent to which the animals are using the safe passages. To achieve these objectives we have been capturing koalas and fitting them with the latest generation GPS transmitters, enabling extremely detailed information to be gained on the daily (and nightly) movements of these animals within their highly altered habitats (See Figure 1). This work is continuing and will be concluded at the end of this year; so only preliminary findings can be reported here. Nonetheless, it is clear already that our approach has yielded the most temporally and spatially detailed data on koala movements ever obtained, and the most valuable picture of the activity of urban koalas to date.

Figure 1: The left hand panel shows a GPS tracker fitted to a Koala and the right hand panel illustrates the kind of detailed tracking information that can be gathered using this technology and how the koala’s range extends to both sides of the highway.

As intimated above, the results of the movement patterns show that while some animals are content to remain within the bushland remnant they live in, the majority – especially males – wander widely and often, frequently moving deep within the suburbs to access important feed trees. These wanderings are, however, comparatively small and modest compared to the activities of wild koalas living away from human settlements (Ellis *et al*. 2002). Unquestionably, urban koalas are significantly constrained by the arrangements of the fragments of forest that remain, yet continue to move widely through the urban matrix.

With regard to movements across roads, in general the aim of the exclusion fencing has been largely successful in keeping koalas away from the road surface. Nonetheless, by virtue of this being a highly developed and human-dominated landscape, most of the fences are limited in extent and often open at intervals to provide road access to nearby properties. Inevitably, some koalas do move across the road surface in places and some do so nightly. However, to date none of the more than 60 tagged koalas involved in this study have been killed on the roads. Furthermore, we are now convinced that the koalas have altered their normal activity cycle to cross the roads at the quietest – and therefore safest – periods of the night.

The most important aspect of this work relates to the use of the structures retrofitted to existing underpasses or viaducts to assist in the safe crossing of roads by koalas. Using a wide array of camera, Radio Frequency Identification (similar to the PIT tags used on domestic pets) and GPS transmitters we have documented a large number of movements under roads and have established that koalas will use such obvious passages as cleared paths under bridges and dry culverts.

Of greater interest and significance is whether koalas will utilize the numerous specially designed walkways constructed under several of the roads. As with most mammals, koalas are extremely reluctant to get their feet wet and even spacious box culverts will not be used if there is any water present. To overcome this, QTMR adapted an approach frequently used in Europe for smaller mammal species with either a wooden platform attached to the sides of the culvert or the floor of the culvert being raised by an additional layer of concrete (See Figure 2). Although the researchers were admittedly rather sceptical as to whether koalas would even find these structures, let alone use them for crossing, our fears were rapidly put to rest when the first walkway was used within a week of installation (see Figure 2, included here as historically, the first pictorial evidence of a koala using a ledge). Since this first shot, this ledge and several others have been in virtually nightly use. While the cognitive abilities of koalas are rarely celebrated, let the record show: koalas can learn new tricks!

A bigger challenge awaits the animals living on either side of Mt Cotton Road, in Redlands, however. At the time of writing (August 2012), the finishing touches to the first ever koala-specific overpass are being completed. This major structure spans an extremely busy arterial road leading from Redland City to Brisbane and will allow the populations of koalas living either side in conservation land to cross in complete safety for the first time. Let's hope they learn this new trick too!

Figure 2: The left hand panel shows the design and layout of a 'safe passage' under a major road. Note the raised animal walkway on the right hand side of the tunnel. The Right hand side panel (infrared image) shows a koala using the raised walkway through this tunnel to safely move from one side of the road to the other at night.

References

Dique DS, Preece H, Thompson J and de Villiers DL (2004). Determing the distribution and abundance of a regional koala population in south-east Queensland. *Wildlife Research* **31**, 109-117.

Ellis W, Melzer A, Carrick FN and Hasegawa M (2002). Tree use, diet and home range of the koala at Blair Athol, central Queensland. *Wildlife Research* **29**, 303-311.

Louv R. (2005). *Last Child in the Woods*. (Workman Publishing: New York.)

McDonnell MJ, Hahs AK and Breusle JH (2009). *Ecology of Cities and Towns*. (Cambridge University Press: Cambridge.)

Miller JR (2005). Biodiversity conservation and the extinction of experience. *Trends in Ecology and Evolution*. **20**, 430-434.

State of Queensland (2005). *Decline of the Koala Coast Koala Population: Population Status in 2008*. Dept. Environment and Resource Management. Brisbane.

Salvaging the conscience: the biomedical research dilemma

J C Schofield
Animal Welfare Office
University of Otago, Dunedin NZ

Abstract

Working in research, at the cage-face, where laboratory animals undergo daily experimentation, causes one to often reflect on the reasons why these animals are used. Episodes of doubt can occur, particularly when the unexpected happens. One must have faith in the system, otherwise continued employment in this field would not be possible, or one would go mad. In my view, the general public have almost no understanding of the subject, and the vast majority seem indifferent. This contrasts with a small minority of passionate abolitionists, with extreme opinions. This paper offers some key strategies which I believe salvage the conscience; which gives me the moral courage to continue. These are presented from the animal's point of view: as if to say to the animal, *'let us reassure you that we have taken every possible step to ensure that you will have a comfortable experience in our research laboratory'*. The biomedical research dilemma: we need the knowledge to improve the human condition, but at the expense of the animal. There are eight such steps to ensure a comfortable experience and they are detailed in this presentation. Perhaps the public should be informed of these steps? Usually when challenged, institutions provide assurances to the public by indicating their compliance with regulations, without any explanation. Perhaps the public would appreciate the details?

I have worked for the last 30 years in a field which is generally not considered an acceptable cocktail party subject. I work as a veterinarian in laboratory animal facility in a medical school and these days I practice avoidance behaviour when asked what I do for a living. This is because an honest answer will commonly elicit a response like; *"but how can you live with yourself doing such cruel things to poor defenceless animals?"* I imagine that such opinions are probably based on literature promoted by the animal rights movement, which continues to parade unsavoury historical practices, such as burn injuries in pigs by blowtorch, or head trauma studies on monkeys. Without wishing to condone such events, probably very few historical medical or veterinary practices would escape criticism if thoroughly investigated. Animal rights activists do not seem to be very interested in progressive developments in this field and would rather continue to mislead the public. However, as a societal watchdog and whistleblower, activists have had a major impact in some areas of research. For they have caused many of us to re-evaluate our ethics, to reconsider and recalibrate our personal stance on what was once considered ethical and humane. There has been important progress in refining experimental techniques to minimise the harm, but sadly, change occurs slowly and sporadically around the world, despite most countries adopting the same basic ethical framework. Political and financial pressures are applied to continue historical practices, despite clear evidence that would seem to demand that certain procedures be abolished. The ethical dilemma: we need the knowledge to improve the human condition, but we gain that knowledge at the expense of animals.
So how do I live with myself? How do I justify to myself, the experimental use of animals within a large biomedical institution where academic freedom is sacrosanct?

Let me start with a brief explanation about how the research system operates. All science starts with a question. For example; "will our new magnesium alloy compound (designed for orthopaedic plates and screws), be biocompatible and retain its strength long enough to allow fracture repair, before dissolving away?" In fact there are at least three questions in that sentence; questions about biocompatibility, strength retention and degradation rates. Most questions build on previous studies, however some questions; the really clever ones, have never been asked before. The ability to conjure up new questions is the hallmark of good science. The questions get argued, debated, analysed and refined. Applying Occam's razor; simple is best. The next step is a literature review to determine what techniques might already exist that might allow the question to be answered. Of course scientists already have a sound working knowledge of their subject through constant reading of their literature. They might be able to immediately identify the technique they need to apply. Alternatively they may need to develop a new technique to answer their question. In either case, the technique itself will need validation, to prove that it can be repeatedly performed with accuracy in their own laboratory. Just because a technique is published by a laboratory in Germany, doesn't necessarily mean it will work well in NZ. This is followed by designing the experimental, with control and treatment groups, including sample size calculations. In our example, there may be four different magnesium alloys to test, plus one control alloy. How many animals should be used for each alloy implant? Can we implant more than one alloy per animal? How long should the implanted alloys remain in the animal before euthanasia and removal? What size of difference between the control and the experimental treatments do we think will be biologically significant? There are also welfare questions to consider; what potential adverse effects might occur? Will the degradation of the magnesium alloy release hydrogen gas? Will these rats start to look like the Michelin Man?

Let me now share the strategies I believe in, which salvage my conscience:

1. Peer review determines that each research proposal has scientific merit. Scientific investigations based on animals are probably the most expensive form of research. Therefore trivial procedures are not undertaken. Animal rights activists' often claim that there is lot of duplication of research. However research funding is limited and studies need to be novel in order to be funded.

2. With the development of new technology, earlier research techniques can be expanded and explored in more detail. For example, in-vivo imaging devices can track the distribution of fluorescent labelled molecules of a drug, in the tissues of a mouse by repeated scans of the same animal. The fluorescing mouse may undergo a procedure which duplicates a previous technique; however, the imaging technology enables the scientist to study the drug's distribution in an entirely new way. Such a study is not duplication.

3. The Three Rs of Russell and Burch; replacement, reduction and refinement are firmly embedded principles in the culture of many research institutions. At Otago, they are regularly applied, often during the design phase of an experiment. Their institutional acceptance empowers the veterinarian to challenge any published technique which a researcher may propose, with the view to improving or refining that technique. Thus the literature is not taken as gospel. The reader may be surprised to learn that there are a significant number of unethical, unacceptable experimental techniques still being published. In my experience, researchers in general, appear to believe that publication (the peer review process) automatically confers ethical acceptability. Regrettably such is not the case. There is great personal satisfaction in saying to a researcher, '*let me suggest a better way of doing that'*. Clearly the unethical publications are produced by scientists without a working knowledge of the Three Rs, and I would argue, the Animal Ethics Committee which approved their proposal are dinosaurs, by accepting the published methodology as gospel.

4. Endpoints in research programmes can play a critical role in welfare. Firstly, there is the study endpoint: for example, rats are treated with drug X at 20mg/kg twice daily for

10days, and on day 12 they are scheduled for euthanasia and tissue retrieval, to determine drug distribution. If a number of animals develop problems during the treatment period, they may not survive to day 12. Their loss can compromise the data collection, there being too few animals left to achieve a statistically significant result. Secondly, there are humane endpoints. These can be decided for most studies, before the work begins, based on accepted international guidelines. Some examples: animals which have lost 15-20% of their body weight (when compared to controls) should be euthanased, even if the animals have not yet reached the end of the study. Animals which lose 10% of body weight in 24hrs should be euthanased. Self mutilation of the feet, following misplaced intramuscular injections in the hind legs, which results in a neuropathy, would require euthanasia. Animals which develop seizures or convulsions should be euthanased. The next endpoint to discuss is 'death as the endpoint'. In the context of biomedical science, this term means that the animal is left unattended to die. No intervention is used to relieve suffering. While there may be scientific justification for this research strategy, few AECs are willing to accept this endpoint these days. It is helpful to have an AEC protocol clearly indicate that death is not the endpoint, to avoid any confusion and to promote animal welfare. The final and comprehensive endpoint; by which any animal which is deemed to be suffering, in the opinion of the veterinarian, will be euthanased, is a great comfort. For this endpoint enables the veterinarian to override scientific opinions which might seek to extend life, in order that the animal reach the study endpoint. When humane endpoints are a condition of approval, their actual implementation is more certain. When humane endpoints are included in the protocol application form as standard welfare benchmarks, their application is almost irrefutable. The veterinarian need only present a copy of the approved protocol to whoever seeks to challenge the veterinarian's opinion and remind the animal user that their animal use is conditional upon upholding the humane endpoint criteria. The only remaining question I like to ask is: *'so after euthanasia, what tissues do you need to collect?*

5. Study design can have a major impact on the outcome of the research. For many projects, the degree of variation in data and the size of the difference between experimental and control groups can be estimated. Estimates can be obtained from similar published studies, or best guess estimates based on experience. This enables statistical tests to recommend the numbers of animals which should be in each treatment group. The veterinarian can assist here, by anticipating morbidity and mortality. The researcher might be advised that their procedure places animals at increased anaesthetic risk and because animals will be subject to repeated anaesthetic episodes (as part of the study design), some complications should be anticipated. *'How many animals can you afford to lose before statistical significance is compromised?'* is a good question to ask. Often the answer is clear*: '.....in that case, you should increase the numbers of animals per group by 10%'.* Poor study design can have serious welfare implications. For example, when a study is poorly conceived, such that unanticipated losses reduce animal numbers to such an extent that no meaningful result is possible, the researcher frequently petitions the AEC for more animals to repeat the study. The AEC can quite reasonably demand to know why the researcher had not been more diligent in design and anticipation of problems. In effect, all the animals used in the first study were wasted. And if these animals suffered a degree of discomfort or distress, all the more reason for concern. Therefore there is great merit in careful study design to take account of all possible adverse effects. Animal numbers should be increased as needed, so that every animal used has scientific value and has contributed to the study, in order to answer the question being asked. Clearly such care demands the consultative services of a range of specialists, including the veterinarian. In my experience, the most successful research programmes are those where the specialists meet to explore and discuss the study so that

all possible problems have been considered and a management plan devised to deal with the most likely complications.

6. The reader will have appreciated the importance of the scientific literature in this process. I have in the past, provocatively stated at conference presentations that in my view, '*scientists worship the literature'* and make reference to (hypothetically) *the Journal of Obstetricalcardiophysiologicalendocrinimmunology*. The literature is used to justify their research proposals. For example, if a technique is published in the Journal of Surgical Research, to induce sepsis in rats, by a research group in Japan, then the same technique must surely be acceptable in Australia or NZ? The scientist will argue and insist that publication therefore allows the technique to be used in this country. However, what if that technique does not provide analgesia for the animals (when analgesics would be expected to be administered), to manage their welfare? The astute AEC will not find the referenced technique acceptable and so the confrontation starts. A fundamental problem with the scientific literature is the lack of specific detail. For example, some published papers do not specify the anaesthetics used, or list the anaesthetic but not the dose rate. Some papers don't define the strain of animal used. Most papers do not give details of the clinical complications or the mortality rates. Publications in which death was the endpoint (the animals are left to die without any humane intervention) seldom provide the scientific justification for this strategy, in my experience. In fact, one wonders how some papers manage to get published. These concerns reflect inadequate editorial and ethical review in my opinion.

7. Monitoring of animals can quickly identify clinical problems which arise from time to time after experimental procedures are performed. In the biomedical field, the research engine is the graduate student. The student performs the surgery and the monitoring, after being trained appropriately. A research study might have groups of 30-50 animals on the post operative monitoring list at any one time in one room and the task of daily monitoring (sometimes twice daily), is the responsibility of the student. The veterinarian relies on the student to report clinical problems. In support are the trained laboratory animal technicians who provide daily care and general husbandry. Our institution prefers to employ technicians who have completed the veterinary nursing course. The most effective monitoring strategies employ animal welfare score sheets to record body weight, water intake (by weighing the water bottle) clinical condition, surgical site and so on. These criteria focus the student's mind on the individual animal. You can imagine that the task of twice daily monitoring a group of 30 animals would be very time consuming, so simple monitoring records that are student-friendly work the best.

8. The training of animal users is one of the most important aspects of managing welfare. Most will end up performing surgery alone, sometimes late into the night; as these 'research engines' have a remarkable capacity for work. The training triad of; see one, do one, teach one, is frequently used and depending on the student's laboratory, the time spent under direct supervision can vary greatly. The vast majority of academics in the basic sciences have not had the benefit of a veterinary or medical education; hence their surgical skills have been developed, usually without any formal training. The standard surgical approaches are not always used and basic surgical anatomy is not generally taught in graduate school in the basic sciences. The potential for a 'home made' surgical technique is ever present. These students are highly motivated and creative individuals who will invent methodology as needed, if they have not been properly trained. The infrastructure needed to ensure that all student animal users receive appropriate training and at the right stage of their project, is in our experience, a significant but essential overhead cost that institutions must meet. The veterinarian alone cannot provide enough

training to achieve a high level of surgical skill for all researchers, in my view. Given the limitations in resources and time, we have learnt that the most practical approach is to provide training in the specific techniques required, rather than general training across a broad range of procedures. For example, training to perform adrenalectomies in rats, not general abdominal surgical training. In our research setting, the institutional veterinarian creates and delivers the appropriate basic training, which is then supplemented by special techniques taught by the academic research staff. There is great satisfaction in observing students perform survival surgeries exactly as directed, particularly with the knowledge that one has been able to refine and improve upon the technique which the student would have otherwise taken from the literature and when that student appreciates the difference, one can only hope that they will pass on their new found knowledge, when they become academics with supervisory responsibilities of their own.

9. A strategy we have developed to assist students focus on their proposed animal use is the Manipulation Techniques Meeting (MTM). The AEC decides which students should be assisted in this way during review of the proposed research application. The meeting is arranged between the veterinary staff and student, with an invitation for the supervisor to attend, although most do not. A copy of the document we use for this meeting is attached. The MTM system is generally applied to procedures which involve major survival surgery. The document is a simple 14 point cockpit drill for students. The veterinarian reviews his/her own understanding of the relevant AEC protocol before the MTM meeting, which is an informal discussion, often over a coffee in the Animal Welfare Office. The student is given a blank copy of the MTM document and invited to discuss the various aspects of: aseptic technique, anaesthetic equipment, surgical anatomy and so on. It quickly becomes apparent whether the student has a working knowledge of these fundamentals. Any lack of understanding requires remedial action. This might take the form of an in-depth discussion, a practical tutorial (to be arranged as soon as possible), refresher attendance at the next formal training session or rarely, the suggestion to postpone the proposed surgery. Such a delay is discussed with the student's supervisor. By the time the student has reached the MTM stage in our training programme, they have met the veterinary staff several times through course attendance and this familiarity encourages a free and frank exchange of views. Often the student will ask for additional veterinary assistance at the MTM, before attempting the surgical procedure alone. The veterinarian annotates the MTM document as required (usually there are several recommendations and additional assistance matters to record on the form), it is signed/dated by the veterinarian and copies given to the student, their supervisor and one retained at the Animal Welfare Office. An interesting benefit of this system is its compliance value. The student has been clearly advised of the conditions set out in the approved AEC protocol, because it is the key reference document for the animal study. Any subsequent deviation from this protocol (as might be discovered by a site visit) can be readily managed by reference to the MTM.

In summary, the use of animals for the advancement of knowledge is justified in my mind, when the project has scientific merit, when humane endpoints are agreed upon and are always implemented, when the techniques used are appropriate and when the personnel performing them are competent, sensitive and compassionate. Institutions which embrace the Three Rs have by definition, empowered their animal care staff to challenge current practices. From the animal's point of view, the most important salvage strategy is careful attention to morbidity and the application of humane endpoints, for these are an insurance policy against suffering.

ANIMAL WELFARE OFFICE CONSULTING FORM

Review meeting with (name) ____

PI ____________________________ AEC # _______________ Dept ________________________________

Date _______________________

- ☐ **Clinical Consultation on Study Design and Animal Models**
- ☐ **Consultation on Animal Ethics Committee protocol related issues**
- ☐ **Manipulation Technique meeting (MTM)**
  A review of practical and clinical animal manipulation issues prior to the implementation of a new AEC protocol, for students and new staff.

Study Design & Animal Model review

Animal Ethics Committee Protocol Review

Manipulation Technique Meeting (MTM)

1. Surgical equipment and provision of aseptic techniques

☐ Steam sterilization
☐ Chemical disinfection
☐ Sterile surgical sponges and suture material
☐ Disinfection of surgical bench area
☐ Fur clip and skin prep with alcohol, iodine or hibitane
☐ Surgical drapes, clear plastic: wet, dry or cloth
☐ Surgical Gloves, or disinfected disposables
☐ Sterile covers on instrument knobs
☐ Spare instruments in surgical packs
☐ Wound retractors available as required

2. Ventilators, gas machines, oxygen supply and gas scavenging OSH concerns

☐ Ventilator required, Tidal Vol set at ____ blood gases or endtidal CO_2
☐ Gas machine in use
☐ Face mask / nose cone or ETT used
☐ Scavenging system in place
☐ Oxygen supply available to support injectable anaesthetics

3. Anaesthetic techniques, drug delivery, anaesthetic monitoring, and peri-operative analgesia

☐ Gas anaesthesia with Isoflurane or other (specify) ________________
☐ Injectable with Ketamine, Xylazine, Medetomidine, Valium, Nembutal
☐ Route of injectable: IP, SC, IM, IV
☐ Dose rate used for injectable agents (specify) ____________________
☐ Monitoring depth by PWR, limb variation in responses
☐ Monitoring by Pulse Oximetry
☐ Preemptive analgesia No / Yes (specify) __________________________
☐ Post-operative analgesia No / Yes (specify)________________________

4. Surgical anatomy and the management of potential unexpected adverse events

☐ Regional anatomy is known
☐ Potential anaesthetic unexpected adverse events are understood
☐ Potential surgical unexpected adverse events are understood

5. Humane endpoints for the study

☐ 10 – 20% body wt loss standards to apply
☐ To list other endpoints that relate to manipulations used

6. **Post-operative monitoring strategies and systems to document animal welfare**
    - ☐ Animal welfare score sheets or equivalent used
    - ☐ Preoprative assessment of behaviour
    - ☐ Gentling animals

7. **Supportive therapy to promote recovery, fluid therapy and heat sources**
    - ☐ Fluid balance: 10% of body weight per 24 hours
    - ☐ Heat pads, heat lamps used
    - ☐ Individual recovery cage
    - ☐ Wound assessment and management

8. **Use of carcinogens, biohazards or radioisotopes in live animals.**
    - ☐ No
    - ☐ Yes, specify controls put in place

9. **Euthanasia methods**
    - ☐ Method does not compromise research data
    - ☐ Method selected CD, Guillotine,CO_2, anaesthetic OD, other

10. **PAR supply, management and controls**
    - ☐ PAR supply from AWO and ordering systems
    - ☐ Controls in place

11. **Blood sampling techniques**
    - ☐ Whole blood ☐ plasma ☐ serum ☐ survival ☐ non-survival
    - ☐ Anticoagulants known ____
    - ☐ Sites ☐ jugular ☐ facial artery ☐ saphenous ☐ orbital
    - ☐ Tail veins ☐ CP-terminal ☐ other
    - ☐ Knowledge of regional anatomy and blood flow
    - ☐ Knowledge of animal blood volume ☐ sample volume known
    - ☐ Use of restraint cones ☐ tubes ☐ tourniquet ☐ other
    - ☐ Needle size ☐ syringe size
    - ☐ Haemostasis methods

12. Drug administration techniques

☐	Methods	IP	PO	IV	SC	IM-NR
☐	mls / kg	10	10	5	5	0.05 NR

☐ anatomical site known

☐ pH ☐ viscosity ☐ sterility ☐ solubility ☐ biocompatability ☐ temperature

☐ skin disinfection used

☐ needle size ☐ syringe size

☐ restraint techniques are known ☐ needle size ☐ syringe size

☐ adverse affects are known

13. Any proposed changes to the current protocol

☐ No

☐ Yes

14. Additional assistance to be provided by the Animal Welfare Office

Proposed recommendations, suggestions:

‘Behind the Scenes of Animal Production’

P. Milton, M. Bunce, M. Haddrill, K. Tweedie, J. Krause, D. Hopwood.
The Animal Resources Centre, W.A.

Abstract

All too often in medical research it is not possible to replace animal models for more ethically aesthetic alternatives that truly model disease pathogenesis in humans. Animal production facilities thus represent an important resource in the supply of laboratory rodents to the research community. But where do these research animals come from? And, what does it take to fill a hypothetical customer’s weekly standing order of mice? These questions and more will be answered as we take you ‘Behind the Scenes of Animal Production’. Not only will we illustrate how refinement and reduction underpin the operation of an animal production facility but also how the principle of ‘economics of scale’ minimises animal wastage in meeting customer needs.

Introduction

In medical research, it is often not possible to replace animal models with alternatives that truly model the pathogenesis of disease in humans. Animal Ethics Committees (AECs) therefore play a pivotal role in ensuring the use of animals in research is reasonable and appropriately justified.

Following AEC review of an animal use application, the placement of an animal order typically represents the start of animal use in a research project. For customer service teams at large-scale animal production facilities, orders raise questions of ‘what’ is required by the customer and ‘when’ the delivery is scheduled but also questions pertaining to ‘how’ the animals are produced, which are not so frequently considered by the broader research community. For AECs, such questions may underpin the three Rs by relevance to reducing animal numbers and therefore wastage and consequently the ability to recycle and reuse. However, from a researcher’s perspective, efficiency of animal supply and availability to supply on demand, are often paramount and they remain unaware of the breeding animals required to produce their orders. In addition, the possibility of establishing an institutional breeding colony is occasionally considered by research facilities as a way to have the researchers’ animals immediately available.

Where do research animals come from? What does it take to fill a hypothetical customer’s weekly standing order of mice? Through answering these questions, the ways in which refinement and reduction underpin the operation of an animal production facility will be illustrated and furthermore, the principles of ‘economy of scale’ and how they apply when minimising animal wastage while still meeting customer needs will be considered.

Factors Affecting Colony Size and Management

Animal production facilities represent an important resource in the supply of laboratory rodents to the research community. Managing breeding colonies in large-scale animal facilities is a complex and multi-faceted operation, particularly when the three Rs form the backbone of strategies to manage colony size. Parameters that may be inherent to breeding colonies or can be a result of external pressures, affect colony size and all must be taken into consideration. The four broad parameters affecting colony size and management considered in this paper include a) the percentage of offspring available for use, b) the colony’s breeding performance, c) the gender required and, d) customers’ ordering frequency (Figures 1a to 1d).

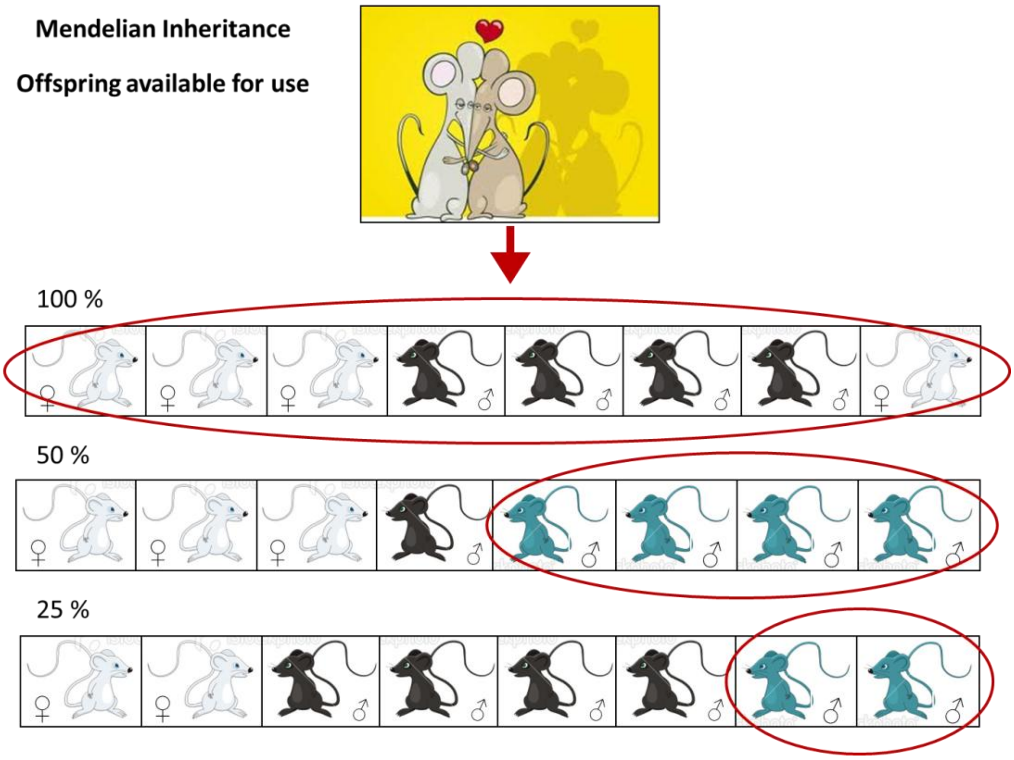
The percentage of offspring available for use is an inherent characteristic of breeding colonies (Figure 1a). According to the principles of Mendelian Inheritance, the details of which are beyond the scope of this paper, only a proportion of offspring may carry the gene of interest and therefore may be available for sale to customers. For instance, 100% of offspring could potentially be available for sale but depending on the parental genotypes, only 50% or 25% of offspring may be available. Production managers apply the principles of Mendelian Inheritance to correlate animal availability with customer demand and to therefore determine best management practices and appropriate colony sizes.

Another inherent characteristic of animal production colonies that affects colony size and management is breeding efficiency (Figure 1b). Breeding pairs with poor breeding performance may produce small and infrequent litters. This is compared to those with good breeding performance, where larger litters may be produced more frequently. Such varied and often unpredictable breeding characteristics must be managed appropriately to minimise excess production and unnecessary animal wastage, which in turn is influenced by customer demand.

Two external parameters that may be particularly variable and difficult to directly control include the gender specifications of the animals required (Figure 1c) and customers' ordering frequency (Figure 1d). Forecasting customer demand comes with years of experience, is highly dependent on research requirements and fluctuates often and without prior notice.

Taken together, there are many combinations of factors affecting colony size and management, particularly given the variety of orders available and the infinite possibilities of customer ordering frequencies. Of the numerous combinations, three will be applied to two animal production scenarios to illustrate differences in colony size, animal wastage and recycling. These combinations are circled in red in Table 1. Each of the three combinations has an average breeding performance and requires the supply of only one gender to the customer but the combinations differ in the percentage of offspring available for use.

a)



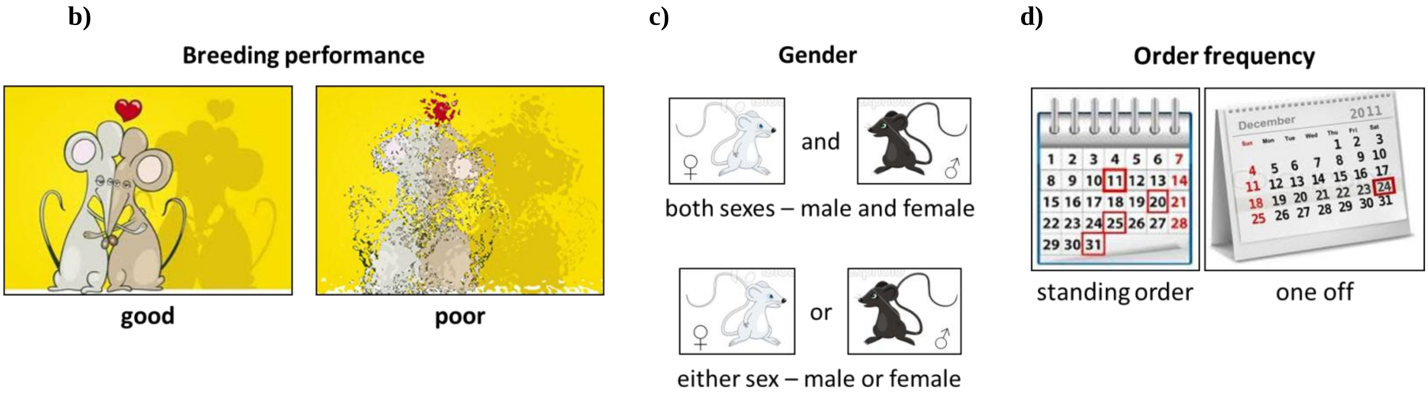


Figure 1: The factors affecting colony size and management include parameters inherent to breeding colonies such as a) the percentage of offspring available for use and b) the breeding performance of the colony. External parameters include those exerted by the customer, such as the required gender c) and d), ordering frequency.

gender	offspring available (%)	breeding performance
M & F	100	good
M & F	100	average
M or F	100	good
M or F	100	average
M & F	50	good
M & F	50	average
M or F	50	good
M or F	50	average
M & F	25	good
M & F	25	average
M or F	25	good
M or F	25	average

Table 1: The possible permutations for three of the four factors that may affect colony size and management. Those combinations circled in red form the basis of two animal production scenarios to be considered.

Scenarios of Animal Production

The three combinations of factors affecting colony size and management will be applied to two relevant animal production scenarios. Not only will these scenarios illustrate differences in colony management, but they will also illustrate the benefit and advantages of dedicated large-scale facilities in supplying animals to the research community.

Scenario one is diagrammatically represented in Figure 2, which illustrates how a large-scale animal production facility, such as the Animal Resources Centre, functions as the central supplier in providing animals to customers. In this scenario, 10 six week old female mice are supplied to 10 different customers every week (Figure 2). All remaining offspring are either sold to other customers or appropriately utilised in other ways.

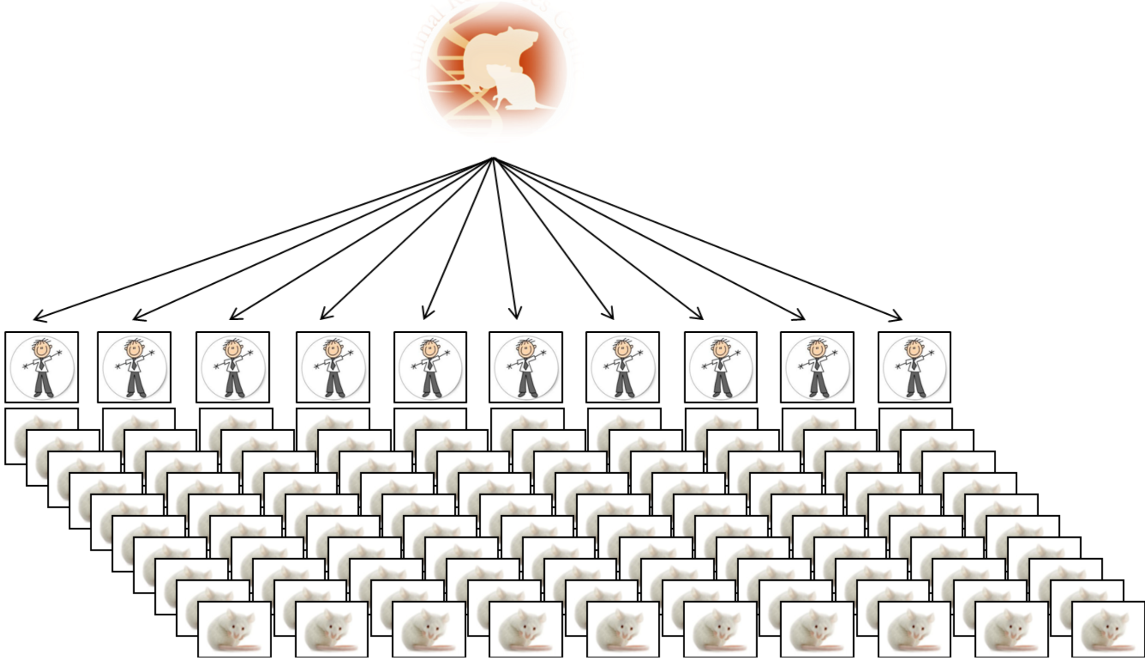


Figure 2: A diagram to illustrate Scenario 1, where a large-scale animal production facility, such as the Animal Resources Centre, supplies 10 female mice at six weeks of age to 10 customers, every week. The remaining offspring are sold to other customers.

During the course of obtaining animals from an animal facility, some researchers from time to time may experience transport difficulties, for example, animals are dead on arrival or there have been delays in delivery. Similarly, research institutes may have put pressure on researchers to establish their own animal colony at the institute due to space being available for use. As a consequence of these experiences, researchers may be influenced to move away from purchasing animals from a central supplier and to therefore establish their own independent breeding colonies. This situation is depicted in scenario two where ten different research institutes across Australia each establish their own colonies and function independently of the other research institutes (Figure 3). As such, each of the ten research institutes produces 10 female mice at six weeks of age, every week for supply to internal researchers at the institute. Since the research institute operates independently to produce only those animals required, all remaining offspring are surplus to need.

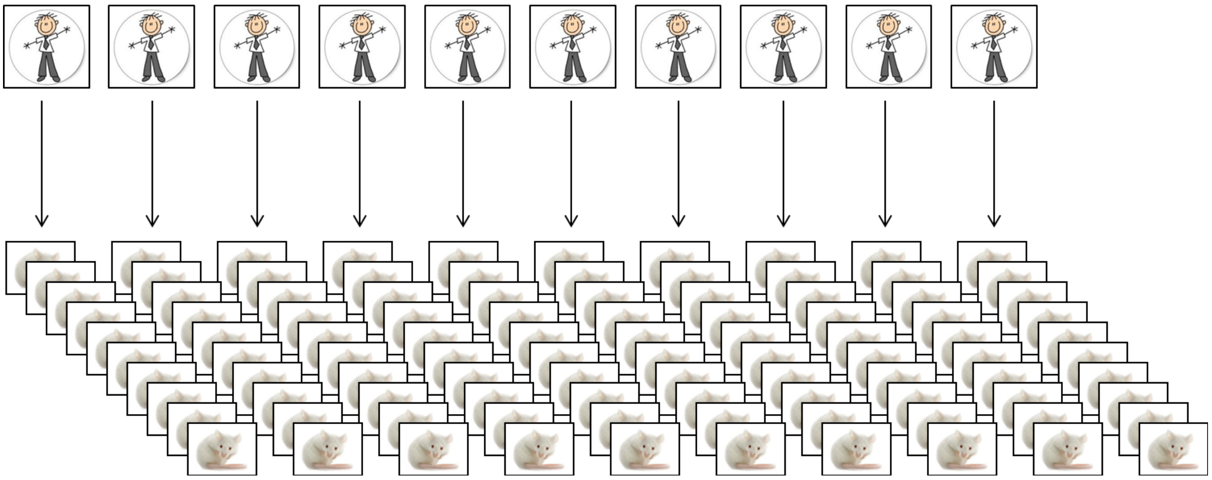


Figure 3: A diagram to illustrate Scenario 2, where ten different research institutes across Australia each establish their own colonies to produce 10 female mice at six weeks of age, every week. Remaining offspring are surplus to need.

Applying the Factors Affecting Colony Size and Management to the Animal Production Scenarios

Noticeable trends are observed when the three combinations of factors affecting colony size and management are applied to the two different animal production scenarios, as summarised in Table 2.

example	scenario	gender	offspring available (%)	breeding performance	number of colonies	total colony size	weekly surplus	monthly surplus
1a	facility	M or [✓ F]	100	average	1	474	7	28
1b	institute				10	470	305	1220
2a	facility	M or [✓ F]	50	average	1	1164	113	452
2b	institute				10	1060	370	1480
3a	facility	M or [✓ F]	25	average	1	3967	943	3772
3b	institute				10	3970	1640	6560

Table 2: Illustration of the differences between a dedicated animal production facility and research institutes when three combinations of factors affecting colony size and management are applied. For clarity, the figures for the research institute scenario represent the total across the ten institutes.

With respect to ‘colony’ size, a colony consists of the production colony to produce the stock animals available for sale and a future breeding colony to replenish breeders in the production colony. The total colony size required to produce the 10 six week old female mice is largely comparable for the animal production facility and the research institutes. Colony size and the number of colonies may be a contentious issue for AECs when considering animal use applications, especially for independent research institutes. Should researchers be required to report the number of animals taken to produce those used in the actual research project? This is an advantage of large-scale production, since the same breeding pairs supply animals for many customers, thus satisfying the three Rs.

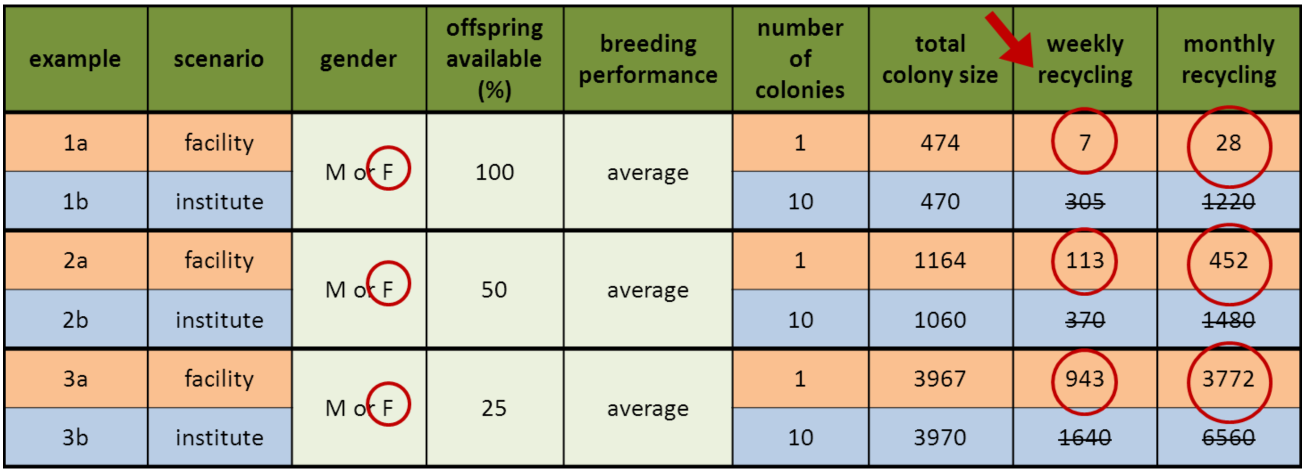
According to total colony size, large-scale facilities and independent institutes are on par in regards to the three Rs. Differences emerge when considering the weekly number of animals that are surplus to need. Significantly more animals are surplus to need for independent institutes compared to a large-scale facility. In addition, the number of animals surplus to need increases when a smaller proportion of offspring are available for use by customers. This trend is further pronounced if breeding efficiency is poor and if considered over a longer period of time. Furthermore, large-scale animal production facilities monitor colonies to guard against overproduction and statistics for each strain are reported to an AEC. The number of animals surplus to need reinforces that large-scale animal production facilities are more efficient at production and supply than a multitude of independent research institutes.

The ability to reuse and recycle is one major advantage of large-scale animal facilities over animal production by research institutes. The efficiency of a large-scale facility to produce animals is emphasised when weekly and monthly surplus in Table 2 is replaced with weekly and monthly recycling in Table 3. Research institutes producing animals solely for internal use may not be able to cater for recycling, so animals surplus to need go to waste. This is compared to dedicated production facilities, such as the Animal Resources Centre, that have the capacity to recycle and are designed for making the most of all surplus non-GM animals, including biological waste. For example, animals that are surplus to need may be sold as aged stock, for time-mating and colony increases, used to generate bi-products such as serum or plasma for customers, as sentinels in the Quality Control program, to confirm genetic integrity or frozen and sold as feed to wildlife carers. The breadth to which surplus animals can be reused and recycled further illustrates the benefit and advantages of large-scale animal facilities in supplying animals to the research community.

Additional Advantages of Dedicated Large-Scale Animal Production Facilities

In addition to avoiding overproduction, there are other advantages of large-scale animal facilities compared to research institutes. These advantages stem from the principles of economies of scale and the fact that dedicated animal production facilities are purpose built for the supply of animals to researchers. The greater centralisation of resources and funding permits a comprehensive range of services and high quality product. Large-scale facilities are often also equipped to facilitate imports and exports between facilities and cryopreservation services. This enhances animal welfare and reinforces the three Rs.

Monitoring health and genetic integrity is an essential part of producing high quality specific pathogen free (SPF) rodents. Dedicated production facilities are often more able to undergo extensive health screening to guarantee an SPF status, aided by highly controlled systems and a business focus on ensuring disease-free status. This is a costly exercise that smaller independent facilities are not often able to afford. On-site veterinarians ensure colony health and staff at large-scale facilities have a wealth of knowledge and expertise in all aspects of animal production. This is complemented by standardised procedures for breeding and maintenance, which ensures consistency of product. These aspects assist in ensuring optimal animal welfare.



example	scenario	gender	offspring available (%)	breeding performance	number of colonies	total colony size	weekly recycling	monthly recycling
1a	facility	M or F	100	average	1	474	7	28
1b	institute				10	470	~~305~~	~~1220~~
2a	facility	M or F	50	average	1	1164	113	452
2b	institute				10	1060	~~370~~	~~1480~~
3a	facility	M or F	25	average	1	3967	943	3772
3b	institute				10	3970	~~1640~~	~~6560~~

Table 3: The differences in the number of animals that can reused and recycled for a dedicated animal production facility and a research institute for each of the three combinations of factors that can affect colony size and management for the two scenarios.

Confirming genetic integrity and strain-specific phenotype characteristics complements the health monitoring arm of quality control. Ensuring strains are true to type and in line with expectations is important for the scientific validity of research. Furthermore, the existence of propagation agreements between international facilities minimises genetic drift and contributes to ensuring strains are comparable on an international and national scale. This is important for researchers as data will be comparable within a field and therefore meaningful and applicable to human health and disease.

For biosecurity and in order to maintain an SPF status, areas within large-scale animal facilities are managed as discrete units to minimise any chance of cross-contamination in the event of a disease outbreak. Such a barrier system is achieved through strictly managing the movement of people, animals and supplies. Staff routines include showering and restriction of their movement in the facility so they work from clean to areas of progressively dirtier health status. This also applies to goods and materials that have been sterilised into the barrier unit. Procedures, location of colonies and movement of animals are also all managed to avoid the possibility of genetic contamination of lines.

Conclusion

Managing an animal facility is a complex multi-faceted operation but such a purpose built facility ensures customers receive a quality product. The three Rs form the backbone of large-scale facility operation, which minimises overproduction and ensures surplus animals are utilised in some capacity. Biosecurity and maintaining an SPF status are paramount to providing a quality product and genetic monitoring ensures strains are true to type. Such consistency is central to the scientific validity of research data, its interpretation and extrapolation to humans.

Fishy Business – The Set Up and Maintenance of a Zebrafish Colony

Doreen Mackie
Edith Cowen University

Abstract

Question: Why did we need to set up a zebra fish colony?
Answer: It was to use the zebra fish as a model for Alzheimer's Disease research

Question: So what's all the fuss about? Surely it's just like setting up an aquarium at home, only more of them!
Answer: If only it was………!

Come along and here the true story!
The questions…the modifications …the certifications…the legislation…the heartache!

No paper received

The Unnatural Relations Between Artistic Research and Ethics Committees

Stuart Hodgetts[1, 2] **and Ionat Zurr**[2]
[1]Spinal Cord Repair Laboratory, School of Anatomy, Physiology & Human Biology, The University of Western Australia, 35 Stirling Highway, Perth, Western Australia 6009
[2] Symbiotica, School of Anatomy, Physiology & Human Biology, The University of Western Australia, Perth, 35 Stirling Highway, Perth, Western Australia 6009

Abstract

SymbioticA is recognised as the first artistic laboratory dedicated to providing artists with support to work with biological material within an academic institute involving access to support, expertise, and relevant technologies. It is proposed that artistic research, involving hands-on engagement with the tools of the life sciences, challenge perceptions and create a zone for discussion about our changing relations to life as material for manipulation. A major role of Ethics Committees is to review research on animals and humans, within established guidelines, by carefully weighing the potential benefit of the project against the potential risks of harm or suffering, but its role is complicated somewhat when the project itself confronts the utilitarian ethics that are inherent within these guidelines as the value of the arts is not measured by any instrumental benefits in achieving other ends. There is a tension between artistic research and the ‘cost / benefit’ analysis governing Australian Animal Ethics Committees, that creates situations which shed light on some of the ethical and philosophical questions with which contemporary society must deal. Any notion of artistic research as being of value for itself—or artistic freedom as an end in itself—can be foreign to Ethics Committee processes and deliberations. Developing research projects and art-works within a scientific context requires readjustment on both sides. Artists are required to articulate their project in a way that makes sense within a science-research framework, in order to communicate to the members of the committees about the project. The blur between the justification of the use of human and animal material in artistic research and the ethical wellbeing of the subjects for the Ethics Committee may also be problematical. The problems and some potential solutions between these issues are discussed.

Discussion

SymbioticA is a Centre of Excellence in Biological Arts at the University of Western Australia (http://www.symbiotica.uwa.edu.au/). It was founded by Oron Catts and Ionat Zurr. Simply, SymbioticA is an artistic laboratory dedicated to the research, learning, critique and hands-on engagement with the life sciences. It endeavours to; identify shifting perceptions of life (trends in the life sciences and their applications), explore possibilities for the purpose of proposing alternative directions in order to initiate cultural debate (by creating contestable futures and evocative objects), to reflect on ethical issues (via interrogation and conveyance of the uneasiness concerning the use of living biological systems), and explore - through hands on experience - embodied experience and its importance.

Symbiotica is in a unique position to offer hands on access to scientific laboratories and tools for artistic research and is the only artistic laboratory of its type in the southern hemisphere. It focuses on development of technical skills and the use of scientific tools. Projects are subjected to stringent ethics and health and safety approvals, in a similar fashion to animal and human research studies in UWA’s academic environment, which in turn created some international precedents in regards to the approvals of the use of living materials/subjects for research that has artistic aims.

Developments in the life sciences and its applied technologies create new ethical and philosophical perplexes. Artists have an essential role in exploring these new terrains by continuing to question and even provoke, also by the medium used, in order to suggest contestable scenarios. Following the Ethics Committees methodologies, which are geared predominantly for biomedical research, becomes a great challenge when applied to artistic research. Weighting a project's benefits versus costs becomes more of a philosophical issue when applied to an artistic or cultural activity and new issues are raised such as the role of the committee as censors; protection of audience sensitivities; the perception of artistic research versus scientific research and allowing ironic and playful research to be recognised as serious research tools.

It generates these types of questions: What is artistic research? How does it differ to "scientific" research? What are its outcomes? Does (should?) it follow same principles? What medium does "artistic research" require (compared to classical media such as canvass, oils, acrylic, textile, clay, photographic, digital etc). Is life itself an artistic medium?

Associate Professor Stuart Hodgetts is Director of the Spinal Cord Repair Laboratory in the School of Anatomy, Physiology & Human Biology and has a long standing collaboration with Symbiotica in projects such as "Pig Wings", the Tissue Culture and Art Project, "Lifeboat", as well as assisting in Symbiotica's Tissue Engineering Workshops. More recently, he has become the official Scientific Adviser/Consultant for Symbiotica. In addition to conducting his own research into stem cell transplantation therapies (and tissue engineering) for the treatment of spinal cord injury, he is also chair of the Animal Users Group at UWA and has extensive experience with Ethics Committees, which gives him a unique perspective in his role for Symbiotica. As Scientific Adviser, he assesses Symbiotica Resident proposals, provides networking with other researchers, directly assists with projects in the laboratory and oversees ethics issues with projects.

Bioart requires the marriage of a biological medium with the process of ethics governing the welfare and humane use of that biological medium (human and/or animal). To provide guidance within Symbiotica toward the successful marriage of two, the feasibility of the proposed work (scientifically, logistically, infrastructure and network) must be assessed with an appropriate ethics application. For a good proportion of the resident artists, this process often becomes part of the artistic process and arguably a medium of itself. It becomes, in effect, training with medium for the artist/resident to perform bioart within UWA.

As an adviser, there is an obligation not to judge the artistic proposal in terms of its scientific merit or whether the artistic use of the biological "medium" is warranted, but more importantly to provide an avenue for the resident to achieve their aims. The Ethics Committee's role should also be consistent with this approach.

Scientists ultimately have a responsibility to the animals they use to publish data generated by that research. Ethics and the AEC system, requires that researchers consider the animal's inability to provide "informed consent" and it relies on the AEC to essentially do this on behalf of the animals used for these research purposes in a manner that is consistent with the "three R's", which include;
Replacement – the use of alternatives to animals should be considered and adopted wherever possible
Reduction - effective and intelligent ways proposed to minimise use of animals
Refinement – the impact of the research on the animals and minimisation of impact on animals

If these guidelines are followed by the Bioartist, does the use of an animal (or human?) for science outweigh its use for art? Who decides? Should this decision be made by Members of the

Ethics Committees Or by others? If the medium is for non-scientific use, can it's use be justified? Is it Outcome based? Is it a hypothesis driven design? Is it simply a waste of animals?

These issues are often perceived similarly *by scientists*. The "appropriateness" or "purpose" of the use of animals is open to scrutiny; the artists may be scrutinized by the public, by scientists and even Ethics Committees. The scientists themselves may also be scrutinized by fellow scientists for being involved with these projects (they are, in fact, guilty by association). However, has the ethical status or wellbeing/welfare of animals ever been compromised because the work is seen to be "unjustified"?

Occasionally, members of two "disciplines" do find some common ground. Research scientists can find merit in some artistic research and artists employing research techniques are arguably "disciples" of science or budding "kitchen" scientists in their own right – i.e. their work can have scientific merit. How does – or should – this impact on Ethics Committees?

Dr Ionat Zurr presented examples of previous Symbiotica projects.

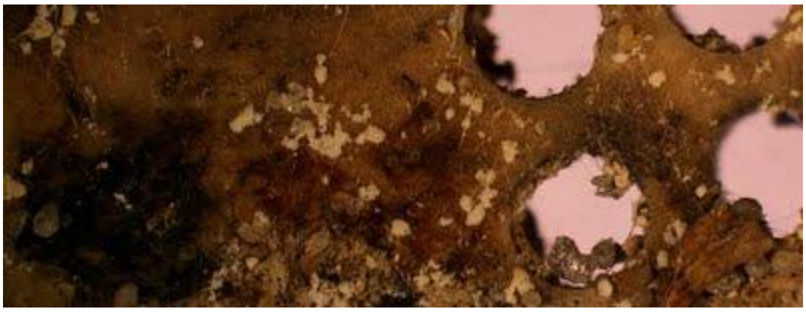
Artist Verena Kaminiarz set out to explore the changing relations humans have with animals when those animals have been designed and engineered as specific human disease models. Through the development of '*May the mice bite me if it is not true'* (2008) the artist also questioned the ethics of the Animal Ethics Committee whose role is to protect the welfare of these new 'living tools.' The work, which was mounted in the Animal Housing facility at the University of Western Australia, concerned the use of mice that have been developed as 'human disease models.' The project focused on four mice, each of which was positioned as a living portrait of a person who had died from a condition that particular mouse had been developed to model. The resulting 'mouse portraits' were of Franz Kafka (lung cancer), Joseph Beuys (natural causes), Felix Gonzalez-Torres (compromised immune system), and Gilles Deleuze (lung cancer). The project mimics some elements of biological research (the location and care the mice receive) and deliberately alters others (the housing, the materials within the mice houses to make the environment richer, and the focus on their identities as 'individuals' by naming each mouse).

'May the mice bite me if it is not true' Verena Kaminiarz 2008-2010
Creating experimental portraits using human disease model mice as a medium.

Kaminiarz used transgenic mice that were considered 'surplus' and 'retired breeders,' each of which was destined to be culled. The housing she designed for the mice was larger and offered the mice a richer and more varied environment in comparison with the standard animal housing in research labs. Although the project posed *no* harm to the animals (rather, the project rescued the animals and allowed them to continue living in comfort) the Committee was reluctant to give its approval. The problem was that no systematic and recognizable scientific procedure was to be employed, no measurement was proposed, and no quantitative data would be generated. In effect, the Ethics Committee was questioning the merits and validity of artistic research in not following the methodology of the scientific research. Naming the animals was also questioned: because it may skew the objectiveness of the researcher by personalizing a relationship with a mouse which would otherwise be regarded as a tool in research. However this was exactly the point in issue for the research project. Kaminiarz aimed to probe the ethics of the instrumentalization of life as raw material for technological ends. In other words, by transforming the mice from a scientific model and individualizing them, she drew attention to the 'de-humanized' use of animals for scientific research.

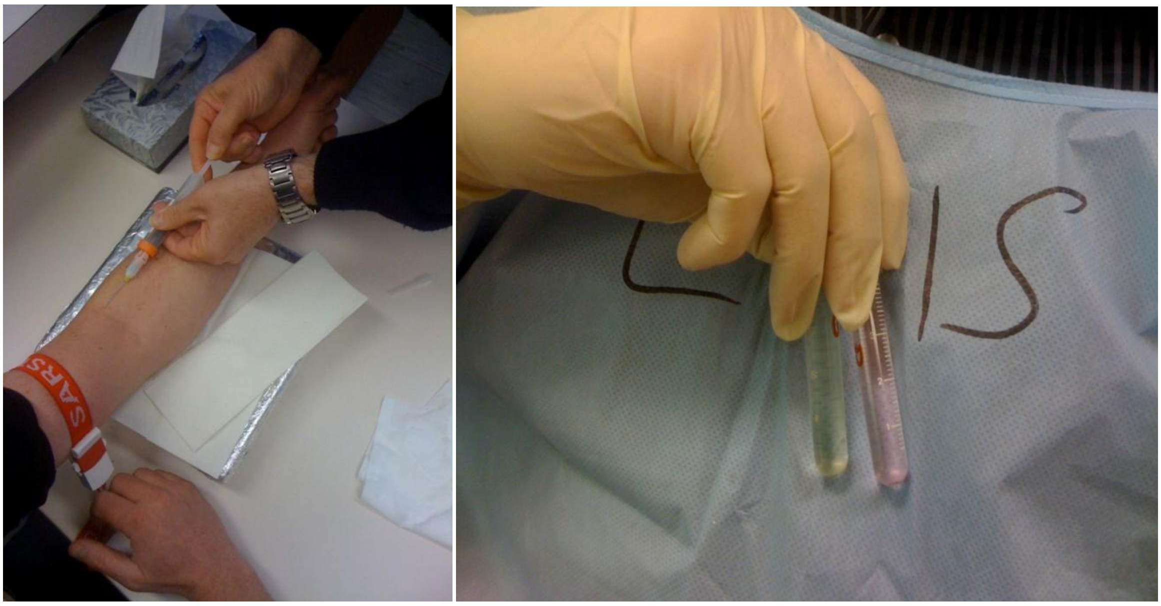
Kaminiarz used transgenic mice that were considered 'surplus' and 'retired breeders,' each of which was destined to be culled. The housing she designed for the mice was larger and offered the mice a richer and more varied environment in comparison with the standard animal housing in research labs.

"Trash Fashion- Designing out waste". The Chair of the Animal Ethics Committee of UWA wrote to PhD student Pia Interlandi (who was working in conjunction with SymbioticA) to congratulate her "on setting a fine example for others to follow" in her "care and respect" for animals. This related to a project developed by Interlandi to investigate forms of ecological burial garments. She had joined with the Centre of Forensics Science (UWA) in dressing two dozen, forty-kilo pig carcasses, with garments developed by Interlandi, prior to burying the animals. The Chair of the Animal Ethics Committee had visited the burial site and wrote to Interlandi that, "We were most impressed by the manner in which you are approaching this project, in particular the care and respect you show for the animals, both in the preparation of the burial shrouds and ceremonies that take place surrounding the burial." He added that "A major element of ethics is respect for the animals that have lost their lives when contributing to important outcomes, but unfortunately this respect is not as common as it should be."



2010 – 2011 London Science Museum:
Exhibition shroud at "Trash Fashion- Designing out waste"

One of SymbioticA international precedents in regards to human ethics approvals is concerned with artists working with their own tissue externally to their body. This kind of research raises a couple of bioethical questions: The artist has to have a biopsy to obtain the tissue; hence there is a need for inflicting harm on a body for non-medical purposes. The artist's relation with the subject matter may raise ethical issues as the artist (or part of the artist) *is* the subject matter. In addition, working with one's own tissue may be risky. This is because the body's immune system will reject cells and tissues coming from another individual. However, if cells/tissue from the same person is re-introduced to the body the body will not recognize the cells as foreign. When working with cells/tissue in vitro (externally to the body) there is always the risk of the cells mutating and therefore, their re-incorporation into the body may carry risks.



Not all this kind of work requires the use of animal derived tissues as artists
will also use their own cells and tissues in some works.

To meet ethics committee requirements Symbiotica have laid out potential benefits to the ‘participants’ (the viewers) in terms of their being “drawn to reassess their perceptions of life in the light of their encounter with a real tangible example of the concept of partial life.” A hope was expressed that this would “assist them in coming to an informed opinion in regard to developments in the bio-medical field” and would “provide them with the opportunity to meditate on what it means to be alive.” With regard to humanity generally, our point was that the project was “part of a larger scale endeavour by artists internationally to deal with new concepts of self and life that our society is being confronted with, in the light of developments in the biomedical field.” Symbiotica argued that “art can play an important role in generating a cultural discussion in regard to these issues: by presenting tangible examples of contestable scenarios, art can act a starting point for a broader philosophical and ethical discussion”.

Censorship? Good art versus bad art? Good experiment versus bad experiment?

There is a tension between artistic research and the ‘cost benefit’ analysis governing the Universities Ethics Committees, that creates situations which shed light on some of the ethical and philosophical questions contemporary society deals with. There is an increase in the use of living matter as technology and in treating life as a raw material that can be manipulated and engineered. Human relationship to life is increasingly confronted with our ability to intervene at all levels of the life processes.

Just as engineers are entering the field of the life sciences to offer engineering solutions and utilitarian applications, so should artists, who offer non-utilitarian artefacts and gestures, participate in this field to problematize, provoke and subvert those dominant understandings and uses of living material.

Symbiotica continues to drive such issues and it’s progress is due not only to a successful marriage between an artistic body and a highly respected research facility that is open towards this symbiotic relationship, but more importantly, for its sometimes brutally honest (and refreshing) ability to make all of us ask such questions of ourselves - as well as others.

Burning questions: Can animal research help change patients' lives?

Fiona Wood
University of Western Australia

Abstract

In the process of developing solutions to solve pathological problems faced by people on a daily basis, we are focused on ensuring safety, and efficacy, as we seek to improve current therapies and develop new ones. This also requires an increased understanding of the impacts of pathology, an essential aspect of solving the problem faced. Increasing patient and volunteer involvement is critical, but the many issues inherent to human research leaves gaps in knowledge and understanding that can be filled through careful and ethical animal work. This is critical to build the bridge between basic science and clinical application that can change patients' lives.

By prior arrangement with organizers, no paper was submitted by this speaker

Improving Outcomes at the Edge of Viability: Research in the Fetal and Preterm Lamb

Jane Pillow

Centre for Neonatal Research and Education,
The University of Western Australia
35 Stirling Hwy, Crawley, 6009, WA

Approximately 8 % of births in Australia occur prematurely, prior to 37 completed weeks' gestation. Infants born at less than 28 weeks gestation are often referred to as extremely low gestational age infants (ELGANs). Although ELGANs account for less than 1 % of the total births each year, ELGANs stay in hospital for up to 5-6 months, accounting for a significant proportion of overall bed days and health resource usage.

There are major challenges involved in caring for ELGAN infants due to the profound immaturity of all of their major body systems. The most immediate challenge occurs at birth, when an infant needs to transition from a reliance on the placental circulation to air breathing to achieve adequate gas exchange. ELGAN lungs are structurally and functionally immature, with low or absent levels of surfactant, an endogenously produced substance that reduces surface tension in the air sacs of the lung. An absence of surfactant that is necessary to prevent the lungs from collapsing and to reduce the work of breathing due to the stiffness of the lungs. Establishment of effective, regular breathing patterns after birth may be further compromised by underdeveloped diaphragm muscle (the "motor pump" of the breathing system). Irregular rhythmicity due to an immature central nervous system can also compromise effective respiration.

Other body organs of ELGANs are also immature. The brain has poor auto-regulation of its blood supply and neurones are not yet fully myelinated. Persistent fetal vascular channels within the heart can impair gas exchange. Immaturity of the intestine impedes absorption of feeds, and normal bowel activity. The kidney has a reduced ability to concentrate urine, resulting in high losses of sodium and bicarbonate. Low placental transfer of maternal antibodies can result in increased susceptibility to infection. Friable skin results in dehydration and skin breakdown.

Clinical research in the ELGAN population is similarly challenging. Sick newborn infants are a highly vulnerable population. Consequently, it is inappropriate to expose ELGANs to high risk or controversial treatments without sound theoretical evidence of a potential benefit and thorough preclinical evaluation. As a scarce resource, ELGAN infants are under high demand for research study enrolment and at risk of exploitation or over-recruitment into research studies. Randomisation of an infant to more than 1 randomised controlled trial may confound outcomes for one or both studies. This results in a human ethical imperative to ensure that only the most promising treatments are subjected to randomised controlled trials in the highly vulnerable ELGAN population. Preclinical research studies have a major role to play in filtering potential treatment interventions worthy of trial in the clinical setting.

In an ideal preclinical research model of preterm birth, the species used for research would reflect the reproductive biology of humans, mimic what occurs clinically in most cases of spontaneous human preterm birth, and have fetal maturation characteristics and postnatal physiological behaviours that are similar to those of humans. The ovine model is used widely in Australia and internationally for this purpose. Advantages of the ovine model include the relatively long gestation and the availability of singleton pregnancies providing opportunities for minimally invasive ultrasound guided antenatal interventions such as intra-amniotic and fetal intramuscular exposures to inflammatory or anti-inflammatory agents. Such studies allow us to increase our understanding of how intrauterine events affect adaptation of the preterm subject to postnatal life. For example, major improvements in respiratory distress syndrome followed preclinical studies demonstrating lung maturation in the womb after maternal treatment with steroids. We have also learnt how fetal exposure to infection whilst in the womb can improve immediate chances of survival after birth, but that this occurs at the expense of adverse outcomes in the longer term.

The preterm lamb is a similar size to the newborn human infant and has similar neonatal physiology and anatomy. Our ability to mechanically ventilate preterm lambs with the same machines as are used in preterm human infants enhances the translational potential of preterm lamb research. Examples of major advances in our understanding of optimal postnatal respiratory strategies for the preterm infant that have arisen from preclinical studies include the introduction of exogenous surfactant therapy and high-frequency oscillatory ventilation. Treatment soon after birth with exogenous surfactant results in improved lung volumes, lung compliance and gas exchange whilst reducing work of breathing and requirement for supplemental oxygen and mechanical ventilation. High-frequency oscillatory ventilation delivers breath volumes less than the anatomical dead-space to gently vibrate the lung whilst keeping the airways open, achieving highly efficient gas exchange without causing overstretching or repeated collapse of the lung. More recently, we have developed preterm lamb models that allow us to understand more about the potential benefits of non-invasive ventilation. Similarly, development of techniques that have allowed us to ventilate the preterm lamb whilst still attached to the placenta have provided the opportunity to understand more about optimal treatment of the preterm infant during the vital period of initiation of ventilation. Elsewhere, the sheep model has been central to the development of an *in utero* treatment (fetal tracheal occlusion) that significantly reduces the respiratory morbidity associated with the presence of a congenital diaphragmatic hernia.

Currently, the major emphasis of clinical research is increasing our understanding of how new treatments impact on long-term outcomes in the ELGAN population. This shift in emphasis is extending to the preclinical environment, where the development of extended care preterm animal models allow evaluation of the potential for long term benefit or harm associated with new treatment approaches. The more rapid infant to adult transition in animals, will permit earlier assessment of potential benefit:harm ratios for new treatments. Given the intensiveness of resource utilisation and cost to the animal of more chronic disease models, it is imperative that this approach is reserved as a final preclinical step for those approaches most likely to be successfully translated to the clinical environment in the highly vulnerable ELGAN population. Further, studies utilising the chronic model need to be highly collaborative, with extensive sharing of data and tissues in order to maximise the research outcomes relative to the costs incurred.

In summary, despite the many physiological challenges of caring for ELGANs, their outcomes have improved substantially over the last 20 years due to improved technology and the progressive translation of preclinical research findings to clinical care and education. Preclinical studies using appropriate animal models have facilitated major improvements in neonatal care over the last 3 decades. The ovine perinatal model has been an especially valuable tool to understand the impact of different maternal, fetal and neonatal exposures on neonatal pathophysiological outcomes. Future research directions will include the development of preclinical animal models that increase our ability to evaluate and improve long term neonatal outcomes associated with the introduction of promising new clinical treatments.

The Importance of Animal Models in Alzheimer's Disease Research

Giuseppe Verdile
Edith Cowen University

Abstract:

According to the 2011Access Economics report[1], there were ~265,000 Australians diagnosed with dementia, a figure that is expected to increase to ~1.1 million by 2050. This dramatic increase will have an annual financial impact on the Australian community that is estimated to be close to $6 billion/year. Alzheimer's disease (AD) accounts for 50-70% of dementia cases. Currently, anti-cholinesterase drugs are the most widely used anti-AD drugs, though only ameliorating some symptoms in about 50% of cases, and only for about 6-12 months. Thus there is an urgent need for preventative or disease modifying therapeutic agents to be developed.

Animal models have played a critical role in providing significant insight into disease mechanisms and are critical in the pre-clinical drug development that targets the disease pathogenesis. The study of AD in humans is often complex due to genetic and environmental factors, which contribute to the development and progression of AD. Although cell culture models can provide information on the mechanisms at the cellular level and can be an initial tool in drug development, *in vivo* animal models are required where genetic and environmental factors related to the human condition can be mimicked but the variation minimized. The limitations of tissue collection and degradation of proteins associated with long post-mortem intervals in humans can also be overcome. Another benefit of the use of animal models is the capacity to assess the biochemical and cognitive behaviour effects of prospective treatments. In humans, only the behavioural effects of treatments can be assessed unless brain tissue is collected post-mortem. Even if human tissue is collected post-mortem following treatment, the genetic and environmental/lifestyle confounding factors hinder analysis.

The most commonly used animal model in AD research is the transgenic mouse expressing genetic mutations that cause the familial form of the disease. The advent of these and other murine models have provided significant insight into the mechanisms by which a key protein, called beta amyloid, accumulates in the brain, in-turn providing targets for the development of therapeutic or preventative drugs. However, there is now a push towards the development of alternative models to minimize the use of the transgenic mice to provide insight into disease pathogenesis and pre-clinical drug development in AD.

[1] Access Economics "Dementia Across Australia 2011-2050", Alzheimer's Australia

No paper received

One researcher's personal and scientific experiences of studying cancer using animal models

Delia Nelson
Curtin University

Abstract:

My childhood in Africa was extremely privileged. I lived on a large property and spent most school holiday in game reserves. I loved wildlife and rescued animals I thought were suffering. As a result my long-suffering mother let me share my bedroom with two monkeys and a cat, a guinea pig lived in the lounge and a mongoose dropped in for dinner. I came to Australia and trained as a biomedical scientist. The thought of animal experimentation never entered my head until I did my honours degree and it was a shock. My dream was to help all living things. Then I saw a friend suffer through cancer and their desperation when told nothing could be done. Several years later I witnessed one of my students go through the same thing. They both experienced a painful death and that is what I hold on to when considering many ethical aspects of my work.

My group is trying to identify ways to treat cancer by involving the immune response. I now understand why so little seems to have changed for cancer patients. It is not easy.

This is why. Anti-cancer immunotherapies aim to generate resolution of all existing tumours, including inaccessible ones and provide long-term protection against recurrence. This is rarely achieved in humans or mice, unless the tumour burden is very small. Thus, our target is to eradicate larger tumour burdens that more accurately represent advanced and/or aggressive human tumours, such as those seen in mesothelioma and lung cancer patients. Our studies have shown that our murine models of mesothelioma and lung cancer can respond to single-agent immunotherapies however, they fail at a defined 'cut-off' tumour burden; i.e. larger tumours become resistant to therapy, similar to the human situation. We have been using this system to define the immune mechanisms required to mediate regression of these larger tumours. We have so far identified one combination therapy that results in the permanent resolution of treated and untreated distal tumours. We are continuing to explore combination therapies and aim to generate sufficiently thorough scientific data that avoid the pitfalls of earlier studies and genuinely warrant translation to humans and other larger mammals.

No paper received

Holistic approaches to animals in teaching: who learns from whom?

Teresa Collins
Murdoch University

Abstract:

Animals used in teaching are a highly valued resource and yet their use stimulates much controversy. The scope for using animals for educational purposes is wide and decisions about their use are made based on the specific learning objectives, the species required and the nature of the use which must involve a detailed cost-benefit evaluation. Our consideration and approval of such use is broadly based on science-based and ethics-based concepts. We need to know what guarantees good welfare of our subjects (the science) and how important the use is, and whether it can be justified (ethics). Scientists are well advanced in assessing the degree of any harm done by identifying the various components of welfare (e.g. physiology and behaviour), but there is a need to take a more holistic approach - that is taking a 'whole animal' approach, such as, using qualitative assessments and seeking the opinions of the wider community. As animals may have good or bad experiences in our hands, we have at the very least, an obligation to treat them considerately and to constantly review whether their use in teaching can be replaced by alternatives. This translates into minimizing the harm we do to them and, importantly, maximizing the good. Animal welfare is a complex entity and the use of animals in teaching provides an ideal experience for veterinary and animal science students and staff to engage in a discussion linking philosophical values and evidence-based science that leads to converging views.

Introduction: attitudes to animals

Controversy about the use of animals for scientific purposes, including for teaching has been at the forefront of the animal welfare debate from the beginning of the modern animal welfare movement in the mid-nineteenth century (Rose and Grant 2008). Perhaps more than any other animal welfare issue, the use of animals in research and teaching is marked by robust public debate and on occasion, by conflict. We are confronted with a range of competing values and passionately held beliefs which challenge and potentially confound our reaching agreement as to the ethical acceptability of our use of animals, particularly in veterinary education.

Societal interest in animals and how they are treated has increased substantially in recent years (reviewed by Bayvel and Cross 2010). Public attitudes toward animal welfare have changed with growing social affluence and the altered role from custodians to companions for those animals with whom we share our home. Traditionally accepted uses of animals for teaching have been increasingly questioned as to whether the welfare status of the animals involved has been compromised and indeed, if animals are required at all.

We need to know what guarantees good welfare for our subjects (science) and how important their use is and whether it can be justified (ethics). The growth of animal welfare science has better defined what matters to an animal and provided a deeper understanding of how pain may be experienced by animals. Scientists are now more advanced when it comes to assessing the degree of any harm done by identifying the various components of welfare (e.g. physiology and behaviour), but there is a continued need to take a more holistic approach - that is taking a 'whole animal' approach, such as using more qualitative assessments and seeking the opinions of the wider community.

Animal welfare means different things to different people, but central to the shared concern for animals is that they are sentient beings – that is they have the capacity to suffer pleasure and pain and have interests that matter (Duncan 2006). It is inescapable that questions about our use of animals in science must involve the wider community and reflect their understanding and attitudes. Animal welfare is a complex entity and the use of animals in teaching provides an ideal experience for veterinary and animal science students and staff to engage in a discussion linking philosophical values and evidence-based science that may lead to converging views.

The scope of animal use in education

In most science and veterinary science courses, there is a level of acceptance that for students to gain a good understanding of biological science, animals may be used and possibly endure unpleasant procedures as they are being viewed as a biological model or learning tool. The scope of animal use for teaching is as extensive as the variety of species used: it may involve observation and basic care for a classroom pet in primary school, basic dissection in high school biology, anatomical dissection and physiological manipulations for veterinary and medical training, studies of animal behaviour and/or as tools to develop specialist veterinary skills, or invasive procedures for diagnosis and treatment.

A utilitarian approach is generally used when regulators need to assess whether animals should be used for scientific education. This involves a cost-benefit analysis where the ‘benefits’ measured by student learning outcomes are weighed up against the ‘costs’ or harms to the animals. As our understanding of the impacts of our handling and use on experimental animals has grown, so has the desire to limit the harms done. In most situations this desire is seen to be aided by promoting Russell and Burch‘s principle of the 3R’s; reduction, refinement and replacement. This ability to weigh up the various options is critical from both a staff and student perspective. Hence, the inclusion of an appropriate form of animal welfare and ethics education should lay alongside any course that utilises animals for undergraduate teaching, particularly a veterinary curriculum.

The need for ethical discourse

It has well accepted that many uses of animals for educational purposes provide a challenging ethical issue. Perspectives on whether such use is justified vary widely and debate will continue with various cultural and historical perspectives. Moore (2001) strongly endorses the use of dissection in science education claiming it enhances knowledge and deepens our appreciation of nature. It is claimed there is no better way to understand the structure and function of an organism than by directly examining the organism (Moore 2001). Strong support for animal-based teaching methods is evidenced by the long standing use of animals in veterinary schools. The Australian Veterinary Association (AVA) and the Association of American Veterinary Medical Colleges (AAVMC) recognizes the important role that animals play in the education of veterinarians in their initial professional training. The AVA endorses the requirements outlined by the NHMRC *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* and is committed to the three R’s (AVA 2009). The NHMRC guidelines state that animals are not to be used for teaching purposes unless there are no suitable alternatives and that any specific training involving procedures that may cause adverse impacts on the animals should be justified on a case-by-case basis (Australian Government NHMRC 2004). The AAVMC states that not all educational objectives can be met through client owned animals, thus it is necessary that some live animals be obtained by donation or purchase and used for instructional activities that may be terminal in nature (AAVMC 2010).

Historically, animal use that may result in death has been an accepted mode of veterinary education worldwide, provided such use was embedded in an educationally valid curriculum and humane animal care was provided. Many alternatives now exist and adoption of these alternatives has either fully or partly replaced much of the need for live animals. One driver for change in the way we use animals for teaching is student expectations. Many students enter a veterinary course with fundamental desire to do good things for animals and this personal ethic may be challenged during their studies. Concurrently, students recognise the need for using animals and for competency on animal welfare related issues (Colonius and Swoboda 2010). It is therefore essential that students have some instruction in animal ethics, can comprehend why such animals are used for their learning and develop the skills to debate the acceptability of any use. Veterinary students are being trained as professionals and in addition to gaining animal handling and surgical skills must learn to uphold their ethical principles.

The veterinary profession is privileged to occupy a position of enormous social influence on animal welfare issues and so it is vital that students understand the various perspectives and attitudes to animals that exist in the community. Equally important is that students develop both a respectful attitude to all animals and the ability to make sound rational decisions about animal use. Thus, students need training in ethical decision making and practice in engaging in a discursive, rational debate about animals. Encouraging students to reflect on and discuss their personal use of animals during their education is an important first step.

Benefits versus costs:

The main ethical principle which guides most animal use in science is this: "*Using animals for scientific purposes is acceptable only when any harm done to the animals is very greatly outweighed by the benefits of their use"* (ANZCCART, 2012).

Despite the specific discipline, the justification to include animals for training need to address how the proposed procedure will enhance student learning. There are three broad categories of learning objectives; cognitive, sensorimotive and affective and teaching methods should be chosen on the basis of these. Students generally react positively when animals are used, are motivated and show enhanced learning and a combination of objectives may be addressed with one animal use. For example, both retention of anatomical knowledge and a positive attitude towards the species may be gained by the presence of a live dog in an anatomy class. However, there is a constant need to weigh up the benefits in terms of students' acquisition of skills and knowledge against the harms including stress or loss of animal life, as well as stress or concern by the students and staff.

For veterinary undergraduates, there remains an accepted need to provide training in clinical competency and veterinary surgery in the latter years of the 5-6 year curriculum. The objective of training in surgery is to produce graduates with surgical skills that meet day 1 graduate attributes as dictated by registration authorities. All Australian Veterinary Boards require that *all* veterinary graduates demonstrate competence in all areas of veterinary science. In the past, students performed a range of surgical procedures under supervision on live anaesthetized unwanted dogs from local pounds or shelters, which were euthanased at the end of the class (Read 2012). However, since 1990s in Australia, the availability of pound dogs has ceased and a combination of factors including rising costs has led to a decrease in the amount of supervision of undergraduate students' surgical training (Read 2012). Increased financial pressure and client expectations have further restricted the opportunities for students to perform surgery such as, wound closures, under supervision whilst on extramural training. Thus, faculty have responded to such changes by developing extensive exercises that promote psychomotor and manual skills using inanimate models, cadaver parts and dog substitutes (for example, DASIE, Rescue critters) in addition to fostering closer relationships with shelter organisations to provide student access to shelter animals for desexing. However, given the somewhat variable supply of shelter animals for desexing, some schools have retained a very limited number of non-survival practicals where species other than dogs are anaesthetised to teach critical skills such as tissue handling and haemostasis (Read 2012).

Other perspectives challenge the need for any live animal use for teaching and state that there is no justification for an animal to be harmed for this purpose. Progressive universities such as the University of Queensland and Murdoch University among others have adapted to this change in attitude and allow students with a substantial ethical objection to a specific animal use to make a claim for exemption. An ethical objection is defined as 'a deep inward conviction of moral injustice; it can only be held after a period of serious reflection' (University of Queensland 2008). Thus, teachers should be sensitive to these objections and provide an appropriate alternative pathway whereby students can achieve their learning objectives without partaking in specific practicals. This view has been sought by some veterinary students with a conscientious objection to the non-survival practicals and provides challenges for faculty staff to ensure surgical competence is gained by all students; both those in the traditional classes and those who seek a program with less direct faculty supervision on campus.

Concurrent to the changing attitudes towards animal use and the further expansion of animal welfare science, there have been an increasing number of courses on animal welfare taught to veterinary students. Animal welfare education is not delivered in a standardized manner across schools however it is thought to be best integrated throughout the curriculum in a systematic way (Colonius and Swoboda 2010). Veterinarians occupy a unique role as animal welfare advocates and hence students' training must examine that role. It is stated that veterinary schools should make efforts to elevate animal welfare as a critical theme within the curriculum (Colonius and Swoboda 2010).

It has been suggested that the harmful use of animals in veterinary education may result in the decreased likeliness of students to view animals as sentient, a decreased empathy towards animals, a decreased propensity to administer peri-operative analgesics and the impedance of normal development of moral reasoning ability (Self et al 1991; Paul and Podberseck, 2000; Levine et al 2005). Furthermore, a study of science students indicated that forcing students to use animals in ways they view as harmful or wrong may cause some psychological stress (Capaldo 2004). Such students may lose their interest in science if not given the option to conscientiously object. Knight (2007) suggests that veterinarians trained without harmful animal use will be more sensitive to welfare issues and may develop higher animal welfare standards. However, the impact of animal use on students may be affected by the adequacy of the training, or lack thereof, of ethical reasoning. Importantly, the question that must be answered is if reduced or no stress for students is always beneficial for them in the long term? Veterinarians as professionals are faced with many challenging dilemmas, including life and death decisions where providing the best outcome for patient may directly conflict with the owner's demands. The stressfulness of ethical dilemmas in veterinary practice is significant; 34% of UK veterinarians in practice reported that they faced 3-5 dilemmas per week (Batchelor and McKeegan 2012) and that they find these dilemmas stressful. These concerns further support the need for increased training and support for veterinary students in dealing with ethical issues.

Comparing Animal Use with the Alternatives

Many alternatives now exist, including computer simulations, high quality videos, 'ethically-sourced cadavers' such as animals that were euthanased for medical reasons, preserved specimens, models and surgical simulators, non-invasive self-experimentation and supervised clinical experiences. Many veterinary schools have included some of these alternatives as effective teaching tools. For example, most veterinary physiology laboratories run interactive computer programs completely replacing the need for any invasive use of animals. Computer simulations are used as replacements for some, but not all, practical classes or they are being used as supplementary resources to prepare students in advance of a class using animals. Consequently, objective studies of veterinary students that compare the learning outcomes generated by non-harmful teaching methods with those achieved by traditional animal use are required.

Studies show conflicting results as to whether the use of alternative teaching methods consistently achieves better learning outcomes than those using animals. Patronek and Rauch (2007) provided the first systematic review; concluding that alternative methods used in biomedical and veterinary science were not significantly different or superior to, the conventional method. This contrasts with other studies reporting the use of well designed alternative programs such as haemostasis models, computer simulations and 'ethically sourced cadavers' can provide learning outcomes that are equal to or superior to, traditional programs that use animals (Olsen 1996, Griffon 2000, Knight 2007). Use of preserved cadavers prior to a live surgical practical with animals may still be favoured; 95.1% of students in one survey agreed this would provide the ideal training (Silva et al 2007).

A recent survey in Europe on animal use in veterinary education indicated that the major factor that would persuade academic staff to introduce alternatives to animal use were good empirical evidence of educational effectiveness, with colleague's recommendation and objections from students also important (Dewhurst and Hemmi 2011). The same survey reported the most common barrier to the introduction of computer-based alternatives was the difficulty in finding suitable resources.

The future

Societal attitudes will continue to suggest that harming or using animals particularly for terminal use, is not justified. Thus veterinary and other science training will need to be continually reviewed and with rising costs anticipated, accept the increased use of new models and cadavers to further reduce, refine and replace animals where possible. The underlying message for both students and staff is to be respectful of all animals, minimise their use and engage in ethics to identify any barriers and work towards a position where the community, faculty and students can agree. Veterinarians must be broadly educated in animal welfare science and understand the complex societal issues associated with the field and be aware of the evolving nature of animal welfare education. Addressing the issue of the use of animals in their own education should provide students with important experience in how to manage the ethical dilemmas that are an inevitable consequence of veterinary practice and enable them to be the respected voice for animals.

References:

ANZCCART (2012) Balancing Harm and Benefit
http://www.adelaide.edu.au/ANZCCART/humane/balance.html

Australian Government. NHMRC. (2004) *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. 7th edn.*
http://www.nhmrc.gov.au/publications/synopses/ea16syn.htm.

Australian Veterinary Association (2009) *Animal experimentation Policy*
http://www.ava.com.au/policy/159-animal-experimentation

Association of American Veterinary Medical Colleges (2010) *Use of Animals in Education Policy* J. Vet. Med. Educ. **37**(10) 114

Batchelor CEM and McKeegan DEF 2012 *Survey of the frequency and perceived stressfulness of ethical dilemmas encountered in UK veterinary practice.* Veterinary Record **170**:19

Bayvel, A.C.D., and Cross, N., (2010) *Animal welfare: A complex domestic and international public-policy issue-who are the key players?* J. Vet. Med. Educ. **37**, 3–12.

Capaldo T (2004) *The psychological effects on students of using animals in ways that they see as ethically, morally or religiously wrong* ATLA. Alternatives to laboratory animals. Vol **32**, SUP1B, pages 525-531

Colonius, T and Swoboda, J (2010) *Student Perspectives on Animal-Welfare Education in American Veterinary Medical Curricula* J. Vet. Med. Educ. **37**(1), 56-60

Dewhurst D and Hemmi A (2011) *A Survey of Animal Use and Alternatives in Higher Education in Europe Altex Proceedings 1/12*, Proceedings of WC8 411-414

Duncan IJH (2006) *The changing concept of animal sentience Applied Animal Behaviour Science* Vol **100**, Issues 1–2; 11–19

Griffon DJ, Cronin P, Kirby B, and Cottrell DF (2000) *Evaluation of a hemostasis model for teaching ovariohysterectomy in veterinary surgery. Vet Surg* **29**(4):309-16.

Knight A. (2007) *The effectiveness of humane teaching methods in veterinary education. ALTEX: Alternatives to Animal Experimentation;* **24**(2):91-109. www.altex.ch.

Levine ED, Mills DS and Houpt KA. (2005). *Attitudes of veterinary students at one US college toward factors relating to farm animal welfare.* J. Vet. Med. Educ. **32**(4), 481-490.

Moore R (2001): *Why I Support Dissection in Science*
Education, Journal of Applied Animal Welfare Science, **4**:2, 135-138

NHMRC (2004) *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*. Commonwealth of Australia, Canberra

Olsen D, Bauer MS, Seim HB and Salman MD.(1996). *Evaluation of a hemostasis model for teaching basic surgical skills.* Vet. Surg. **25**(1), 49-58.

Patronek GJ and Rauch A. *Systematic review of comparative studies examining alternatives to the harmful use of animals in biomedical education.* JAVMA; **230**(1):37-43.

Paul E. and Podberscek A. (2000). *Veterinary education and students' attitudes towards animal welfare.* Vet Rec **146**, 269–272.

Read R (2012) *Proceedings of the Australian Veterinary Association Annual Conferences, AVA Annual Conference, Canberra, Education/Welfare*, May

Rose M and Grant E (2008) Australia's *ethical framework for animals used in research and teaching.* AAWS International Animal Welfare Conference http://www.daff.gov.au/animal-plant-health/welfare/aaws/aaws_international_animal_welfare_conference

Self DJ Olivarez M, Baldwin DC and Shadduck JA (1996). *Clarifying the relationship of veterinary medical education and moral development.* J. Amer. Vet. Med. Assoc. **209**(12), 2002- 2004.

Silva RMG, Matera JM and Ribeiro AAC *New Alternative Methods to Teach Surgical Techniques for Veterinary Medicine Students despite the Absence of Living Animals. Is that an Academic Paradox?* Anatomia, Histologia, Embryologia, Vol **36** (3) 220-224(5)

University of Queensland (2008) *School of Veterinary Science Guidelines on Ethical Concerns on Use of Animals in Teaching*
http://www.humanelearning.info/assets/resources/CO%20Policy%20U%20QLD%202008.pdf

Animal Ethics Committees and Biomedical Research in Singapore

Mark Vinson F. Vallarta
Compliance Veterinarian
Institutional Animal Care and Use Committee
National University of Singapore

Abstract:

Singapore's thrust to become Asia's biomedical hub has indeed spurred growth for the small city-state. As part of this development, the number of animals used for scientific purposes has continually increased. Singapore is home to more than 20 research facilities where animals are used in scientific experiments.

In 2003, the National Advisory Committee on Laboratory Animal Research (NACLAR) was formed to establish national guidelines for the use of animals for scientific purposes. The NACLAR Guidelines, based on the principles of the 3 Rs – Replacement, Reduction and Refinement, were set down to ensure humane and responsible care and use of animals for scientific purposes in Singapore.

Under the Animals and Birds (Care and Use of Animals for Scientific Purposes) Rules 2004, all research facilities which use animals for scientific purposes have to obtain a license from the Agri-Food and Veterinary Authority of Singapore (AVA) and are required to establish their own Institutional Animal Care and Use Committee (IACUC) – the equivalent of an Animal Ethics Committee (AEC).

As Singapore continues to endeavor to attract top scientific talents and serve as a melting pot for world-class biomedical research activities, the use of animals for research, teaching, and testing will continue to increase and AECs will have to keep pace with changes to maintain its crucial role in ensuring that best practices in the welfare and care of animals used for scientific purposes are met.

No paper received

Testing times – Developing awareness while fostering research: Improving health, welfare and production in South African Merinos

Cloete, A.S.[1], Hough, D.[2] and Cloete, S.W.P.[1,3]
[1]Western Cape Department of Agriculture, South Africa,
[2] Biochemistry Department, Stellenbosch University, Stellenbosch, Western Cape, South Africa,
[3] Department of Animal Science, Stellenbosch University, Stellenbosch, Western Cape, South Africa.

Abstract:

Although a well-structured and regulated system, comparable to those supported by ANZCCART has recently been developed in South Africa (SA) for medical research and pre-clinical studies on animals, there is currently no specific legislative framework for supporting the role of an AEC overseeing research on “agricultural animals” (outside academic institutions). This implies that agricultural research institutions in South Africa are currently functioning (at best) in a self-regulated or potentially unregulated fashion, with the South African National Standard for the Care and use of animals for scientific purposes, SANS 10386:2008 (Edition 1) as guideline. Despite this, ethical research in the agricultural field is thriving in SA, as illustrated in the second part of this presentation.

The measurement of the steroid hormone, cortisol (in fish and most mammals such as sheep, cattle, goats and pigs) or corticosterone (in birds, rodents, amphibians and reptiles), has become the gold standard for the assessment of stress in animals. The measurement of cortisol (or corticosterone) reflects the activity of the hypothalamic-pituitary-adrenal (HPA) axis, which may also be used as a tool to genetically select animals with superior robustness. The aim is to have animals that are genetically superior in their ability to overcome stress as this can result in improved health, welfare and production. In South Africa, a project has been undertaken to improve the HPA axis activity of Merino sheep by identifying genetic markers that increases the efficiency of the cortisol response. One such a genetic marker that has already been identified is the *CYP17* genotype, which encodes an enzyme that is crucial in the synthesis of cortisol. Furthermore, selection for the number of lambs born per mating opportunity ultimately leads to a higher cortisol response. The implications of this superior cortisol response in Merinos continue to be investigated in terms of gestation length, maternal behaviour, lamb behaviour, lamb growth, behavioural responses to psychological stress and recovery from physiological stress. In addition to the measurement of cortisol, the concentration of more than 20 steroids are measured to setup steroid profiles, which may be more informative for characterizing the associations between the HPA axis activity and traits of health, welfare and production.

Background on Animal Ethics Committees in South Africa

The NSPCA Research Ethics Unit reported a trend in South Africa away from the use of rodents to the use of production animals and in particular the porcine model for human pharmacology and physiology studies, as well as vaccine production. There is also an increase in wildlife anaesthetic and conservation research, with the adoption of the national standard by wildlife research establishments.

In South Africa, no legislative provision is presently in place for the functioning of Animal Ethics Committees (AECs) evaluating animals used in Agricultural research and teaching. These include the acts that are administered by the national Department of Agriculture, Forestry and Fisheries (DAFF), namely the Animal Protection Act (Act 71 of 1962) (APA) (50 years old) and the Performing Animals Protection Act (Act 24 of 1935) (72 years old), as well as the act that is administered by the Department of Justice, namely the Societies for the Prevention of Cruelty to Animals Act (Act No. 169 of 1993) that governs the organization and management of the National Council of Societies for the Prevention of Cruelty to Animals (NSPCA). In 2009, DAFF produced a framework for the development of a comprehensive policy for the care and protection of animals, which refers to animals used in research and training. However, it has not materialised into an official DAFF policy to date. Thus, apart from the APA, which can deal with any offence in terms of animal welfare, there is currently no specific legislative framework for AEC in South Africa overseeing research on "agricultural animals".

The Medical Research Council (MRC) published an excellent document in 2004, namely "*Guidelines on Ethics for Medical Research: Use of Animals in Research and Training*", which was adapted for use by the Western Cape Department of Agriculture (WC DOA) Departmental Ethics Committee for Research on Animals (DECRA) evaluations, but not all the criteria were directly applicable to "agricultural animal research". The Standards Division of the South African Bureau of Standards (SABS) published a South African National Standard for the Care and use of animals for scientific purposes during 2008, SANS 10386:2008 (Edition 1). This Standard which is based on international standards is the only document available in South Africa that, amongst others, also specifically addresses the use of "agricultural research animals". Although the Standard is not legislatively compulsory, many AECs subsequently adopted it as the research ethics standard, including DECRA.

The Department of Health (DOH) National Health Act (Act 61 of 2003) is the only South African Act which makes mention of research on animals, whereby the National Health Research Ethics Council (NHREC) was established and is responsible:

1. to **determine guidelines** for the functioning of health research ethics committees;
2. **register and audit** health research ethics committees;
3. **set norms and standards for conducting research on humans and animals**, including norms and standards for conducting clinical trials;
4. institute such **disciplinary action** as may be prescribed against any person found to be in violation of any norms and standards, or guidelines, set for the conducting of research in terms of this Act; and
5. advise the National and Provincial Departments of Health on any ethical issues concerning research.

An "Animal Health Ethics Workgroup" drafted the guidelines, norms and standards on behalf of NHREC, incorporating the MRC Guidelines, as well as the SABS Standard, making it legally compulsory. The requirement in terms of "animals" in the National Health Act is, however interpreted to focus only on animals used in pre-clinical studies as models for human health purposes. All health AECs involved in pre-clinical studies therefore have to be accredited with the NHREC and are therefore well regulated. This however, means that all "agricultural animal research" and related AECs are currently excluded from this process.
However, should the interpretation change at a later stage to include all animals, NHREC could become a body comparable to ANZCCART in Australia and New Zealand. Until then, agricultural research institutions in South Africa stay at best, self-regulated or potentially unregulated.

Despite these regulatory challenges, important and ethically responsible agricultural research is being conducted at the WC DOA, of which, the most significant recent example is the measurement of steroid hormones as a tool to improve health, welfare and production in South African Merinos. The research conducted at WC DOA is reviewed by DECRA for ethical approval.

WC DOA projects: Improving health, welfare and production in South African Merinos

The ability of an animal to cope with stress is not only important for the welfare of that animal, but also reflects the animal's ability to express its genetic production potential in a variety of environments (robustness). The importance of incorporating traits of both fitness and production in selection criteria was realized by the research group at the WC DOA in collaboration with the Biochemistry group at Stellenbosch University. A project at the Institute for Animal production (WC DOA) was undertaken in 1986 that involved a divergent selection program where selection was based on the ability of ewes to wean multiple offspring per joining. Two distinct Merino flocks were established that showed a marked divergent response in overall reproduction in the lines selected. The annual rate of genetic improvement in total weight of lambs weaned over three lambing opportunities, in the line selected for multiple rearing ability (H-line), was 1.8% of the overall phenotypic mean (Cloete et al., 2004). A corresponding decline of 1.2% of the overall mean was found in the line selected against their ability to rear multiples (L-line). Genetic selection responses were slightly asymmetric, but resulted in a cumulative difference of 21.2 kg of lambs weaned over three lambing opportunities between the two lines in ewe progeny born in 1995. Furthermore, this line difference was supported by responses in other fitness and production related traits, where the H-line mostly outperformed the L-line in terms of lamb output (Cloete et al., 2004), behavioural stress responses, meat quality (Cloete et al., 2005a), live weight and wrinkle score (Cloete et al., 2005c), lamb survival and mothering ability (Cloete and Scholtz, 1998; Cloete et al., 2005b). Ewes from the H line also had a reduced susceptibility to breech blowfly strike (Scholtz et al., 2010). Moreover, the collective data suggested a probability that L-line animals were more susceptible to stress than H-line contemporaries on the genetic level (Cloete et al., 2005a). A subsequent study compared the activities of the hypothalamic-pituitary-adrenal (HPA) axis between the H- and L-lines in terms of cortisol responses to insulin-induced stress, which confirmed that the H-line had an improved capacity to overcome physiological stress compared to the L-line (Hough, 2012). Furthermore, the cortisol responses of these sheep were related to some behavioural responses to isolation stress (Hough, 2012). The implications for the observed difference in HPA axis activity are presently being investigated and its application in animal breeding will subsequently be explored to select animals that are more robust.

Cortisol is a steroid hormone that is released from the adrenal gland upon stimulation of the HPA axis in response to stress (hypothalamus releases corticotrophin releasing factor, which stimulates the release of adrenocorticotrophic hormone from the pituitary that stimulates the adrenal gland to secrete cortisol). Steroid hormones are vital in the regulation of reproduction, stress management and mineral balance. Androgens, for example, affect muscle weight, bone growth, and serve as precursors of reproductive hormones such as estradiol and testosterone. Reproductive steroid hormones facilitate reproductive endocrine functions. Aldosterone is involved in the regulation of electrolyte homeostasis. Cortisol and corticosterone play a central role in stress responses where their catabolic activity in carbohydrate metabolism mobilizes energy resources. Cortisol, like other steroid hormones, cannot be stored in the body/cells and has to be synthesised *de novo* from the common steroid precursor, cholesterol. The increased supply of any steroid hormone upon a physiological demand is therefore controlled by the enzymes that facilitate their synthesis or the factors that regulate the enzyme activities. This concept has placed the steroid biosynthesis pathway in the spotlight for the research group at WC DOA to identify genetic markers for robustness. One such a genetic marker that has been identified to date is the *CYP17* genotype, which encodes an enzyme that is crucial in the synthesis of cortisol (Hough, 2012).

The measurement of the cortisol (in fish and most mammals such as sheep, cattle, goats and pigs) or corticosterone (in birds, rodents, amphibians and reptiles), is considered to be the gold standard for the physiological assessment of stress and welfare in animals. However, the interpretation of the measurement of only one steroid hormone is not always straightforward, due to the complex interplay between the various adaptation mechanisms that are involved. An important tool that has recently become available to the WC DOA is the simultaneous quantification of more than 20 steroids with ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) to setup steroid profiles for each animal (Hough, 2012).

This UPLC-MS/MS method is more sensitive than conventional immunoassays, requires less sample volume and allows for a holistic view of the steroid biosynthesis pathway. Steroid profiling has proven to be valuable in clinical studies to screen for steroid-related endocrine disease in humans (Miller and Auchus, 2011). The research group at WC DOA intends to utilize steroid profiling to identify animals for breeding purposes. Although the main focus is to identify which steroid profiles are associated with high robustness, its association with other traits (such as traits of reproduction and production) will also be investigated. The implications of this superior cortisol response in Merinos continues to be investigated in terms of gestation length, maternal behaviour, lamb behaviour, lamb growth, behavioural responses to psychological stress and recovery from physiological stress.

One of the present studies at WC DOA involves the regulation of maternal and lamb behaviour by steroid hormones. Maternal behaviour is a function of endocrine and experiential factors, where hormonal control appears to be the main regulator of maternal behaviour (Poindron and Levy, 1990; Kendrick and Keverne, 1991). The exposure to ovarian steroids is essential in sheep for the induction of maternal care at parturition (Poindron et al., 1984). The expression of maternal behaviour in sheep is also modulated by the expression of corticotrophin releasing factor (CRF) from the paraventricular nucleus in the hypothalamus, which varies with the stage of parturition (Broad et al., 1995). CRF agonists also potentiate the expression of maternal behaviour (Keverne and Kendrick, 1991). Maternal behaviour therefore seems to be elicited by neuroendocrine events, specifically dependent on steroid hormone priming and interaction with modulating factors such as CRF (Dwyer et al., 2004).

Furthermore, maternal behaviour has also been correlated with stressful behaviour, where ewes with more ‘calm’ temperament displayed superior mothering behaviour compared to ewes with a ‘nervous’ temperament (Murphy et al., 1994; Murphy, 1999; Dwyer et al., 2004). However, the inferior maternal behaviour may be a reflection of an increased production of glucocorticoids (mainly cortisol) in response to stress. Very high levels of glucocorticoids, as a result of stress during parturition, have been shown to be detrimental to maternal care postpartum (Tu et al., 2005; Weinstock, 2005). In contrast, moderate glucocorticoid concentrations have been positively correlated with postpartum maternal care (Dwyer and Lawrence, 2000; Pickup and Dwyer, 2001; Rees et al., 2004).

Steroid hormones are not only involved in the onset of maternal behaviour, but also the quality of maternal care (Pryce et al., 1998; Dwyer et al., 1999; Dwyer et al., 2004). Various studies have identified individual and breed differences in steroid hormones as correlated with maternal care, but further elucidation about the underlying mechanisms responsible for these variations is required (Pryce et al., 1998; Dwyer et al., 1999; Dwyer et al., 2004; Rees et al., 2004; Bickell et al., 2011). The identification of the factors that contribute to the variation in steroid hormone concentrations and their correlation with maternal behaviour is ultimately seen to assist with genetic selection for robust animals. The study of hormone levels during the peripartum period generally utilizes immunoassays to determine the concentrations of estradiol, progesterone and cortisol (Dwyer et al., 2004). The research group at WC DOA sets out to analyse steroid profiles (~20 steroids in one analysis) with UPLC-MS/MS. This allows for a holistic view of steroidogenesis that aids in the identification of the factors that add to variation in maternal behaviour. Furthermore, the H- and L-line is an ideal animal model to study, since it was demonstrated that the H-line expresses higher maternal care (Cloete et al., 2005b), less stressful behavioural responses to isolation stress (Cloete et al., 2005a) and a superior acute cortisol response to physiological stress (Hough, 2012) compared to the L-line. Against the background of this discussion, it would be worth the while investigating the steroid profiles in the H- and L-lines during the peripartum period in relation to maternal behaviour.

A rapid increase in foetal cortisol in the last 10 – 15 days of gestation is necessary for the final maturation of foetal organs and is also responsible for the onset of the hormonal cascade that results in parturition. Recent data also suggests that the gestation length for H-line lambs is shorter than for L-line lambs (unpublished data). It is possible that the H-line lambs produce more cortisol in those final days of gestation than the L-line, which leads to a shorter gestation length. However, further investigations are required at this time and the implication for the final maturation of foetal organs is unknown.

From this discussion it should be clear that steroid hormones are pivotal for both the survival and welfare of the animal. While serious diseases can manifest when insufficient steroid hormones (or too much) are produced, steroid profiles also hold potential to select animals with superior robustness, reproduction or other production related traits. This approach still requires a great deal of research, but has the advantage that both robustness and production related traits are considered during selection. This concept is crucial to reach sustainable breeding goals and represents ethically responsible research.

Conclusion

The welfare of the livestock population in South Africa and specifically of the WC DOA, which relates to our direct sphere of influence, as well as the mechanisms to ensure ethical evaluation and adequate monitoring of research on animals, is a high priority of the WC DOA. Our goal is to strive to be in line with internationally acceptable standards for ethical research and teaching, albeit voluntarily and by virtue of self-regulation at this point in time.

Acknowledgements

I want to thank the NSPCA Research Ethics Unit for information regarding the research tendencies in South Africa.

References:

Bickell, S., Poindron, P., Nowak, R., Ferguson, D., Blackberry, M., & Blache, D., 2011. Maternal behaviour and peripartum levels of oestradiol and progesterone show little difference in Merino ewes selected for calm or nervous temperament under indoor housing conditions. *Animal* 5, 608-614.

Broad, K.D., Keverne, E.B., & Kendrick K.M., 1995. Corticotrophin releasing factor mRNA expression in the sheep brain during pregnancy, parturition and lactation and following exogenous progesterone and oestrogen treatment. *Molecular Brain Research* 29, 310–316.

Cloete S.W.P. and Scholtz A.J., 1998. Lamb survival in relation to lambing and neonatal behaviour in medium wool Merino lines divergently selected for multiple rearing ability. *Australian Journal of Experimental Agriculture* 38, 801-811.

Cloete S.W.P., Gilmour A.R., Olivier J.J. and van Wyk J.B., 2004. Genetic and phenotypic trends and parameters in reproduction, greasy fleece weight and live weight in Merino lines divergently selected for multiple rearing ability. *Australian Journal of Experimental Agriculture* 44, 745-754.

Cloete, J.J.E., Cloete, S.W.P., & Hoffman, L.C., 2005a. Behaviour of Merinos divergently selected for multiple rearing ability in response to external stimuli. *Small Ruminant Research* 60, 227-236.

Cloete, S.W.P., Olivier, J.J., Van Wyk, J.B., Schoeman, S.J., & Erasmus, G.J., 2005c. Genetic parameters and trends for hogget traits in Merino lines divergently selected for multiple rearing ability. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* 16, 24-27.

Cloete, S.W.P., Scholtz, A.J., Cloete, J.J.E., & Van Wyk, J.B., 2005b. The ability of Merino ewes and lambs to reunite after separation, as affected by divergent selection for ewe multiple rearing capacity. *Australian Journal of Experimental Agriculture* 45, 1131-1137.

Dwyer, C.M., & Lawrence, A.B., 2000. Maternal behaviour in domestic sheep (Ovis aries): constancy and change with maternal experience. Behaviour 137, 1391–1413.

Dwyer, C.M., Dingwall, W.S., & Lawrence, A.B., 1999. Physiological correlates of maternal–offspring behaviour in sheep: a factor analysis. *Physiology and Behavior* 67, 443–454.

Dwyer, C.M., Gilbert, C.L., & Lawrence, A.B., 2004. Prepartum plasma estradiol and postpartum cortisol, but not oxytocin, are associated with interindividual and breed differences in the expression of maternal behaviour in sheep. *Hormones and Behavior* 46, 529–543.

Hough, D., 2012. *Comparison of two CYP17 isoforms: Implications for cortisol production in the South African Merino.* Ph.D. (Biochemistry) dissertation, University of Stellenbosch, Stellenbosch.

Kendrick, K.M., & Keverne, E.B., 1991. Importance of progesterone and estrogen priming for the induction of maternal behavior by vaginocervical stimulation in sheep: effects of maternal experience. *Physiology and Behavior* 49, 745–750.

Keverne, E.B., & Kendrick, K.M., 1991. Morphine and corticotrophin-releasing factor potentiate maternal acceptance in multiparous ewes after vaginocervical stimulation. *Brain Research* 540, 55–62.

Miller W.L. and Auchus R.J., 2011. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocrine Research* 32(1), 81-151.

Murphy, P.M., 1999. *Maternal behaviour and rearing ability of Merino ewes can be improved by strategic feed supplementation during late pregnancy and selection for calm temperament.* PhD thesis, The University of Western Australia.

Murphy, P.M., Purvis, I.W., Lindsay, D.R., Le Neindre, P., Orgeur, P., & Poindron, P., 1994. Measures of temperament are highly repeatable in Merino sheep and some are related to maternal behaviour. *Proceedings of the Australian Society of Animal Production* 20, 247–250.

Pickup, H.E., & Dwyer, C.M., 2001. Does variation in the onset of maternal behavior affect the strength of association between ewes and their lambs? In: *Proc. 35th Int. Congress ISAE*, vol. 95. J.P. Gardner, J.A. Mench, S.P. Heckin (Eds.) Center for Animal Welfare at UC Davis, Davis, CA.

Poindron, P., & Levy, F., 1990. Physiological, sensory and experiential determinants of maternal behaviour in sheep. In *Mammalian parenting: biochemical, neurobiological and behavioral determinants* (ed. NA Krasnegor and RS Bridges), pp. 133–156. Oxford University Press, New York.

Poindron, P., LeNeindre, P., Lévy, F., & Keverne, E.B., 1984. Les méchanisms physiologiques de l'acceptation du nouveau-né chez la brebis. *Biol. Behavior* 9, pp. 65–88.

Pryce, C.R., Abbott, A.H., Hodges, J.K., & Martin, R.D., 1988. Maternal behaviour is related to prepartum urinary estradiol levels in the red-bellied tamarin monkeys. *Physiology and Behavior* 44, 717–726.

Rees, S.L., Panesar, S., Steiner, M., & Fleming, A.S., 2004. The effects of adrenalectomy and corticosterone replacement on maternal behavior in the postpartum rat. *Hormones and Behavior* 46, 411–419.

Scholtz, A.J., Cloete, S.W.P., Van Wyk, J.B., Kruger, A.C.M., & Van der Linde, T.C.de K., 2010. Influence of divergent selection for reproduction on the occurrence of breech strike in mature Merino ewes. *Animal Production Science* 50, 203-209.

Tu, M.T., Lupien, S.J., & Walker, C.D., 2005. Measuring stress responses in postpartum mothers; perspectives from studies in human and animal populations. Stress 8, 19–34.

Weinstock, M., 2005. The potential influence of maternal stress on development and mental health of the offspring. *Brain Behavior and Immunity* 19, 296–308.

NSPCA Annual Report 2010-2011. http://www.nspca.co.za

A review of the effects of space allocation and housing density on measures of wellbeing in laboratory mice

Alexandra Whittaker
University of Adelaide

Abstract:

In the majority of countries where there are legislative requirements pertaining to the use of animals in research, figures are quoted for minimum cage sizes or space allocation to be provided per animal. These figures are generally based on professional judgement and are in common usage. However, there is a growing trend and expectation that welfare science should inform regulatory decision-making. In addition, the increased globalisation of the animal-related research environment would imply the need for greater harmonisation of requirements across jurisdictions. Given the importance of the potential welfare influences of cage size on the animals themselves, this paper presents the latest scientific knowledge on this topic in one of the most commonly used animals in research, the mouse. A comprehensive review of studies in laboratory mice was undertaken, examining the effects of space allocation per animal and animal density on established welfare indicators. To date, animal density studies have predominated, and the effects of space allocation *per se* are still relatively unclear. There is also considerable controversy surrounding the interpretation and collation of data, obtained using a number of different welfare science approaches. This information will guide those involved in facility management or legislative review, and provide a more solid foundation for further studies into the effects of routine husbandry practices on animal welfare.

No paper received

The Importance of the Experimental Environment on the Stress Response in Sheep

Maggie Honeyfield – Ross
University of Auckland
New Zealand

Abstract:

Acclimatisation to experimental environments is important not only for animal welfare but also for good results. A large part of our work involves metabolic and endocrine balance, which can be very affected by stress. Sheep are not known for their easy going temperament so undue stress can harm experimental outcomes of interest.

The hypothalamic-pituitary-adrenal axis (HPAA) helps mediate stress responses. Corticotrophin releasing hormone (CRH) and arginine vasopressin (AVP) are released from the hypothalamus in response to stress, stimulating the pituitary to release adrenocorticotrophic hormone (ACTH). This acts on the adrenal gland to release cortisol, which inhibits the action of insulin, affecting blood glucose concentrations. Plasma cortisol concentrations vary during the day/night cycle and are also influenced by feeding and sleep, so it is important that the animal is introduced to a routine including a dark/light cycle, regular feed times and a good night's sleep.

A CRH+AVP co-challenge is performed to assess HPAA responsiveness. The dose is administered intravenously and blood samples are taken regularly over the next 120 min. The rise in plasma ACTH and cortisol concentrations is then measured. High cortisol levels normally 'feed-back' to inhibit release of CRH, AVP and ACTH.

Major stress factors for sheep are human contact, being alone, pain and excitement at the possibility of food. Acclimatisation to human contact, pain minimisation (catheters rather than repeated jugular puncture), and ensuring visual contact with other sheep is essential to reducing the animals' background stress and ensuring that CRH/AVP testing yields representative results.

As a senior research technician working for the Liggins institute at the University of Auckland, I am based on a sheep research farm and involved with a variety of experiments. Most of our research is biomedical but over the last year or so we have started diversifying into some agricultural based experiments. Without going into too much detail, our biomedical research involves looking at the growth and development of the foetus *in utero* and often following their subsequent development postnatally.

As I'm sure you all know, even if you haven't had much to do with sheep, they are not known for their easy going nature. They scare easily and are not always comfortable around people. However, accurate experimental outcomes are the name of the game and undue stress can interfere with this so we need to provide a stress-free, welfare friendly environment for these sheep. A large part of our work involves studying metabolic and endocrine balance, which can be profoundly affected by stress, so it is in our best interests to have happy, healthy and well looked after animals.

As mentioned previously endocrine balance is something we commonly study, in particular assessing hypothalamic-pituitary-adrenal axis (HPAA) responsiveness. Corticotrophin Releasing Hormone (CRH) and Arginine Vasopressin (AVP) are released from the hypothalamus in response to stress, stimulating the pituitary to release adrenocortitrophic hormone (ACTH). This acts on the adrenal gland to release cortisol which inhibits the action of insulin, “releasing glucose” ready for the ‘flight’ response (See Figure 1). The increased cortisol levels normally feedback to inhibit release of CRH, AVP and ACTH. The release of CRH from the hypothalamus can be influenced by stress, physical activity, illness, cortisol levels in the blood and the sleep/wake cycle.

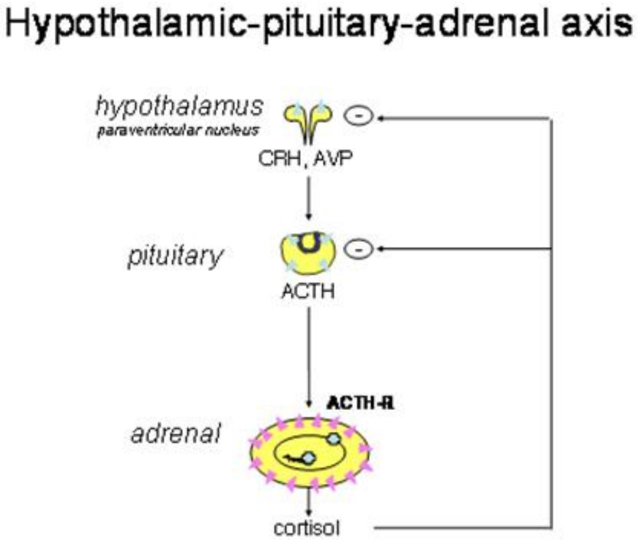


Figure 1: Schematic representation of how the Hypothalamic – pituitary – adrenal axis works

We have a number of processes that we use to try and prevent our sheep from being overly stressed when being used for research. Getting sheep used to people, living indoors, and our indoor feed, all takes time and repetition. As most of our research involves foetuses and then following the lambs into adulthood, our acclimatisation has to begin with the pregnant ewe. Assuming a normal pregnancy, we need to ensure our pregnant ewes are happy and healthy so we get “normal” foetuses and lambs to study.

We use 5 year old ewes because these sheep are experienced mums and probably haven’t had too many problems lambing. Our ewes are being handled regularly before they are even mated. We do foot, teeth and udder checks on all our ewes to a make sure they are in good health and should therefore have another successful pregnancy. In keeping with the 3R’s(that is replacement, reduction, and refinement), we try to only use experienced healthy ewes so we have as few pregnancy/lambing/lactation issues as possible.

As mentioned, our ewes are being handled from before they are even mated and this is continued right through the pregnancy. We have a relatively small farm so the ewes are moved with and without dogs on a regular basis. They are pregnancy scanned twice and shorn, so get regular people contact right through pregnancy.
Approximately ¾ of the way through the pregnancy the ewes start being introduced to our indoor feed. The indoor feed is presented as pellets which have been specifically designed for sheep and so they are a nutritionally balanced food source. These pellets are fed to the ewes, in the paddock for 2 weeks prior to them coming inside, so when the ewes come into the feedlot for lambing they are acclimatised to this feed and don’t go on a hunger strike. In saying that, there can be a few animals that just don’t acclimatise and are overly stressed, so these ones will be put back out onto pasture and subsequently sold. When the ewes come inside, they initially

go into a group pen for around a week. This allows them to get used to being inside and to more regular contact with people.

We always bring in a few more animals than required for the project. Once they have been inside for a week we can go through the ewes again and check bodyweights, body condition scores, temperament and the udders. This way we are doing our absolute best to pick the ewes that will hopefully have the fewest problems and be the least stressed by the change in their living conditions. Apart from the importance of the 3R's, it is never nice losing a sheep or lamb during lambing or having to euthanise an animal, so it is really important to select the best subjects for the project.

The selected animals then get moved into individual pens. These pens are approximately 1.2 x 1.4m in size and are made from wire so that the sheep can each other. A big stressor for sheep is being alone, so as you can see from the picture the pens have be designed to minimize any stress associated with isolation. We are also very careful to ensure that we never have a sheep alone in the feedlot. If it is a quiet time and we have no experimental sheep in the building but have to use it to house a sick sheep, we make sure we bring in a buddy for it. While we have sheep inside (pregnant or not) we minimise distressing noises or disturbances. We want the sheep to be able to express normal behaviours such as napping and ruminating.

The sheep are let out of their pens on a daily basis during cleaning to "stretch their legs" and also when they are walked around to the scales for regular weighing. The majority of the lambs born get regular body measurements and weights taken, often monthly until they are 18 months. This plays a big part in acclimatising the sheep to people and the feedlot. They become desensitised to these stressors and it doesn't become such an issue for them when they are required to stay inside for the duration of experiments.

Training of staff is a vital part of ensuring the sheep are happy and healthy. It is not only for the welfare of the sheep but also for the safety of the staff. Knowing how to best restrain a sheep or how to get a sheep to move where you want it to, are both important aspects and being able to do these things effectively is imperative for both the sheep's well being and for the safety of staff. It is also important to train new staff on health issues specific to sheep so that sick animals can be detected early and treated accordingly.

There are factors that occur on a daily basis that will stress/excite the sheep and cause their cortisol levels to fluctuate even though these various measures have been taken to help them become acclimatised to living indoors. Feeding is a big one. Sheep seem to thrive on routine, so we feed around the same time every morning. Obviously this is an exciting time for the sheep, they call out, play with their feed buckets and some even climb their gates. So this sort of thing needs to be taken into account if we are going to be doing a challenge looking at the HPA axis. Another factor to consider is the day/night cycle. In healthy individuals, plasma cortisol levels rise rapidly after waking, generally peaking within 30-45 minutes. We try to control these factors by having our lights on timer, so the light/dark cycle stays consistent. Minimising pain is also key when trying to have an animal as stress-free as possible. Many of the challenges we perform on our sheep involve taking multiple blood samples, so rather than poking a needle in the sheep each time, we place a jugular catheter into each side of their neck. Using a local anaesthetic to minimise the pain, this procedure only takes 5-10 minutes and ensures that when sheep partaking in numerous challenges where blood is being collected, it is a pain free experience.

One of the experiments that we conduct to assess the responsiveness of the HPA axis is called a CRH/AVP challenge. This involves giving a very small bolus of CRH and AVP intravenously and taking blood samples at various time points for the following four hours. More often than not, our sheep are just lying in their pens resting while these challenges are going on and don't even bother getting up for us when we go in for a blood sample. Once collected, the samples are centrifuged, frozen and analysed at a later date.

To illustrate the kind of data we obtain from these trials, I have included an example of some results from a CRH/AVP challenge (See Figure 2). In this particular experiment maternal nutrition around time of conception was being investigated. The red line represents the ewes that were on a reduced plane of nutrition and the blue line represents the ewes that we on a normal plane of nutrition. The challenge was done 5 times over a 3 month period. The middle three graphs are when the nutrition levels were different between groups. There appears to be two things going on here. Firstly, being undernourished appeared to put more stress on the body leading to a higher production of ACTH. Secondly, the adrenal gland in the undernourished group doesn't seem to be responding normally to the ACTH as expected so that they would also have higher cortisol levels. In the first and last graphs, the feed levels are the same and as shown in these graphs, ACTH and cortisol production are very similar. This suggests that the stressor in this case was inadequate nutrition.

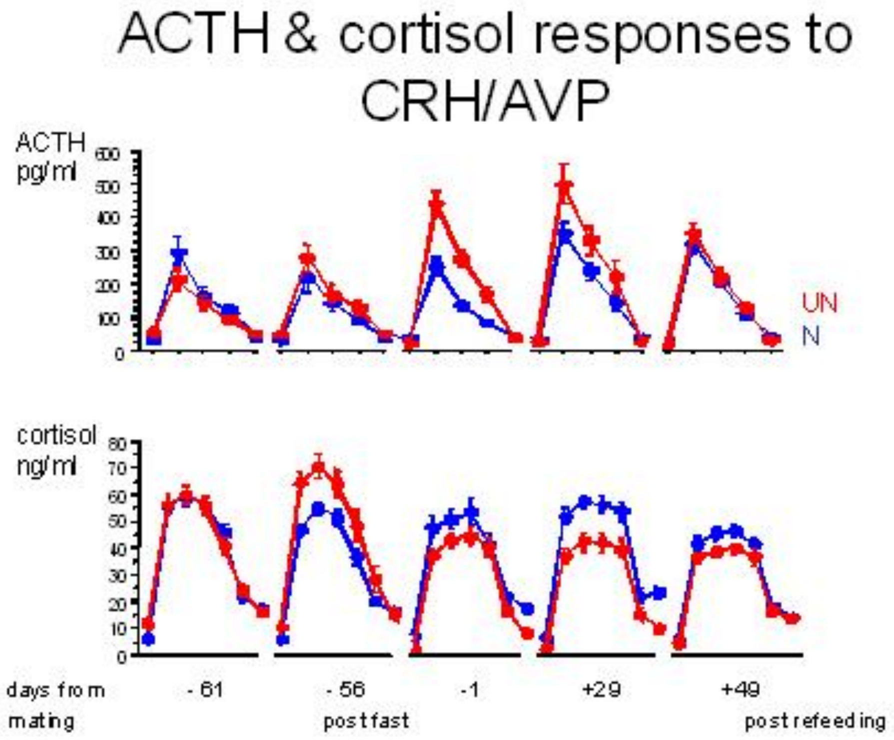


Figure 2: Assesses the HPAA responsiveness during a CRH/AVP Challenge

There are a lot of factors that must be considered and a lot of time required to ensure our sheep are the happy, stress-free subjects we need for this work, and of course lots of hands on care and interaction from the day they are born is a big contributing factor.

Developing Monitoring Guidelines for Fish Welfare

Miriam Sullivan and Dominique Blache

School of Animal Biology, The University of Western Australia

Abstract:

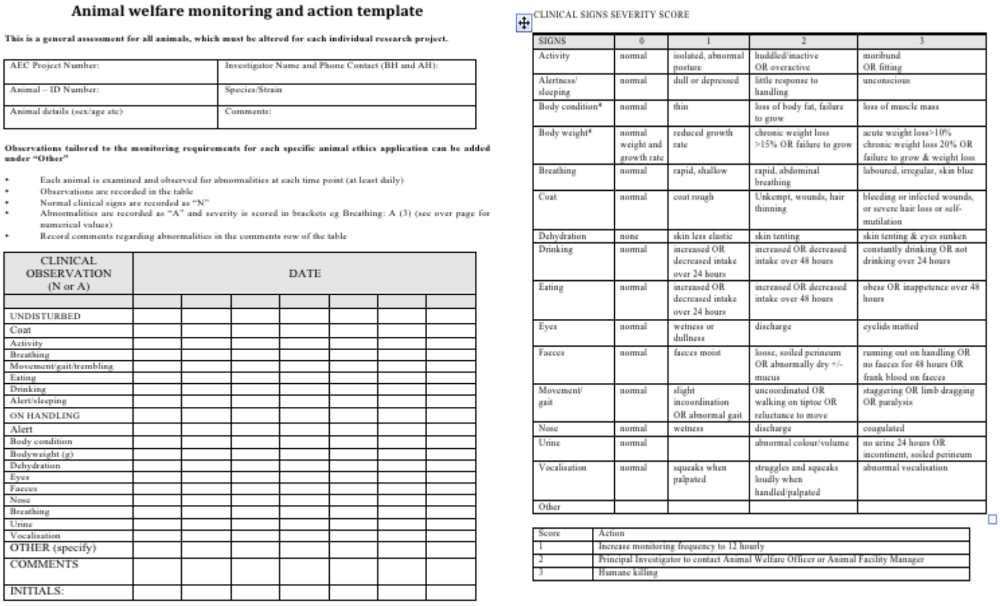
The NHMRC guidelines for wellbeing of animals used in scientific research aspire to treat all vertebrate animals equally. In practice however, the welfare monitoring templates provided as examples have far lower standards for fish compared to mammals. Mice for example, require individual monitoring and action is taken when a score of 1 is recorded. By comparison, fish are monitored in schools and no action is required until a score of 4 is reached. We propose an improved monitoring sheet for fish with additional measures of health and earlier intervention. In particular, this monitoring schedule is more sensitive to species-specific problems such as parasite and disease outbreaks that can cause high mortality rates among fish if not detected early.

Background

The *Australian code of practice for the care and use of animals for scientific purposes*, published by the Australian National Health and Medical Research Centre (NHMRC), requires all researchers to routinely monitor animal health and wellbeing (National Health and Medical Research Council, 2004). Researchers need to justify the monitoring parameters they will follow to the satisfaction of their AEC: How will monitoring be done? How often will monitoring occur? When you will intervene if there is a problem? What action will you take? Who is responsible? (Section 2.2.16).

All of these questions can be addressed by using appropriate score sheets for animal monitoring. Score sheets are a form of marking rubric used to quantitatively score the animal's health, with set scores triggering specific interventions by the researcher. First suggested by Morton and Griffiths (1985), score sheets help to standardize the process of measuring animal health and welfare, increase objectiveness of monitoring, minimize differences in judgment between researchers, helping to measure the impact of procedures and can be tailored to species, strain and procedure specific needs (Hawkins et al., 2011). A good score sheet uses indicators that are quick and simple to measure, non-invasive, reliably recognizable, specific and are relevant to animal welfare (Hawkins et al., 2011, Van der Meer et al., 2001). For example, the template provided by Deakin University (Figure 1) lists 15 health measures against which mice should be scored on a daily basis. A normal mouse is scored at zero; action is taken when a mouse receives a score of one; and a mouse that scores three is humanely killed. In the UK, most researchers use subjective 'clinical observation sheets', while score sheets are used by just 32% of laboratories (Hawkins, 2002).

For model species such as mice, which have a long history of being used in research, there are many templates available to help researchers create their own monitoring rubrics. Comparatively, templates for fish monitoring are far less detailed and normally look at observations of the entire school, rather than individual fish. Even when more detailed criteria are provided, the scores to initiate action may be set too high. For example, in the best example found, a mortality rate of >1% per day scores a three, which is within the range of 0-4, assigned for 'normal' health. In this case, all the fish could potentially die within one year and the researcher would not be required to take any action at all.



Animal welfare monitoring and action template

This is a general assessment for all animals, which must be altered for each individual research project.

AEC Project Number:	Investigator Name and Phone Contact (BH and AH):
Animal – ID Number:	Species/Strain
Animal details (sex/age etc)	Comments:

Observations tailored to the monitoring requirements for each specific animal ethics application can be added under "Other"

- Each animal is examined and observed for abnormalities at each time point (at least daily)
- Observations are recorded in the table
- Normal clinical signs are recorded as "N"
- Abnormalities are recorded as "A" and severity is scored in brackets eg Breathing: A (3) (see over page for numerical values)
- Record comments regarding abnormalities in the comments row of the table

CLINICAL OBSERVATION (N or A)	DATE						
UNDISTURBED							
Coat							
Activity							
Breathing							
Movement/gait/trembling							
Eating							
Drinking							
Alert/sleeping							
ON HANDLING							
Alert							
Body condition							
Bodyweight (g)							
Dehydration							
Eyes							
Faeces							
Nose							
Breathing							
Urine							
Vocalisation							
OTHER (specify)							
COMMENTS							
INITIALS:							

CLINICAL SIGNS SEVERITY SCORE

SIGNS	0	1	2	3
Activity	normal	isolated, abnormal posture	huddled/inactive OR overactive	moribund OR fitting
Alertness/ sleeping	normal	dull or depressed	little response to handling	unconscious
Body condition*	normal	thin	loss of body fat, failure to grow	loss of muscle mass
Body weight*	normal weight and growth rate	reduced growth rate	chronic weight loss >15% OR failure to grow	acute weight loss>10% chronic weight loss 20% OR failure to grow & weight loss
Breathing	normal	rapid, shallow	rapid, abdominal breathing	laboured, irregular, skin blue
Coat	normal	coat rough	Unkempt, wounds, hair thinning	bleeding or infected wounds, or severe hair loss or self-mutilation
Dehydration	none	skin less elastic	skin tenting	skin tenting & eyes sunken
Drinking	normal	increased OR decreased intake over 24 hours	increased OR decreased intake over 48 hours	constantly drinking OR not drinking over 24 hours
Eating	normal	increased OR decreased intake over 24 hours	increased OR decreased intake over 48 hours	obese OR inappetence over 48 hours
Eyes	normal	wetness or dullness	discharge	eyelids matted
Faeces	normal	faeces moist	loose, soiled perineum OR abnormally dry +/- mucus	running out on handling OR no faeces for 48 hours OR frank blood on faeces
Movement/ gait	normal	slight incoordination OR abnormal gait	uncoordinated OR walking on tiptoe OR reluctance to move	staggering OR limb dragging OR paralysis
Nose	normal	wetness	discharge	coagulated
Urine	normal		abnormal colour/volume	no urine 24 hours OR incontinent, soiled perineum
Vocalisation	normal	squeaks when palpated	struggles and squeaks loudly when handled/palpated	abnormal vocalisation
Other				

Score	Action
1	Increase monitoring frequency to 12 hourly
2	Principal Investigator to contact Animal Welfare Officer or Animal Facility Manager
3	Humane killing

Figure 1. *Example of a score sheet for mouse monitoring (Deakin University, 2012).*

There is an urgent need for an adequate monitoring sheet to be generated for use with fish in research because 1) under NHMRC guidelines fish are accorded that same respect as any other vertebrate (National Health and Medical Research Council, 2004); 2) in practice, both researchers and Animal Ethics Committees need to pay greater attention to fish welfare and 3) fish are becoming increasingly popular as an experimental animal. In 2009, over 1.5 million fish were used for teaching and research in Australia, the most popular of any laboratory animal (Humane Research Australia, 2009). This paper describes the development of a new monitoring sheet (Appendix 1) as part of a project on welfare of aquarium fish. We aimed to produce a monitoring sheet that was:

1. Useful for monitoring individuals;
2. Uses non-invasive indicators that are sensitive to fish health;
3. Specific to the needs of the species;
4. Could be used as a template by other researchers and / or their AEC.

1. Used for monitoring individuals

The monitoring sheet prescribes weekly monitoring for individual fish, temporarily increasing to daily morning following stressful procedures.

This is a very easily achievable level of monitoring for our study, in which only 20 fish are used. Since monitoring takes less than two minutes per fish (providing no problems are identified), it is easy to check four fish each weekday while they are being fed.

2. Non-invasive indicators that are sensitive to small changes in fish health.

Handling can be extremely stressful for fish (Brydges et al., 2009), so it is important that the indicators used can be monitored by visual observation. All of the indicators chosen are behavioural or physical and are easy to recognise and monitor.
All of the indicators are based on known signs of distress in fish. For example, a reduced response to stimuli is associated with pain (Sneddon et al., 2003); increased breathing and loss of equilibrium with stress (Sneddon et al., 2003, Newby and Stevens, 2008); and social isolation

with parasitic infections (Croft et al., 2011). Three of the indicators (colour, fins and skin) relate to the skin of the fish as this is one of the most sensitive markers of health- stress alone can cause skin lesions in some fish (Noga et al., 1998, Johansen et al., 2006). These monitoring indicators include both behavioural and physical measures used by specialist fish vets (Loh and Landos, n.d.).

Diagnosing illness in fish can be difficult. Often by the time a problem is obvious, it may too late to save the fish (Johansen et al., 2006). It is therefore important to detect and take action on problems as early as possible. The monitoring sheet has an extremely low score threshold (two) to prompt an increase to daily monitoring and implementing treatment. It also requires the researcher to contact an expert for treatment of any fish scoring four or above.

Obtaining expert advice is important, as the specific cause of a problem is often complicated. Subclinical issues, such as poor water quality, may not kill the fish, but do make it susceptible to more obvious diseases and infections (Loh and Landos, n.d., Johansen et al., 2006). If the underlying stressor isn't treated promptly, then opportunistic diseases will continue to affect more fish.

3. Specific to the needs of the species

This monitoring sheet is specifically designed for goldfish (*Carassius auratus*). This example was selected as goldfish monitoring looks closely at the colour, skin and fin condition of the fish. This is specifically to pick up early indicators of common goldfish parasites such as whitespot (*Ichtyopthirius* spp.) and flukes (*Gyrodactylus* and *Dactylogyrus* species) (Loh and Landos, n.d.). There are over 20, 000 species of fish and although animal ethics reports often do not distinguish between them (Johansen et al., 2006), they can have vastly different needs.

4. Could be used as a template for other researchers.

The monitoring sheet should be useful for most scientific research that follows the three R's of animals ethics (replacement, refinement and reduction) (Russell and Burch, 1959): scientists should already be using a minimal numbers of animals in a controlled environment. It is relatively easy for scientists to adapt the guidelines for other species, for example, including 'erratic swimming' under the movement section would help to detect herpes virus in koi (Matsu et al., 2008).

Limitations

One of the main problems with score sheets is that they are time consuming to implement, especially with large number of animals. One solution is to make daily visual observations of animals, but only fill out individual scoring sheets when a problem is noted. Van der Meer et al. (2001) argues that this system is more subjective and less formal, meaning that some problems could be overlooked. However, it may be a useful system for monitoring large numbers of shoaling fish where it is impractical to tag individuals. Another option is to use a representative sample of the population (Johansen et al., 2006). For example, in aquaculture research large, opaque tanks that make visual observations difficult are often used, but 1-5% of the shoal could be captured regularly for detailed monitoring.

Another limitation is that many of the indicators used are 'iceberg indicators'. These are indicators that could be indicative of multiple causes (Hawkins et al., 2011). For example, fish breathing at the surface could be suffering from a lack of dissolved oxygen, high nitrogen levels or parasitic infection of the gills. These indicators are still important for monitoring because they are sensitive to welfare, but should be interpreted with caution. The intervention point for a score of 2-3 is based on a statement that researchers should increase monitoring and implement treatment, thereby allowing animal technicians to use their own judgment and experience to address iceberg indicators.

Johansen et al. (2006) offers a good overview of important environmental parameters to monitor, as well as other options for measuring fish welfare. Our monitoring sheet does not provide room to record the temperature, pH and water quality. These environmental factors are extremely important determinants of fish welfare, however they should be monitored on a daily basis and recorded separately to observations of individual fish health.

Conclusions

The health and welfare of individual fish used in research is often overlooked. We have presented a monitoring sheet that is easy, non-invasive and quick to implement. Although this form of monitoring will not be suitable for all fish research, we encourage other researchers to use our score sheet as a template to improve fish welfare.

Acknowledgements

Dr Richmond Loh, for veterinary advice and aid concerning goldfish disease and treatments

References

Brydges, N. M., Boulcott, P., Ellisa, T. & Braithwaite, V. A. 2009. Quantifying stress responses induces by different handling methods in three species of fish. *Applied Animal Behaviour Science,* 116**,** 295-301.

Croft, D. P., Edenbrow, M., Darden, S. K., Ramnarine, I. W., Oosterhout, C. V. & Cable, J. 2011. Effect of gyrodactylid ectoparasites on host behaviour and social network structure in guppies *Poecilia reticulata*. *Behavioural Ecology and Sociobiology,* Online ahead of print.

Deakin University. 2012. *Animal Welfare Monitoring Template* [Online]. Available: http://www.deakin.edu.au/research/integrity/animal/forms.php [Accessed 6 July 2012].

Hawkins, P. 2002. Recognizing and assessing pain, suffering and distress in laboratory animals: a survey of current practice in the UK with recommendations. *Laboratory Animals,* 36**,** 378-395.

Hawkins, P., Morton, D. B., Burman, O., Dennison, N., Honess, P., Jennings, M., Lane, S., Middleton, V., Roughan, J. V., Wells, S. & Westwood, K. 2011. A guide to defining and implementing protocols for the welfare assessment of laboratory animals: eleventh report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory Animals,* 45**,** 1-13.

Humane Research Ausytralia. 2009. *Statistics: Animal use in research and teaching, Australia* [Online]. Available: http://www.humaneresearch.org.au/statistics/ [Accessed 6th July 2012].

Johansen, R., Needham, J. R., Colquhoun, D. J., Poppe, T. T. & Smith, A. J. 2006. Guidelines for health and welfare monitoring of fish used in research. *Laboratory Animals,* 40**,** 323-340.

Loh, R. & Landos, M. n.d. *Fish Vetting Essentials*.

Matsu, K., Honjo, M., Kohmatsu, Y., Uchii, K., Yonekura, R. & Kawabati, Z. I. 2008. Detection and significance of koi herpesvirus (KHV) in freshwater environments. *Freshwater Biology,* 53**,** 1262-1272.

Morton, D. B. & Griffiths, P. M. H. 1985. Guidelines on the recognition of pain, distress and discomfort in experimental animals and a hypothesis for assessment. . *Veterinary Record,* 116**,** 431-436.

National Health and Medical Research Council 2004. Australian Code of Practice for the care and use of animals for scientific purposes. Australian Government.

Newby, N. C. & Stevens, E. D. 2008. The effects of the acetic acid "pain" test on feeding, swimming, and respiratory responses of rainbow trout (Oncorhynchus mykiss). *Applied Animal Behaviour Science*, 114**,** 260-269.

Noga, E. J., Botts, S., Yang, M. S. & Avtalion, R. 1998. Acute stress causes skin ulceration in striped bass and hybrid bass (Morone). *Veterinary Pathology*, 35**,** 102-107.

Russell, W. M. S. & Burch, R. L. 1959. *The principles of humane experimental technique*, London, `Methuen.

Sneddon, L. U., Braithwaite, V. A. & Gentle, M. J. 2003. Novel object test: examining nociception and fear in the rainbow trout. *Journal of Pain*, 4**,** 431-440.

Van Der Meer, M., Rolls, A., Baumans, V., Oliver, B. & Van Zutphen, L. F. M. 2001. Use of score sheets for welfare assessment of transgenic mice. *Laboratory Animals*, 35**,** 379-389.

Appendix One. Template score Sheet for monitoring individual fish

AEC Project No:	**Investigator Name and Contact:**
Animal ID:	**Species:** Goldfish

Signs	**Clinical Observation (N or A)**											
Date / Time												
Activity												
Behaviour												
Buoyancy												
Breathing												
Colour												
Eating												
Fins												
Movement												
Skin												
Other (Specify)												
Total and Comments												
Initials												

- Examine each animal for abnormalities and record observations in the table
- Observations to be made **weekly** EXCEPT-
- Daily monitoring for three days following introduction to tank and experimental procedures
- Normal clinical signs recorded as ‘N’
- Abnormalities recorded as ‘A’ and severity indicated in brackets eg. Skin A(2)

- Record comments concerning abnormalities in the comments section

Contact Details of Researchers

CLINICAL SIGNS SEVERITY SCORES

SIGNS	0	1	2
Activity	Normal	Dull or slow OR overactive	Little response to stimuli OR overreacts. **Lethergic and non-responsive.**
Behaviour	Normal	Keeping slightly apart from other fish	Isolated from other fish
Buoyancy	Normal	Swimming is a little off-balance	Loses equilibrium when not swimming
Breathing	Normal	Gill beat rate is a little faster or slower than normal	Breathing heavily or **breathing excessively at the surface.**
Colour	Normal	Slightly dulled or **with black or white spots.**	Dull or **extensive black or white patches.**
Eating	Normal	Increased OR decreased over **24** hours	Increased or decreased over **48** hours
Fins	Normal	A little frayed at the edges	Split or ragged fins
Movement	Normal	Rubbing against ground	Keeping still near the bottom of the pond
Skin	Normal	Thickened mucous or small spots	Trailing mucous, spotting or swelling; **skin lesions**.

Intervention Criteria		
Total Score	**Health**	**Action Required**
0-1	Good	Continue routine monitoring
2-3	Fair	Increase to daily monitoring. Implement treatment if practical.
4-8	Poor	Contact expert, treat immediately. Consider pain relief or euthanasia.
9+	Very Poor	Euthanise

Contact Details of Researchers

Education of Undergraduate Animal Science Students in Professional Practice – Can we ensure compliance and educational outcome?

Jo-Anne Chuck
University of Western Sydney

Abstract:

The University of Western Sydney has 80 students per year which complete a Field Project where animals are used for research involving clients from industry, research organisations, and government agencies. In many cases the activities are low risk but often fall under the jurisdiction of the ACEC. From an institutional perspective, ensuring compliance around welfare issues is problematic as the expertise and animal facilities are often controlled by the client. In research organisations where an ACEC exist, reciprocal approval is used, however in the majority of cases no committee exists. This has lead to the situation where together with the client and student, staff from the institution are named as investigators (teachers) with little or no expertise or control in the project. The volume of applications also makes it impossible for formal agreements to be arranged for these short term projects and for the inspection of all animal holding facilities and field work sites as per the Code of Practice. To address this we have been delineating the roles and responsibilities of institutional staff, clients and students in these situations. This has been paramount for satisfaction of both the ACEC and stakeholders so the important educational outcomes can be achieved for these students.

Introduction

The Australian university sector is rapidly expanding in student numbers and diversity with the government aiming to have 40 % of all 25-34 year olds having a bachelor's degree by 2024 (Transforming Australia's Higher Education System, 2009). At the University of Western Sydney (UWS) a 33% increase in undergraduate student numbers has occurred in the last ten years with some of these students being "first in family" to go to university and have had little prior exposure to the discipline area in which they are enrolled.

There has also been a change in the usage of animals for teaching at the university reflecting the change in demand for courses. As demand for traditional agriculture degrees has fallen, students have moved into more broadly-based animal science-focused degrees (Old and Spencer (2011)). These students have varied interests and thus need to be exposed to a broader range of animals compared with those attending university in the past. They are looking for experience in agricultural, companion and wildlife animal species with the view to obtain wide ranging careers such as wildlife officers and animal handlers in wildlife or companion animal organisations, as well as traditional agricultural positions.

In the past UWS staff have taught a "systems thinking" approach and provided 'real-world' experiences in subjects. This involved students working on projects in an area of their career interest with an external client. A final report is provided to the client after they have developed, planned, conducted and analysed data on the project. This type of engaged learning or learning in context has educational benefits especially promoting deep learning and graduate attributes such as communication and confidence in working within the profession. Such completion of the project subjects is an essential learning component in the animal science degrees. This is supported by employer surveys which indicate that graduates are expected to have discipline knowledge but more importantly be able to effectively communicate, work in teams, use critical reasoning and analytical skills as well as having knowledge of the industry (Graduate Outlook Report 2011).

There has been a recent increase in the popularity of students wanting to undertake such projects. As the majority of animal-based projects require animal care and ethics approval through the institution, the number of applications each year has placed increased pressure on students, academic staff and the committee.

Usually the projects are straight forward but on occasion do perturb the animals. Examples of projects include wildlife surveys, development of behavioural enrichment in captive settings, husbandry modifications and improving pest animal eradication programs. From an animal care and welfare perspective the degree of risk of various projects also varies.

This paper discusses the ongoing process of working with academic staff and the Animal Care and Ethics Committee (ACEC) to work through issues regarding the approval process and the need to gain successful learning outcomes for students. This is complicated as the delineation of animal care and welfare responsibility between the client and university staff is problematic and needs to be clear while still maintaining beneficial outcomes for the client. In addition, mechanisms for meeting the legislative requirements for animal care and ethics approval are also discussed with the view to ease the relationship tensions between all parties.

The committee's perspective

Some of the issues raised as a result of these changes over the last 4-5 years:

- Increased workload as a result of the increased number of applications, and because student numbers and participants change, project amendments are very common leading to an increase in administrative load.
- The quality of applications is often poor. Students and clients have very little experience with committee dealings and the academic in charge of the subject has to deal with large volumes of applications.
- Committee members have resigned as they feel they cannot spend adequate time on applications.
- Committee does not have expertise that covers all species and all types of projects due to the diversity of clients.
- Increased pressure to meet deadlines to ensure students are not disadvantaged.
- Concerns regarding lines of responsibility for the project as the students and academic staff are members of the university; however the conduct of the project is managed by external personnel (i.e. the client).
- Delegated academic staff do not have expertise in the project but are responsible for the administration of the unit or subject.
- Administrative follow up on progress and/or final reports can be difficult since clients and students may no longer be associated with the university at that point of time.
- Non-institutional applicants (i.e. the client) cannot directly submit applications to the committee so must be overseen by a staff member.
- Committee relies on the disclosed expertise of the client and must assume that animal interaction and/or holding facilities are compliant with Department of Primary Industry codes of practice/guidelines or other reporting codes for the species involved as inspections are not possible due to travel distances and number of sites.

The university academic staff perspective

- Staff are looking for educational outcomes to allow the student to move from being a novice to that of a professional in the industry. This includes students developing skills in research and animal behavioural assessment as well as self-responsibility, project management and communication skills.
- The need to rely on external clients due to large numbers of students with diverse interests.
- No incentive to be involved given that staff are time poor and peer appreciation is measured by publication and grant outcomes.
- Increased pressure to reduce the number of practical or hands-on style subjects in favour of less face-to-face teaching with more innovative, flexible and online content.
- Lack of academic staff expertise in completing applications, animal specific knowledge or project-based knowledge.
- Academic staff are often unable to attend ACEC training sessions due to time constraints.
- Lack of clear delineation of the boundaries of responsibility. This has resulted in staff being reluctant to be involved and may place a risk to other higher stake teaching or research projects.
- Increased pressure to gain approval in a timely way so as not to disadvantage students. This is exacerbated by the need for the ACEC to have face-to-face meetings to approve applications, in contrast with Human Ethics projects whereby no approval is required when publishing outcomes do not occur.

The students

- Students are naive to the process.
- Not all students are adequately prepared as they may not have completed all the subjects where the approval process is taught.
- Students seek assistance from academic staff that are time poor and/or that may not have expertise in some animal species or research project areas.
- Some students have equated the process of applying for an animal research authority with that of a traditional university assessment task, with a naive expectation of workload and timelines.
- Students do not realise the time lines for the application process – this includes time taken to complete the application, academic staff and client to review (approval and sign off) and for university executive to sign off applications. After submission there is time required for meetings and feedback to be provided by committee. This can be complicated further if additional approvals are required such as National Parks and Wildlife or Fisheries licences.
- Despite a compulsory animal ethics workshop running each year, some students perceive they will not require ethics approval to conduct their project and don’t attend. Sometimes students receive incorrect advice from clients indicating they don’t need ACEC approval or unexpected changes occur during the project which then necessitates an application.
- Lack of understanding regarding animal welfare terminology e.g. what is considered ‘invasive’ research? For example spotlighting (the use of a filtered flashlights to cause nocturnal animals to stare into the light beam to aid identification) and call playback (use of recorded animal calls), are regarded as ‘invasive’ techniques.
- Students become easily demoralised due to the time taken by the process, regardless of whether or not any amendments are required.

- Students lack expertise in relaying information between academic staff and clients, and this sometimes leads to misinformation being relayed, leading to confusion and frustration.

The client

- Offer diverse projects both from the perspective of species being used and the project setting.
- They often lack a research background or knowledge about the University's obligation to meet ethics approval. They may however be very knowledgeable of other legislative requirements such as the Exhibited Animals Protection Act 1986, etc., that may impact the primary use of the animals.
- Lack understanding of the educational learning outcomes and standards expected.
- Enthusiasm varies from client to client.

Moving forward

The committee takes the stance that they place trust in the academic staff member who supervises the student. As such the staff member acts as a 'quasi' inspector. Often the staff member does not interact with the client directly or complete inspections. Although this may be considered a conflict of interest, there is an element of trust in all ethics applications and project executions. The client becomes the 'person in charge' of the student as per the Code of Practice and 'has responsibility for the care and use of animals from the time of acquisition until completion of the project' (*Australian Code of Practice for the Care and Use of Animals for Scientific Purpose, 2004*). The committee assumes that the code is compatible with the other requirements and not putting the client, academic staff, student or university at risk. It is also assumed that the training and qualifications of the client are sufficient for the research being undertaken and they have enough experience to competently monitor the student.

Although the committee is aware of its role in training students this does not extend to editing and reworking applications for approval. Generic advice however is given to improve applications. Due to the volume and quality of human ethics applications, the university has a sub-committee for this purpose. This may be something the ACEC needs to consider in the future given the time constraints of academic staff and the committee. An online application process may also expedite the process. For low risk activities (e.g. observational studies) this is already in place. Further online support in the form of exemplars and information sheets will also reduce confusion around identifying projects which require approval and the use of specific terminology.

From the teaching perspective, we would like to see more participation in workshops for the ongoing education of staff and students. This also demystifies the process and starts conversations with stakeholders in an open forum. Inclusion of staff that have committee experience or interactions would be highly beneficial. This is with the view to increase the quality of applications before being tabled as well an educational outcome. Currently the application process is not seen as an educational outcome by the students, something that could be stressed in the future.

Conclusions

More students are being trained in professional competencies as part of their education and for animal science students, this means working with animals. Only by working with staff who understand the educational outcomes that these projects are trying to achieve, can the committee facilitate successful applications moving ahead. All ethical processes succeed, based on trust. The model presented in this paper whereby large numbers of different projects are undertaken by diverse clients, centres on this trust between all parties involved. Through education of all involved, the committee can ensure high standards of animal welfare and that this is line with current community views on animal use.

The committee and academic staff, students and clients often have a stressful relationship which needs to be managed long term. For the students however, every year a new cohort needs to be managed and educated. This is very different from the education of longer term staff and clients. Some of this therefore needs to be managed at the course level. Only through open dialog, clear communication and increased education can this process improve. We hope this case study can be used in similar situations by other institutions.

References

Australian Code of Practice for the Care Animals for Scientific Purpose. (http://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/ea16.pdf)

Graduate Outlook Report 2011. (http://www.graduatecareers.com.au/Research/ResearchReports/index.htm)

Old, J. M. and Spencer, R-J. 2011: Development of online learning activities to enhance student knowledge of animal behaviour prior to engaging in live animal handling practical sessions. *Open Journal of Animal Sciences* 1: 65-76.

Transforming Australia's Higher Education System, 2009 (http://www.deewr.gov.au/HigherEducation/Documents/TransformingAusHigherED.pdf)

Animal trapping and animal welfare

Michael C Calver[1]

School of Biological Sciences and Biotechnology,
Murdoch University, Murdoch,
Western Australia 6150, Australia

Abstract:

While much of the discussion on the use of animals in research focuses on animals as models of human disease, many research projects study free-ranging domestic animals or wildlife. Often, the research benefits animals through better husbandry or management.

Trapping and tagging of animals is frequently integral to this research, so such interference in animal's lives is a justified concern, irrespective of whether or not the ultimate goal is improved animal welfare. However, animal welfare need not conflict with wildlife research, because good wildlife studies rarely seek to harm animals (although pest control can be a significant exception). Biologists want to contain costs and therefore don't want to use animals unless it is essential, sometimes choosing to study invertebrates rather than vertebrates, replacing fieldwork with computer simulations or models, or interrogating existing data more efficiently through meta-analysis. When animals are used, reducing the numbers to the minimum needed for conclusive results saves cash. Finally, harming animals in any way that alters their behaviour or survival will bias results, so biologists constantly refine their techniques to avoid harm. All this is familiar to animal welfare workers as the 'three Rs' of Replacement, Reduction and Refinement.

This paper explores the common ground between animal welfare and wildlife research through three examples that illustrate how studies of free-ranging wildlife and domestic animals lead to findings of direct benefit to animals through better husbandry or management. Specific applications of replacement, reduction and refinement in wildlife studies are then discussed with reference to useful sources of information and checklists for proposed procedures that may be valuable to members of Animal Ethics Committees.

Key words: precautionary principle, urban wildlife, pet cats, *Felis catus***,** cat regulation, wildlife protection

Introduction

Although wildlife studies have been within the scope of the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* since 1983 (Rose 1999), the sixth edition of 1997 (NHMRC 1997) was the first to give the topic its own section. The higher profile prompted a flurry of advice for Animal Ethics Committees (AECs) on how to respond to wildlife proposals. Grigg (1999) suggested that AECs should address animal welfare issues and not meddle with the merit of the research; Gott (1999) proposed that AECs have a unique role in educating researchers in animal welfare; and Albrecht (1999) advocated that curiosity-driven research on wildlife should not be permitted. Some of the early decisions of AECs may not have been ideal and some researchers were deeply concerned about potential restrictions on their fieldwork. Fulton and Ford (2001) and Tidemann and Vardon (2002) complained of stays in executing approved projects pending last minute questioning or legal injunctions, committee members lacking the experience to evaluate wildlife projects and a tendency for committees to place the welfare of individual animals ahead of the welfare of species. Dyson and Calver

(2003) noted further problems with delays, workloads and costs (both in preparing applications and serving on committees) and perceptions that the integrity of researchers was challenged by animal welfare scrutiny (although they believed that the last point would fade with familiarity).

Balancing these concerns, are some significant advantages in having an AEC review and consider wildlife research projects (Dyson and Calver 2003). Firstly, a transparent mechanism for demonstrating responsibility and accountability in research, aids in community acceptance and avoidance of violent direct activism against researchers, their equipment and / or their institutions. Applications need to be intelligible to lay members of committees so from the beginning, the researchers need to communicate more broadly than just to other specialists, thereby achieving an important goal for broad transmission of the benefits of their work (Recher 1998, Jacobson 1999, Jacobson *et al* 2006). Similarly, the AEC also provides feedback to the researchers from other specialists such as veterinarians or statisticians that may benefit the project. This process extends to teaching applications too, because students involved in any projects must be familiar with the animal welfare implications (Monamy 1999, Monamy and Gott 2001, NHMRC 2004). AECs have considerable autonomy, so there is scope for developing processes tailored to specific institutions and their circumstances. Lastly, adherence to all the AEC's conditions for approval, offers researchers some protection if there are complaints about approved procedures. If the researcher has followed approvals scrupulously, the researcher is entitled to use the AEC approval as a viable defense.

Now, over a decade and one further edition of the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (NHMRC 2004) since the 1997 watershed, much of the conflict between researchers and AECs has subsided (at least openly in the research literature). While contentious issues of practice remain, many researchers and AEC members would be sympathetic to the position of Lunney (2012a, p.1): 'It is in researchers' interests to publicly defend the essential role of science in conserving our native fauna, and to conduct our work within a well-managed framework.' The need for considering animal welfare in wildlife research is also gaining a place in teaching materials and advice for instructors in wildlife biology (e.g., Monamy and Gott 2001, Dickman and Crowther 2009, p.27) and in techniques manuals or other advice for wildlife professionals (e.g., Gott 1999, Dein *et al*. 2005).

It may be that AECs and researchers are recognising that they have common ground, because good wildlife studies rarely seek to harm animals. The notable exception here might be some pest control investigations, although even then, the prevention of undue pain or distress to the animal remains a core factor in gaining AEC approval. In the interests of efficiency and cost reduction, wildlife biologists want to minimise animal use, sometimes to the point of replacing work with vertebrates by using invertebrates, or replacing fieldwork with computer simulations or models. Meta-analysis, which combines the results of multiple, independent and published studies to permit a more rigorous test of a hypothesis than any of the studies could provide individually, also obviates more empirical animal research. At the least, reducing numbers to the lowest needed for conclusive results saves cash. When studies do go ahead, techniques that alter animal behaviour or survival bias results, which is a major incentive to refine techniques to minimise harm. AEC members recognise this as the 'three Rs' of Replacement, Reduction and Refinement.

Despite that common ground, one long-standing complaint remains. Lunney (1999, 2012b,c) noted that many members of AECs know little about native vertebrates or the techniques used to trap or mark them in field studies. In an attempt to address the issue, in this paper I have provided some background on the kinds of wildlife studies that may be done, before considering how the 3Rs can be applied in the context of such work. I have also suggested some resources members may find useful to improve their understanding of native vertebrates and the techniques used to study them, as well as synthesising from the literature some recommendations from wildlife biologists for improving animal welfare during trapping and tagging studies that could be useful in assessing applications to AECs.

Three examples of studying free-ranging animals

Techniques used in wildlife studies range from minimally disturbing approaches such as observation, through progressively more intense methods including trapping, marking and major manipulation of habitat or animal numbers in field experiments. While it is easy to spot concerns with the more invasive methods, even superficially low-impact techniques such as observation may cause problems, as illustrated by concerns over the potential consequences of tourism activities such as whale-watching (Orams 2000). Dein *et al*. (2005) stress that investigators should be alert to the many indirect effects of their activities including, but not limited to: abandonment of habitat or young, ceasing breeding activities, increased vulnerability to predation, damage to habitat and transmission of disease.

The following descriptions of field studies illustrate a diversity of techniques and readers will be quick to spot the range of animal welfare issues implicated. These are not discussed explicitly as part of the examples, although some of them return when discussing the application of the 3Rs to wildlife studies. The main purpose of the examples, though, is to illustrate some of the research questions tackled by wildlife biologists.

Great white shark

The great white shark *Carcharodon carcharias* occurs mainly in subtropical to temperate waters, especially off South Africa, Australia, New Zealand, California and some Pacific Islands, and is acknowledged to be very widely distributed (Antunes and Balbino 2012). Adult great white sharks may exceed six metres in length and weigh up to three tonnes. Their lifespan is uncertain, but may be up to 40 years (Bannister 1989). They are formidable predators of marine mammals and occasionally fish, marine reptiles and marine birds (Estrada *et al*. 2006, Johnson *et al*. 2006). Although humans are sometimes attacked, this most likely occurs when the shark mistakes a person for a seal (West 2011). It is more common for great white sharks to be eaten by people. According to Cole (no date) quoted in Bannister (1989, p.78), the flesh is ‘reddish … and … fat, soft and appetizing’.

In common with other large, long-lived animals, the great white shark has a low reproductive rate. It reaches sexual maturity at nine – ten years of age and females probably reproduce only every two years, with a gestation of up to a year (Bannister 1989). This means that populations recover slowly from substantial losses and the species’ range may be contracting steadily (McPherson and Myers 2009). More substantial losses occurred in the 1970s and the great white is now listed as ‘vulnerable’ by the International Union for the Conservation of Nature (IUCN). The causes of the decline are uncertain, but may include overfishing for food, sale of jaws and teeth as souvenirs, capture for aquarium display, destruction of important coastal habitat and persecution campaigns following attacks on people (Fergusson *et al*. 2009).

Rather surprisingly given its size and the concern over attacks on people, there is very limited knowledge of the dispersal patterns of great white sharks. However, recent developments in satellite-based telemetry techniques enabled researchers to tag sharks, tracing their movements for many months or years (e.g., Bruce *et al*. 2006).

Some of the most interesting results come from detailed satellite tagging studies of great white sharks off South Africa in 2002 and 2003 (Bonfil *et al*. 2005). Over periods of up to six months, some tagged sharks made return migrations of over 2,000 km along the coast. Others were far more restricted, travelling short distances or remaining within a limited domain. There were also examples of remarkable transoceanic migration, with one female being tracked north up the South African coast for over 700 km before turning across the Indian Ocean to Western Australia, where she reached Exmouth Gulf 99 days later. Her average speed was 4.7 km/h, a very fast sustained speed for sharks, and she crossed open ocean rather than following island chains. After spending time off Western Australia, she returned to South Africa, arriving nine months later.

One significant implication of these results is that interbreeding between widely separated populations is possible. It is also clear that females make long distance migrations, not just males. Such long distance migrations also pose a substantial challenge for conserving great white sharks. Migrating sharks pass through international waters and enter the coastal jurisdictions of different governments. If there is no international collaboration in management, conservation efforts in one country may be countermanded by excessive mortality elsewhere.

Pussycat, pussycat, where have you been?

Domestic cats *Felis catus* are loved family pets in between a quarter and a third of Australian households and they are popular elsewhere too (Chaseling 2001). However, surveys suggest that across the world only between 10 and 33% of them are totally confined, with the rest being either kept in at night and allowed to roam by day or completely unrestricted in their movements (Calver *et al*. 2011). This freedom to roam is unusual in domestic animals and can lead to frequent encounters between cats and wildlife (Sims *et al*. 2008), not to mention risks to cats themselves through fighting with other cats or collisions with cars (Rochlitz 2003a,b, 2004). Therefore, the techniques of the wildlife biologist can be focused on cat's roaming habits with the twin aims of protecting wildlife and reducing risks to roaming cats.

Radiotracking studies of domestic cats in Australia, New Zealand, North America and Europe have found considerable variations in the distances travelled by unrestrained animals, although cats from rural landscapes have larger home ranges than those in the suburbs (e.g., Kays and DeWan 2004; Biro *et al*. 2004). At one extreme, contented suburban cats may roam over areas as small as 0.2 ha, while at the other extreme, cats in rural areas may have home ranges exceeding 20 ha. The maximum linear distances travelled by pet cats are also highly variable, ranging from under 200 m for some suburban cats to over 2 km for rural animals (Lilith *et al*. 2008, Metsers *et al*. 2010). The greater area covered by rural cats is probably related to opportunity, because the overall density of cats is lower, whereas suburban cats are more likely to encounter antagonistic neighbours. While males generally have larger home ranges than females, these differences are harder to confirm with statistical tests – many authors who have attempted these studies complain of small sample sizes.

One important implication from these results is that roaming cats are more at risk of injury or death by misadventure as they fight other cats or cross busy roads. In the case of 4,591 Swedish cats registered for life insurance, Egenvall *et al*. (2009) reported 3% of cats dying of unspecified trauma and 9% dying in traffic accidents. In Cambrésie, France, Moreau *et al*. (2003) attributed the 66 accidental deaths out of the 259 cat deaths they investigated to roaming behaviour. Road fatalities (40.9%) and poisoning (39.5%) were the main causes of death. Considering non-fatal injuries, data from one Swedish insurance company between 1999 and 2006 found that trauma was the most common cause for presenting a cat for veterinary treatment (Egenvall *et al*. 2010). The categories 'bite/cut/wound', 'foreign body stomach/intestines' and 'hit by car/train/vehicle' were the largest causes of trauma – all likely to be associated with roaming.

Encounters with wildlife may also increase when cats roam, raising the issue of predation on wildlife by a domestic pet. Whether or not such predation threatens population viability is contentious, but it distresses some cat owners and leads some municipalities to enforce restrictions on roaming cats (Calver *et al*. 2011). Although unpopular with both cat owners and non-owners, cat exclusion zones around sensitive wildlife habitat may be enforced to protect wildlife. Roaming studies in different landscapes suggest that these may only need to be as little as 360 m wide, but in other areas may need to exceed 2 km to prevent incursions (Lilith *et al*. 2008, Metsers *et al*. 2010).

That feral cat!

Pet cats live in close association with a household and are supported by people, whereas feral cats are completely self-supporting in independent populations. While there is uncertainty around the impacts of pet cats on wildlife, the impact of feral cats on wildlife populations has been established through controlled experiments. One such experiment took place at Heirisson Prong in Western Australia's mid-west (Risbey *et al*. 2000).

Heirisson Prong is a finger of land extending into the Indian Ocean. In the 1990s, a predator-proof fence was established across the prong and the exotic predators the red fox *Vulpes vulpes* and the feral cat were eliminated from the seaward side of the fence by shooting, trapping and poison baiting. To reduce the chance of foxes crossing the fence into the protected 'predator-free' zone, poison baits were distributed by air each year over an area of 120 km^2 to the landward side of the fence to create a buffer zone. While foxes take poison baits readily and their numbers can be controlled, feral cats rarely take baits. With fox numbers reduced, feral cats were free of predatory and competitive pressure and actually increased in numbers in the buffer zone (Risbey *et al*. 2000).

The numbers of the potential native prey species, such as the little long-tailed dunnart *Sminthopsis dolichura*, the ash grey mouse *Pseudomys albocinereus* and the sandy inland mouse *Pseudomys hermannsburgensis*, as well as the introduced house mouse *Mus musculus*, were monitored using pitfall trapping in the 'predator-free' zone and the buffer zone before the predator control and for two years afterwards. Numbers of the ash grey mouse and the sandy inland mouse were consistently higher in the 'predator-free' zone, while the other two species oscillated at low levels. By contrast, in the buffer zone where fox numbers were low but cat numbers had increased, there was a steady decline in the trap success of all prey species (Risbey *et al*. 2000).

One implication of the study is that fox and feral cat numbers are interactive, so if only foxes are controlled, feral cats are likely to increase in numbers. A second implication is that numbers of small mammals, including native species, decline when subject to heavy predation by feral cats. Thus, control of feral cat numbers is important for conservation of native species, at least in this environment in Australia.

The three Rs in wildlife biology

The three examples of wildlife studies presented here show many features that characterise this field of biology. All centred on an applied aspect, be it conservation of great white sharks, regulating the roaming behaviour of pet cats to enhance their welfare and reduce their interactions with wildlife, or determining whether or not native wildlife are endangered by introduced predators. There were curiosity elements too, although they were subordinate to the applied ones. How far do great white sharks or pet cats really move? Do predators really regulate numbers of their prey? The studies also illustrated the range of interventions possible in studies of wildlife. While none solely used observations to gather data without trapping or handling animals at all, the radiotracking studies were characterized by a relatively low level of intervention. In contrast, the culling of predators in the Heirisson Prong study was a major intervention and the methods of culling – shooting, trapping and poison baiting – are themselves contentious. Two of the examples were focused on particular species and one on a larger assemblage. While there would appear to be no alternative to studying these species directly to achieve the research aims, it is still possible to consider the 'three Rs' of animal welfare in wildlife biology projects.

Replacement

Field biology can be dauntingly expensive. Spiller and Schoener (1998) reported that the materials for their study of lizard predation on spider communities in the Bahamas weighed 'several thousand pounds' and were flown to the study site. Not everyone can command such funds, so wildlife biologists are constantly looking to cut costs. If this involves doing without vertebrate animals' altogether, replacement has been achieved.

Some classic studies in wildlife biology did exactly that by substituting simulations using human beings or computer modeling, instead of working with animals. For example, Holling (1959) had a 'predator' (a blindfolded secretary) 'hunt' for discs of sandpaper 'prey' on a desk, using varying prey densities and time constraints. This simulates real situations such as birds fossicking for insects. Holling used the results to derive the concept of the functional response, which is the relationship between the number of prey eaten by an individual predator per unit time and the density of its prey. Simulations have also been used effectively to elucidate other aspects of predatory behaviour (e.g., Gendron and Staddon 1984, Knill and Allen 1995).

Some of the classic simulation studies have been adapted as teaching exercises (Calver and Wooller 1998). They are particularly useful in teaching because they reduce costs, avoid the risks inherent in using live animals and allow teachers to introduce discussion on the ethical value of substituting simulations for animals in the classroom, in line with Monamy (1999) who recommended integrating the three Rs into teaching in wildlife biology. Some exercises use students as experimental subjects, while others use the 'behaviour' of inanimate objects (e.g., the ingenious taxicab exercise of Bishop and Bradley 1972). If suitable facilities are available, teachers can choose from several excellent computer simulations (Finn et al. 2002). The availability of resources has reached a point where it is questionable as to whether or not students need to work with live vertebrates, unless there are specific educational objectives regarding handling and tagging of animals associated with the aims of the course, in addition to the understanding of basic principles or using live animals to motivate students (Hochuli and Banks 2008).

Theoretical modelling can generate predictions about animal behaviour without even requiring the simulation step (e.g., Alpern *et al*. 2011). This is distinct though, from models of population dynamics of particular species in the wild. Gott (1999) points out that computer modelling is not a substitute for field biology in such cases, because the models must be informed by rigorous field data. However, once those data are available modelling can increase their utility (e.g., Akcakaya *et al*. 1995).

If the aim of a study is to elucidate a behavioural or ecological principle rather than study a particular species, replacement of vertebrates may be possible if there is a suitable invertebrate model (e.g., Paine 1976). Such an option will usually be cheaper and will largely avoid many of the ethical issues associated with vertebrate field research.

Lastly, before carrying out any new experimental or survey field study at all, it is useful to ask if the question could be answered from the literature using a meta-analysis. This eliminates further animal work and enhances the value of studies already completed. For example, one important theoretical issue in wildlife biology is whether predators can regulate the numbers of their prey. According to the 'doomed surplus' hypothesis, predators take animals that would otherwise die of different causes and so predators do not regulate prey numbers. Salo *et al*. (2010) tested this in a worldwide meta-analysis of experiments where the numbers of vertebrate predators were changed by either introductions or culling and the numbers of their vertebrate prey monitored. They concluded that predators do limit prey populations, because overall prey numbers change markedly when predator numbers are changed.

Reduction

Some fieldwork has only descriptive aims and deciding on the number of animals to be used is subjective. However, if observations are to be the basis of statistical tests, or if the study follows an experimental protocol, more sophisticated approaches can be taken to determine the number of subjects needed (Gott 1999, Monamy and Gott 2001). One solution, as advised by Green (1979, p.31) is to: 'Carry out some preliminary sampling to provide a basis for evaluation of sampling design and statistical analysis options. Those who skip this step because they do not have enough time, usually end up losing time.' That sage advice also rings true for laboratory experimentalists and clinicians, who are accustomed to using *a priori* statistical power analysis to determine the minimum number of independent replicates they will need to test hypotheses rigorously. The same approach is equally applicable to field experiments in wildlife biology.

Obviously, it is wasteful and questionable from a welfare standpoint to include more animals than are needed to test a study's predictions, while too few may lead to an ambiguous result and the use of animals to no purpose. *A priori* statistical power analysis assists in finding the ideal number. It requires:

1. a pilot study, as recommended by Green (1979), to determine the likely variability between independent replicates during the study,
2. a decision on the size of change or 'effect size' that the researcher wishes to be able to detect if it occurs,
3. setting a significance level for the statistical test (often 0.05 in biology), and
4. setting the power of the test (the probability that, under the specified test conditions, a significant result will be detected if it actually occurs, 80% is often recommended in the life sciences) (Thomas and Krebs 1997).

It is then possible to calculate the required sample size to test an effect under the above conditions. Steidl and Thomas (2001) reviewed power analysis from a field biologist's perspective and while some of the specialist software is expensive, the freeware package G*Power (http://www.psycho.uni-duesseldorf.de/aap/projects/gpower/) is adequate for most needs.

Refinement

Technological advances in field equipment and the sophistication of computer analyses are revolutionising many areas of wildlife biology. Some methods, such as camera 'trapping', use motion triggered cameras to monitor animals without the need to trap them, although careful calibration of techniques is needed (Kalle *et al*. 2011). In some cases, natural markings on animals can be used for identifying them individually by sight, without needing to trap them at all (Webster *et al*. 2010). Prices on the necessary hardware are falling rapidly, so these approaches are no longer restricted to well-funded researchers. Dein *et al*. (2005) argue that such remote methods should be considered wherever possible to minimise disturbance, but also caution that some remote methods such as monitoring from aircraft may also be disruptive.

Even if cameras are not employed, observational field protocols that seek to detect animals by sight or by hearing their calls, can be used to census populations without trapping animals. Recent empirical studies have shown that these methods can be biased by differing likelihood of detecting animals of different species. Correcting for the possibility of detection bias is therefore an important part of many studies (Alldredge *et al*. 2008).

If animals must be handled and tagged, technology is again providing solutions to welfare concerns. One of the most significant of these is deliberate injury to animals when marking them (mutilation marking), or changes to an animal's behaviour because of being marked. Studies using marked animals routinely assume that marks do not change an animal's behaviour or survival, so wildlife biologists and AECs have common cause on this point. If animals are large enough, they can be microchipped using the same technology applied to pets for identification. This leaves no visible external mark and presumably causes no changes in behaviour (Gott 1999). With smaller animals such as reptiles and amphibians toe clipping, in which the tips of digits are removed to give an individual a unique coded mark, continues to be debated (Perry *et al*. 2011). One alternative for amphibians is the Visible Implant Alphanumeric (VIA) tag, which is implanted internally under clear or translucent tissue. The tag is visible externally for easy identification. If the tag can only be placed under pigmented tissue it is not visible in ambient light, but will fluoresce under UV light (Heard *et al*. 2008).

In a novel approach to choosing between techniques on welfare grounds, Parris *et al*. (2010) compared four different methods for collecting frog DNA on the basis of population growth rate post-sampling (an estimate of species welfare) and suffering alone or suffering plus longevity (two measures of individual welfare). In so doing, they addressed a long-standing tension between individual welfare and species welfare (Tidemann and Vardon 2002).

Resources

With so many recent developments in techniques and analyses in wildlife research, AECs may need to consult widely to obtain expert advice on some of the wildlife projects they consider. However, one or two carefully chosen resource books may inform members unfamiliar with wildlife research of the principles behind many techniques and their applications. The Wildlife Society's comprehensive *Techniques for wildlife investigations and management* is now in its sixth edition (Braun 2005) and is an ideal starting point. *Ecology and field biology* (Smith and Smith 2001) is also in its sixth edition and includes clear descriptions of many key techniques in its appendices. Lastly, *Environmental biology* (Calver et al. 2009) introduces Australian biodiversity and ecosystems (with succinct coverage of some study design issues) for those curious about the background to why wildlife studies are important.

Further resources can come from a more open exchange between AECs regarding standard operating procedures developed (Gott 1999). An internet database where AECs could deposit and access such documents would reduce duplication and exchange information effectively (e.g., Minteer and Collins 2005). It will also be helpful if AECs share research papers they may encounter that describe protocols for improving animal welfare in wildlife research studies. For example, Recher *et al*.'s (1985) compilation of practices to reduce bird mortality when mist netting deserves to be known more widely.

There are also specific practices recommended in the literature for improving animal welfare in field studies (Table 1). These may assist as a checklist for AECs when evaluating proposals. Finally, Appendix 1 includes examples of some of the common traps and survey devices for terrestrial wildlife.

Concluding remarks

Those with long memories will recognise this paper as an example of the 'Microsoft genre' – a reworking and repackaging of work that has gone before. It is true that many of the ideas presented here were developed by contributors to Mellor and Monamy (1999) and expanded on

Suggestion	Reference
Limit the number of traps set to one that can be checked in a reasonable time frame given topography, vegetation, distance between traps, likely capture rates and researchers available. For mist nets, set the number of nets within the capacity of the team to cope with the captures – processing is about four minutes/bird.	Monamy and Gott (2001) Recher et al. (1985)
Limit days of consecutive trapping so that animals caught repeatedly are not stressed unduly or precluded from foraging normally	Monamy and Gott (2001)
Plan explicitly to minimise non-target captures and consider their welfare.	Monamy and Gott (2001)
Trap types can be chosen to minimise injury or reduce the chance of predators robbing traps.	Monamy and Gott (2001)
Some traps may need to be insulated to prevent heat or cold stress. Do not trap in inclement weather.	Monamy and Gott (2001) Recher et al. (1985)
Shelter may need to be provided within traps.	Monamy and Gott (2001)
Drain holes may be needed in case of rain.	Monamy and Gott (2001)
Heat, cold, rain, predation and injuries from struggling or during handling are hazards to mist netted birds. All can be reduced by checking nets frequently (even handling, because birds in the nets for longer may become more severely entangled, requiring longer handling to free them). Intervals of 10 – 20 minutes were recommended.	Recher et al. (1985)
Lift the lower shelf of the mist net above ground in swampy terrain to prevent birds drowning in shallow water.	Recher et al. (1985)
If the lower shelf of a mist net extends to the ground, remove fallen branches or stones against which a bird may injure itself.	Recher et al. (1985)
Observe released animals and note behaviour suggestive of distress – if released animals appear distressed, protocols may need to be changed.	Recher et al. (1985)
Conserve and protect habitat at all times.	Dein *et al*. (2005)
Take all possible precautions to reduce the risk of disease transmission, including the cleaning/sterilisation of traps, animal holding bags and other field equipment.	Dein *et al*. (2005)
Monitor the condition of radiotelemetry collars, especially those including elastic, and replace or repair damaged collars.	Fuller *et al.* (2005)

Table 1. Suggestions for improving animal welfare when trapping.

thereafter by Monamy and Gott (2001) and Lunney (2012a,b.c). However, the early friction that arose between some AECs and wildlife researchers (Fulton and Ford 2001, Tidemann and Vardon 2002) suggests that those important ideas need to be restated.

In general, wildlife researchers like and respect the animals they study and are predisposed to think favourably about animal welfare. They also have good practical reasons to do so, because they need to contain the costs of their research and ensure that their procedures do not alter the behaviour or survival of their study animals. This should allow researchers and AECs to meet in dialogue over the importance of replacement, reduction and refinement. Researchers can assist the dialogue by considering animal welfare implications in their publications and in their teaching, while AECs can consider consulting specialists in relation to complex applications and broadening members' knowledge of native vertebrates.

References

Albrecht, G. (1999). A case for some consequences of employing an ecological ethic to guide wildlife research. In. *The use of wildlife for research*. D. Mellor and V. Monamy (eds). Australian and New

Zealand Council for the Care of Animals in Research and Teaching (ANZCCART), Glen Osmond, South Australia. Pp. 115-118.

Alldredge, M.W., Pacifici, K., Simons, T.R. and Pollock, K.H. (2008). A novel field evaluation of the effectiveness of distance and independent observer sampling to estimate aural avian detection probabilities. *Journal of Applied Ecology* **45:** 1349-1356.

Alpern, S., Fokkink, R., Timmer, M. and Casas, J. (2011). Ambush frequency should increase over time during optimal predator search for prey. *Journal of the Royal Society Interface* **8:** 1665-1672.

Antunes, M.T., Balbino, A.C. (2012). The great white shark *Carcharodon carcharias* (Linnaeus, 1758) in the Pliocene of Portugal and its early distribution in eastern Atlantic. *Revista Espanola de Paleontologia* **25:** 1-6.

Bannister, K. (1989). *The book of the shark*. The Apple Press, London.

Biro, Z., Szemethy, L., and Heltai, M. (2004). Home range sizes of wildcats (*Felis silvestris*) and feral domestic cats (*Felis silvestris f. catus*) in a hilly region of Hungary. *Mammalian Biology – Zeitschrift fur Saugetierkunde* **69:** 302–310.

Bishop, J.A. and Bradley, J.S. (1972). Taxi-cabs as subjects for a population study. *Journal of Biological Education* **6:** 227-231.

Bonfil, R., Meyer, M., Scholl, M.C., Johnson, R., O'Brien, S., Oosthuizen, H., Swanson, S., Kotze, D. and Paterson, M. (2005). Transoceanic migration, spatial dynamics, and population linkages of white sharks. *Science* **310:** 100-103.

Braun, C.E. (Ed.) (2005). *Techniques for wildlife investigations and management*. The Wildlife Society, Bethseda, Maryland, USA.

Bruce, B.D., Stevens, J.D. and Malcolm, H. (2006). Movements and swimming behaviour of white sharks (*Carcharodon carcharias*) in Australian waters. *Marine Biology* **150**:161-172.

Calver, M.C., Grayson, J., Lilith, M. and Dickman, C.R. (2011). Applying the precautionary principle to the issue of impacts by pet cats on urban wildlife. *Biological Conservation* **144:** 1895-1901.

Calver, M.C., Lymbery, A.J., McComb, J.A. and Bamford, M.J. (2009). *Environmental biology*. Cambridge University Press, Melbourne.

Calver, M. C. and Wooller, R. D. (1998). A non-destructive laboratory exercise for teaching some principles of predation. *Journal of Biological Education* **33**: 1-4.

Chaseling, S. (2001). Pet populations in Australia. Dogs increasing and cats decreasing - why is it so? In. *10th Urban Animal Management Conference*. D. Murray (ed.). Australian Veterinary Association, Melbourne. Available from: http://ccac.net.au/files/Pet_pops_in_Aust_UAM01Chaseling.pdf. Accessed July, 2012.

Dein, F.J., Toweill, D.E. and Kenow, K.P. (2005). Care and use of wildlife in field research. In. *Techniques for wildlife investigations and management*. C. E. Braun (ed.). The Wildlife Society, Bethseda, Maryland, USA. Pp. 185-196.

Dickman, C. and Crowther, M. (2009). Science and the environment. In. M. C. Calver, A.J. Lymbery, J.A. McComb and M.J. Bamford (eds). *Environmental biology*. Cambridge University Press, Melbourne. Pp. 23-42.

Dyson, S.E. and Calver, M.C. (2003). The value of Animal Ethics Committees for wildlife research in conservation biology - An Australian perspective. *Pacific Conserv Biol***9:**86-94.

Egenvall, A., Nødtvedt, A., Hägström, J., Ström Holst, B., Möller, L. and Bonnett, B.N. (2009). Mortality of life-insured Swedish cats during 1999-2006: age, breed, sex and diagnosis. *Veterinary Internal Medicine* **23I:** 1175-1183.

Egenvall, A., Bonnett, B.N., Hägström, J., Ström Holst, B., Möller, L. and Nødtvedt, A. (2010). Morbidity of insured Swedish cats during 1999-2006 by age, breed, sex, and diagnosis. *Journal of Feline Medicine and Surgery* **12:** 948-959.

Estrada, J.A., Rice, A.N., Natanson, L.J., and Skomal, G.B. (2006). Use of isotopic analysis of vertebrae in reconstructing ontogenetic feeding ecology in white sharks. *Ecology* **87:**829-834.

Fergusson, I., Compagno, L.J.V. and Marks, M. (2009). *Carcharodon carcharias*. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.1. <www.iucnredlist.org>. Downloaded on 15 July 2012.

Finn, H., Maxwell, M., and Calver, M. (2002). Why does experimentation matter in teaching ecology? *Journal of Biological Education* **36:** 158-162.

Fulton, G.R., and Ford, H.A. (2001). The conflict between animal welfare and conservation. *Pacific Conservation Biology* **7:** 152-160.

Fuller, M.R., Millspaugh, J.J., Church, K.E. and Kenward, R.E. (2005). Wildlife radiotelemetry. In. *Techniques for wildlife investigations and management*. C. E. Braun (ed.). The Wildlife Society, Bethseda, Maryland, USA. Pp. 377-417.

Gendron, R.P. and Staddon, J.E.R. (1984). A laboratory simulation of foraging behavior: the effect of search rate on the probability of detecting prey. *American Naturalist* **124:**407-415.

Gott, M. (1999). Wildlife research in the field: welfare aspects of an essential discipline. In. *The use of wildlife for research*. D. Mellor and V. Monamy, (eds.) Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART), Glen Osmond, South Australia. Pp. 28-33.

Green, R.H. (1979). *Sampling design and statistical methods for environmental biologists*. John Wiley and Sons, New York.

Grigg, G. (1999). Wildlife research: why is it conducted? In. *The use of wildlife for research*. D. Mellor and V. Monamy, (eds) Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART), Glen Osmond, South Australia. p11-21.

Heard, G.W., Scroggie, M.P., and Malone, B. (2008). Visible Implant Alphanumeric tags as an alternative to toe-clipping for marking amphibians - a case study. *Wildlife Research* **35:** 747-759.

Hochuli, D.F. and Banks, P.B. (2008). Selection pressures on Zoology teaching in Australian universities: Student perceptions of Zoological education and how to improve it. *Australian Zoologist* **34:** 548-553.

Holling, C.S. (1959). Some characteristics of simple types of predation and parasitism. *The Canadian Entomologist* **91:** 385-398.

Jacobson, S.K. (1999). *Communication skills for conservation professionals*. Island Press, Washington DC.

Jacobson, S.K., McDuff, M.D, Monroe, M.C. (1999). *Conservation education and outreach techniques*. Oxford University Oxford, Washington DC.

Johnson, R.L., Venter, A., Bester, M.N. and Oosthuizen, W.H. (2006). Seabird predation by white shark, *Carcharodon carcharias,* and Cape fur seal, *Arctocephalus pusillus pusillus*, at Dyer Island. *South African Journal of Wildlife Research* **36:** 23-32.

Kalle, R., Ramesh, T., Qureshi, Q. and Sankar, K. (2011). Density of tiger and leopard in a tropical deciduous forest of Mudumalai Tiger Reserve, southern India, as estimated using photographic capture-recapture sampling. *Acta Theriologica* **56:** 335-342.

Kays, R.W. and DeWan, A.A. (2004). Ecological impact of inside/outside house cats around a suburban nature reserve. *Animal Conservation* **7:** 273-283

Knill, R. and Allen, J.A. (1995). Does polymorphism protect? An experiment with human predators. *Ethology* **99:** 27-138.

Lilith, M., Calver, M. and Garkaklis, M. (2008). Roaming habits of pet cats on the suburban fringe in Perth, Western Australia: What size buffer zone is needed to protect wildlife in reserves? In. *Too close for comfort: contentious issues in human-wildlife encounters*. D. Lunney, A. Munn and W. Meikle (eds). Royal Zoological Society of New South Wales, Sydney, Australia, pp. 65-72.

Lunney, D. (1999). Is ethics opposed to science? A wildlife scientist's viewpoint. In: The use of wildlife for research. D. Mellor and V. Monamy (eds). Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART), Glen Osmond, South Australia. Pp. 119-128.

Lunney, D. (2012a). Ethics and Australian mammalogy: reflections on 15 years (1991 - 2006) on an Animal Ethics Committee. *Australian Mammalogy* **34:** 1-17.

Lunney, D. (2012b). Wildlife management and the debate on the ethics of animal use. I. Decisions within a State wildlife agency. *Pacific Conservation Biology* **18:** 5-21.

Lunney, D. (2012c). Wildlife management and the debate on the ethics of animal use. II. A challenge for the animal protection movement. *Pacific Conservation Biology* **18:** 80-98.

McPherson, J.M. and Myers, R.A. (2009). How to infer population trends in sparse data: examples with opportunistic sighting records for great white sharks. *Diversity and Distributions* **15:** 880-890.

Meek, P. (2003). Home range of house cats *Felis catus* living within a national park. *Australian Mammalogy* **25:** 51-60.

Mellor, D. and Monamy, V. (eds). (1999). *The use of wildlife for research*. Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART), Glen Osmond, South Australia.

Metsers, E.M., Seddon, P.J. and van Heezik, Y.M. (2010). Cat-exclusion zones in rural and urban -fringe landscapes: How large would they have to be? *Wildlife Research* **37:** 47-56.

Minteer, B.A., and Collins, J.P. (2005). Ecological ethics: Building a new tool kit for ecologists and biodiversity managers. *Conservation Biology* **19:** 1803-1812.

Monamy, V. (1999). The ethics of zoology: wildlife research as a case study. In. The use of wildlife for research. D. Mellor and V. Monamy (eds). Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART), Glen Osmond, South Australia. Pp. 6-10.

Monamy, V., and Gott, M. (2001). Practical and ethical considerations for students conducting ecological research involving wildlife. *Austral Ecology* **26:** 293-300.

Moreau, D., Cathelain, P. and Lacheretz, A. (2003). Comparative study of causes of death and life expectancy in carnivorous pets (II). *Revue de Médecine Veterinaire* **154:** 127-132.

NHMRC (1997). *Australian code of practice for the care and use of animals for scientific purposes.* 6th edition. (http://www.nhmrc.gov.au/guidelines/publications/ea1)

NHMRC (2004). *Australian code of practice for the care and use of animals for scientific purposes*. 7th edition. (http://www.nhmrc.gov.au/guidelines/publications/ea16.)

Orams, M.B. (2000). Tourists getting close to whales: is it what whale-watching is all about? *Tourism Management* **21:** 561-569.

Paine, R.T. (1976). Size-limited predation: an observational and experimental approach with the *Mytilus-Pisaster* interaction. *Ecology* **57:** 858-873.

Parris, K.M., McCall, S.C., McCarthy, M.A., Minteer, B.A., Steele, K., Bekessy, S. and Medvecky, F. (2010). Assessing ethical trade-offs in ecological field studies. *Journal of Applied Ecology* **47:** 227-234.

Perry, G., Wallace, M.C., Perry, D., Curzer, H., and Muhlberger, P. (2011). Toe clipping of amphibians and reptiles: Science, ethics, and the law. *Journal of Herpetology* **45:** 547-555.

Recher, H.F. (1998). Public and political: the challenge for ecologists. In. *Ecology for everyone: communicating ecology to scientists, the public and the politicians*. R. Wills and R. Hobbs (eds). Surrey Beatty and Sons, Sydney. Pp. 9-15.

Recher H.F., Gowing, G. and Armstrong, T. (1995). Causes and frequency of deaths among birds mist-netted for banding studies at two localities. *Australian Wildlife Research* **12:** 321-326.

Risbey, D.A., Calver, M.C., Short, J., Bradley, J.S., Wright, I.W. (2000). The impact of cats and foxes on the small vertebrate fauna of Heirisson Prong, Western Australia: II. A field experiment. *Wildlife Research* **27:** 223-235.

Rochlitz, I. (2003a). Study of factors that may predispose domestic cats to road traffic accidents: part 1. *The Veterinary Record* **153:** 549-553.

Rochlitz, I. (2003b). Study of factors that may predispose domestic cats to road traffic accidents: part 2. *The Veterinary Record* **153:** 585-588.

Rochlitz, I. (2004). Clinical study of cats injured and killed in road traffic accidents in Cambridgeshire. *Journal of Small Animal Practice* **45:** 390-394.

Rose, M. (1999). Wildlife and the Australian code of practice. In. The use of wildlife for research. D. Mellor and V. Monamy (eds). Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART), Glen Osmond, South Australia. Pp. 22.

Salo, P., Banks, P.B., Dickman, C.R., and Korpimäki, E. (2010). Predator manipulation experiments: Impacts on populations of terrestrial vertebrate prey. *Ecological Monographs* **80:** 531-546.

Sims, V., Evans, K.L., Newson, S.E., Tratalos, J.A. and Gaston, K.J. (2008). Avian assemblage structure and domestic cat densities in urban environments. *Diversity and Distributions* **14:** 387-399.

Smith, R. and Smith, T. (2001). *Ecology and field biology: hands on field package*. Pearson Education.

Spiller, D.A., and Schoener, T.W. (1998). Lizards reduce spider species richness by excluding rare species. *Ecology* **79:** 503-516.

Steidl, R. J. and Thomas, L. (2001). Power analysis and experimental design. In *Design and analysis of ecological experiments*. S. M. Scheiner and J. Gurevitch (eds). Oxford University Press, Oxford. Pp. 3-13.

Thomas, L. and Krebs, C. J. (1997). A review of statistical power analysis software. *Bulletin of the Ecological Society of America* **April 1997:** 126-139.

Tidemann, C.R., and Vardon, M.J. (2002). Animal welfare v wildlife research? *Pacific Conservation Biology* **8:** 71-72.

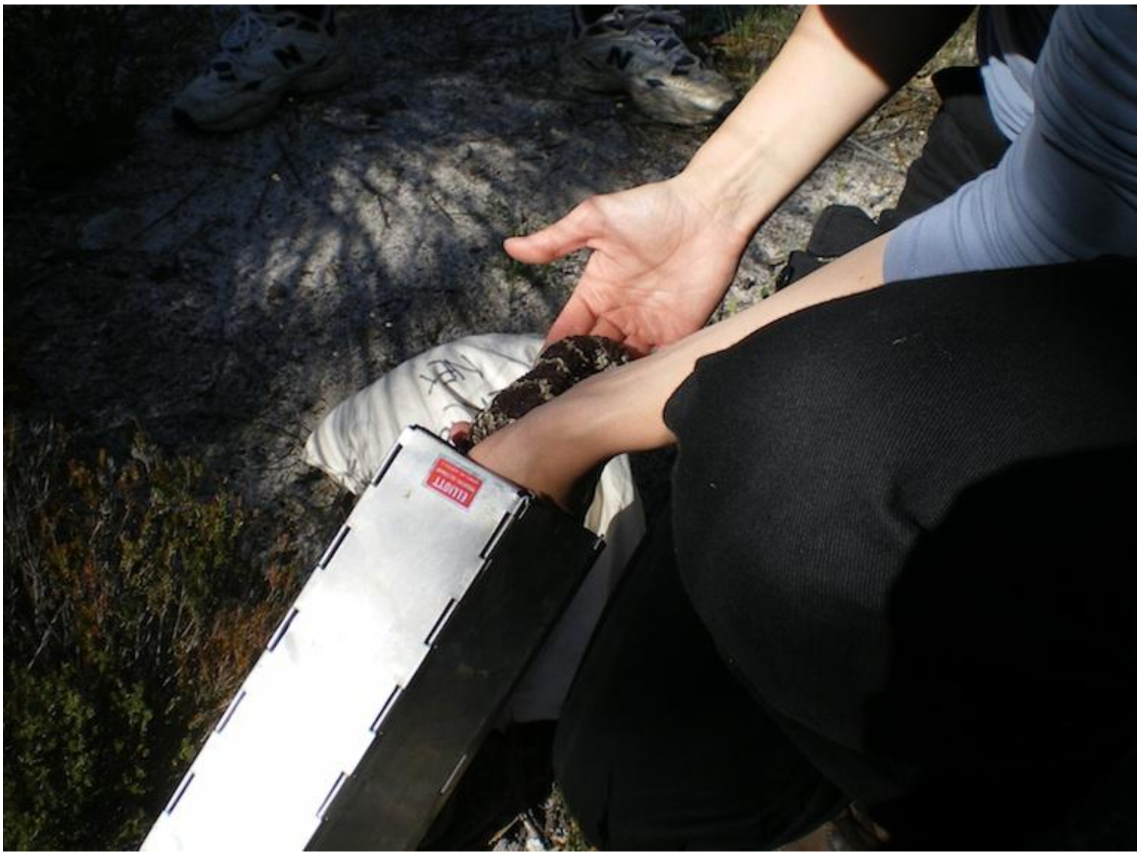
Webster, T., Dawson, S. and Slooten, E. (2010). A simple laser photogrammetry technique for measuring Hector's dolphins (*Cephalorhynchus hectori*) in the field. *Marine Mammal Science* **26:** 296-308.

West, J.G. (2011). Changing patterns of shark attacks in Australian waters. *Marine and Freshwater Research* **62:** 744-754.

Appendix 1. Examples of common traps and wildlife monitoring devices

1.1 Cage traps are used to catch medium-sized mammals (left - courtesy Mike Bamford) or larger ones (centre – courtesy Peter Adams). Traps should be positioned in sheltered places and insulation provided from rain and cold (right - courtesy Kate Bryant).

Appendix 1.2 Small mammals, amphibians and reptiles are often captured in pitfall traps. These are holes lined with either a plastic sleeve (e.g., a section of 15cm diameter storm water pipe) or a 9l bucket. Shelter may be placed inside the trap and some provision such as raised platforms or drain holes must be taken in case of rain collecting in the trap. The trap line is marked by a fly wire drift fence. Small animals are stopped by the fence, move along it and encounter a trap (courtesy Kate Bryant).



Appendix 1.3 Elliott traps, which come in a range of sizes, are used to catch small mammals and reptiles. Traps should be positioned in sheltered places and insulation provided from rain and cold (courtesy Kate Bryant).

Appendix 1.4 Sometimes individual animals have unique natural markings, analogous to human fingerprints, which can be used in identification from photographic records. For example, bottlenose dolphins may have unique marks on the dorsal fin that can be used to identify and trace individuals (courtesy Lars Bejder).

Appendix 1.5 Animals can also be monitored using motion sensitive camera stations (courtesy Mike Bamford).

Appendix 1.6. Mist nets are fine mesh nets suspended between fixed poles to catch birds and bats. The net runs across the photograph, with the mesh most visible against the background of the person at the top left. It's hard to see the mesh elsewhere, which is why it's effective (courtesy Kate Bryant).

Appendix 1.7 Many different tagging methods are available. Clockwise from the top left these examples show: leg banding of birds (courtesy Mike Bamford), fluorescent dust tagging of a small mammal (the trail of dust can be used to trace its movements) (courtesy Rodney Armistead), flipper tagging of a turtle (courtesy Mike Bamford) and a microchip implant in a medium-sized mammal (courtesy Mike Bamford).

Appendix 1.8 Radio collars allow animals to be located and their movements monitored. They come in different sizes and designs to suit many different animals. These examples show a domestic cat (left), a feral camel in inland Australia (middle) and a feral pig from south-western Australia (right). Photographs courtesy of Maggie Lilith (cat), Peter Spencer (camel) and Peter Adams (pig).

The Role of Public Perception in Pest and Wild Animal Management – Changing it for the Better.

Dr Peter Mawson
Director of Animal Health and Research
Perth Zoo

Abstract:

In recent times there have been a number of high profile media items depicting strong, adverse public reactions to pest or over-abundant wildlife management and wildlife euthanasia events. Strong opposition and adverse media representation of events have resulted in changes to original plans, and even their abandonment. This has often resulted in poorer welfare outcomes in the longer-term for the animals.

Achieving the intended animal management goals and obtaining better community understanding is dependent on a clear understanding of the level of public awareness about key pertinent facts and achieving balanced or reasonable coverage in the media.

Once these key facts are understood, wildlife managers need to develop clearly worded education/communication campaigns that demonstrate a transparent sharing of information along with a willingness to engage in informed discussion. While this may not result in full acceptance of pest animal and wildlife management activities, it can achieve a more balanced and informed debate within the community.

I have worked for 27 years in a range of jobs in state government agencies researching over-abundant native and exotic animals in Western Australia and developing improved methods for their control and management. In the later part of that time I also gained extensive experience in interacting with mainstream media outlets and in responding to general enquiries from members of the public and interested stakeholders on matters to do with over-abundant and pest animals. During that time I have seen many changes in how particular messages can be delivered and how those same messages are received and interpreted.

In an effort to demonstrate the types of problems that arise when engaging with the public on wildlife and pest animal management issues, I have provided below four recent issues that received considerable media coverage and used them to highlight the key lessons in managing public perception. I have included published quotes taken from the media articles that show some of the range of public perceptions on wildlife management and animal welfare issues.

<u>Case 1:</u> The first example concerns the management of over-abundant eastern grey kangaroos (*Macropus giganteus*) in the Australian Capital Territory (ACT). The issue is not especially new, but following an extended dry period in the ACT the kangaroo population had moved into urban areas in search of green feed and water where they subsequently caused problems of over-grazing grassed areas, eating planted gardens and colliding with vehicles. When good rains finally fell in the region, the kangaroos did not return to their former haunts and bred at a higher rate, exacerbating the problem. The ACT Parks Authority decided that culling was necessary to reduce kangaroo numbers in some areas. At the same time ACT Parks and the Australian National University (ANU) (and others) had been developing a program at Mulligans Flat where a fenced reserve was being managed to protect threatened fauna and to allow for the re-introduction of locally extinct species such as the eastern or Tasmanian bettong (*Bettongia gaimardi*). The fenced area enclosed a resident population of kangaroos that needed to be managed to avoid over-crowding. The preparations at Mulligans Flat had been in place for several years (see http://www.tams.act.gov.au/play/pcl/parks_reserves_and_open_places/national_parks/mulligans_flat) and there had been extensive media coverage of that program and its objectives. The situation changed dramatically when opponents of the proposed culling program for the eastern grey kangaroos damaged the predator-proof fence at Mulligans Flat in an attempt to release kangaroos from the reserve that were to be culled. More than 30 sections of fence were damaged. The matter worsened when researchers and students from the ANU began receiving death threats because of their perceived (but incorrect) involvement in the kangaroo culling program. Media coverage of the issue included quotes attributed to unnamed protestors who said that they were unrepentant about the vandalism and death threats as they thought they had nothing to lose.

Case 2: A neonatal humpback whale (*Megaptera novaehollandiae*) abandoned in Sydney Harbour in August 2008 attracted world-wide media attention. Not surprisingly it was not possible to save the whale calf (dubbed "Collette" by the media, but was later determined to be a "Colin") and it was euthanased by veterinarians several days after it was first encountered. Media coverage at the end of the process included comments from members of the public such as "I don't understand why they didn't let it die quietly." A comment from a spokeswoman for the NSW Environment Minister Verity Firth said "... among the lessons was a need to communicate better and explain decisions to the community ...".

Case 3: In September 2008 an adult humpback whale stranded on the mid-west coast of WA. After assessment by conservation staff, and on advice from veterinarians, it was euthanased using a controlled implosion created with explosives (see Coughran *et al.* 2012). The event received domestic and international media coverage including comments from readers such as "...presumably, it was too large to euthanize, but to detonate an explosive in its brain, then have to shoot it when the explosive didn't work, then plant another explosive?" A Western Australian media outlet ran a comment from an 'expert', Laurie Levy, a former whale rescuer who ran the Whale Rescue Center in Victoria in the 1980s, who said "I'm disappointed that they went ahead with it". Another media article included the more conservative comment "The sad truth is, there has been a spate of whale strandings recently, prompting authorities to make difficult and controversial decision."

Case 4: In October 2011 the Victorian government introduced a bounty on fox and wild dog scalps. The four-year and $4 million program was designed to help control fox and wild dog numbers in Victoria. After the first 10 months more than 100,000 fox scalps had been handed in. Media articles produced at the time the 100,000 scalp milestone had been reached, included comments from the Chief Executive of the Invasive Animal Cooperative Research Centre saying that history had shown that bounties were ineffective. In response the Victorian Minster for Agriculture and Food Security was quoted as saying "The Invasive Animal CRC should take their research findings to the main street of any town affected by foxes and see what the public think of their research. ... We trust the real-life experience of farmers on the effectiveness of the Coalition's fox bounty." Another comment published in the media said "Some 80,000 foxes have been destroyed thanks to the bounty and are not savagely mauling lambs and calves as a result."

In order to obtain the best possible outcome in any wildlife management or pest animal control program it is useful to work out answers to a few key questions first.

1. Who are the 'public'?
2. How are they getting the message?
3. Are they getting the message they need?
4. What happens when they get the wrong message?
5. What can we, as managers, do to improve how we communicate with the world?

Who are the 'public'?

The public can be anybody from a casual passer-by to the vocal minority and the silent majority. In most cases involving wildlife and pest animals, the local community in the area where the activity is actually occurring, and especially those who are directly affected, are the most important people to communicate with in the short-term. After that the wider community and then the rest of the world need to be considered. Included in that wider community are policy makers, government and regulating authorities. Key non-government stakeholders such as RSPCA and the larger animal welfare peak bodies may also need to be included in any communication strategy being undertaken. It is important to consider government and non-government organizations separately, as the manner in which they are engaged is often different to the way an individual in the community is engaged.

How are they getting the message?

The advent of modern electronic communications such as the internet and the mobile telephone means that a diverse range of people with an equally diverse range of backgrounds and prior knowledge and opinions of particular situations, can be made aware of issues, can comment on them and can object to them very quickly. Modern media outlets have also embraced the electronic communication revolution and taken it further by now providing 'evolving story lines' and '20-minute updates'. This trickle feed of information is relatively new to most researchers and conservation managers. While it provides an opportunity to prolong a story in the media and potentially engage a larger audience, it can also create problems, in that there is less opportunity to provide all of the necessary or desired information in one concise media event. If all of the necessary facts are not delivered until the story line has fully evolved, then there is a risk that some members of the public will miss parts of the message because they do not see all of the snippets of information, or they react before they have been provided with all of the facts. Going off half-cocked is nothing new, but modern electronic communication strategies have the capacity to make this outcome the norm rather than the exception.

Are they getting the message they need?

Many people in wildlife and pest animal management, deliberately or otherwise, do not engage with the community in the first place. Modern communications and media strategies provide a fertile environment for speculation, assumption and falsehoods to fill the void created by an absence of facts and it is not surprising that adverse outcomes arise as a result. Providing the facts about a situation (willingly or begrudgingly) after a protest or an adverse or unflattering media article has appeared can also have negative outcomes: you are likely to appear defensive and less credible when you are playing catch-up after the public or media have raised an issue or concern. The public have become so accustomed to seeing politicians and bureaucrats defending themselves in the public arena that they may assume that wildlife and pest animal issues are being managed with an intent to hide relevant details. The reality is that in most cases there is no intent to deceive and exclude the wider public from such issues, but the delivery of the relevant facts has not been attempted or achieved.

Being able to answer any and all questions that might be raised by the public or put by the media is important. It should not be surprising that public support is not forthcoming if you cannot answer reasonable questions. We expect our children to do their homework in order to pass tests or exams, and this approach is equally applicable to engaging with the public and the media. We should anticipate the likely questions, particularly those relating to controversial topics, and have the necessary information at hand to answer them.

Releasing a 1-2 page media statement on a wildlife or pest animal issue is seldom enough to engage the public or to satisfy their concerns. It is very difficult to start a conversation with a media statement and media statements that only mention the names of important persons, such as Ministers or Directors General, make it very difficult for the public to contact anyone to obtain further information or to discuss their concerns.

It is not uncommon for reporters to seek and obtain comment from so called experts, who for the benefit of the article or report, have a view different to that being advocated in the wildlife program or animal welfare issue. There is no compelling need to respond to each and all alternate view unless a compelling scientific viewpoint has been raised. If the science underpinning the proposed program is sound, it should be able to withstand scrutiny and if not, then there is possibly a need for genuine review of the facts. Most people can work out for themselves that the views of an expert who is not directly involved in the research pertinent to the issue at hand are probably not as relevant. If it is felt necessary to respond to the views of an independent expert, it is important to limit comment to the science that has been raised and not make the response a personal attack.

Following the release of a media statement, stakeholders or others not directly involved in the issue at hand, may take the opportunity to raise a similar but separate issue as part of a response. This hijacking of the communication process can lead to considerable confusion about exactly what the issue is and who is running the program and may lead the public to form the view that the official information provided by the program leader is incorrect or in some way deficient. Sometimes these hijackings are intentional, but you can't blame passionate people for taking advantage of media interest in a particular subject and wanting to use that opportunity for all it is worth.

What happens when the public get the wrong message?

If the public have clearly taken the wrong message from a media statement or media coverage of an issue, it is important to correct the problem as quickly as possible. Having said that, it is equally important not to rush in with a poorly prepared response because that may only add to the confusion and give the public more reason to think that the program manager really doesn't know what he or she is doing.

The public often receives a message in the manner it was intended but has concerns about some aspects of the proposed program. If they cannot contact anyone to discuss those concerns or they feel that they are being ignored, then their concerns about how they are being treated become the focus, rather than the information that was originally provided. That is to say, the science becomes a secondary issue to the public's feelings. When things get to this stage, program leaders and government can get worried about 'public perceptions' and can water down planned activities or withdraw their support entirely. The science behind the intended activity remains unchanged, but the likelihood of success diminishes dramatically to the point where the good science is sacrificed to appease the concerned members of the public. If repeated attempts at engaging with the public lead to this same type of outcome there is the likelihood that decision makers will shy away from attempting similar management actions in the future. While this may be an ideal outcome for some sections of the community, it can also lead to adverse animal welfare outcomes for the over-abundant animal or pest animal populations under consideration.

What can managers do to improve how we communicate with the world?

There are lots of things that managers can do to communicate more effectively with the world. The most important thing is to plan well ahead of any event, whenever possible. Given the wide range of things that can influence the approval process for media statements, it pays to develop a media or communication strategy for planned events that can be guided by people/staff with media expertise. It is important to present the facts relevant to the program clearly and concisely. It is also important to do it in as many formats as necessary. For example the text you would develop for an item to be posted on a website will be different to that presented in a media release and quite different again to the information provided to a Minister, CEO or Vice Chancellor as part of a briefing note.

Wherever possible you should try and enlist the support of independent stakeholders, especially non-government ones, prior to announcing a program. It looks more professional and comes across much better if you can truthfully say that you have already discussed the proposed program with relevant stakeholder groups and that you have their support or at least acceptance of what is proposed. If you can present a united position you stand a much better chance of public acceptance and of being able to implement the proposed program.

It is also important that managers are prepared to talk to concerned people openly, calmly, honestly and as often as necessary. In order to do this, managers should designate one person (or more) who has a detailed knowledge of the issue or the proposed management program and is a competent communicator. It will also be highly likely that after engaging with a small number of people the outcome is that you will agree to disagree. Reaching an agreement to disagree needs to be done carefully so that both parties can end the conversation with a level of respect for one another’s views, rather than a feeling of having ‘won or lost’ the argument: it isn’t about arguing, it’s about communicating.

In the same vein as being prepared for specific media events, there is a standing opportunity for wildlife managers to use dedicated web pages to provide information on a range of topics that have previously been dealt with successfully in the past, or examples of research projects designed to deliver improved animal welfare outcomes. Being able to refer to a series of such programs or projects and to have a history of being open and accountable goes a long way to demonstrating a positive attitude and a willingness to share information. It is also very handy to be able to refer back to such matters when dealing with contemporary issues as a way of showing that the particular issue at hand is not occurring for the first time and has been the subject of ongoing research and management. Given the lead time required to obtain all the approvals necessary to have new material posted on corporate websites, this approach is one that should best be considered as a tool for proactive rather than reactive management.

Hearts and brains aren’t enough

As a conclusion to this contribution I offer a few words of advice to managers. It is not enough just to be passionate about animal control or animal welfare issues. Neither is it enough to be right about a particular issue. You need a human face to present the issue to the public who is available, knowledgeable and can engage in any type of conversation and still want to come to work tomorrow and do it all again.

References

Coughran, D. K., Stiles, I. and Mawson, P. R. (2012). Euthanasia of beached humpback whales using explosives. *Journal of Cetacean Research and Management* 12(1); 137-144.

Introducing the Controversies

Leisha Hewitt

University of Western Australia

No Abstract Received

No Paper Received

Humane euthanasia of pigs

Barbara Frey
Murdoch University

Abstract:

Euthanasia of pigs is problematic given their highly fearful nature and the challenges of physical restraint with minimal stress. This paper reviews current research on euthanasia including common practice in industry as well as in research. A summary of possible options is provided for various classes of pigs, considering each approach from the perspective of its practical, ethical and humane merits.

No Paper Received

Lethal sampling of stingrays (Dasyatidae) for research

Owen R. O'Shea[1, 2], Mark G. Meekan[1] and Mike van Keulen[2]

[1] Australian Institute of Marine Science, 35 Stirling Highway, Crawley, Western Australia, 6009, Australia
[2] Murdoch University, 90 South Street, Murdoch, Western Australia, 6150, Australia

Abstract:

For some species certain biological and ecological data, such as diet, age and growth estimates can only be obtained through lethal sampling of study animals. Traditionally, commercial fishermen have provided samples of rays caught in nets for use by biologists; however, by-catch exclusion devices now prevent medium and larger-bodied rays (>100 cm disc width) from being collected in trawl nets. This means that other methods must be used for lethal sampling. We obtained a large suite of biological and ecological data from 170 wild-caught stingrays collected from Ningaloo Reef, Western Australia over two years. Our sampling program was designed to minimize or eradicate any pain and suffering to the animals, while ensuring the safety of researchers undertaking the sampling process. Small rays (< 100 cm disc width; W_D) were caught in beach seines and euthanized immediately by destruction of the brain and severance of the spinal cord with a reinforced, serrated steel knife. Larger rays were euthanized by firing high-powered spears directly into their brains from close range while free diving. Of the 170 rays sampled in this manner, 94 % (159) were killed instantly or within an estimated 10 - 30 seconds of capture. The design and application of this lethal sampling program was deemed successful in terms of ensuring the safety of researchers as well as minimising suffering to rays. Pain perception in elasmobranchs has been quantified by few studies; however, research suggests that certain neural apparatus associated with pain sensation is lacking in rays. Our study has provided critical data on the biology and life history of stingrays that could not be obtained by any other means.

Keywords: Animal ethics, coral reefs, rays, scientific sampling, destructive techniques

Introduction

Ecological research often involves the collection of certain life history data that requires invasive and sometimes destructive sampling methods. Although lethal collection may come at a cost to a population, such sampling provides essential data that cannot be obtained in any other way (Heupel and Simpfendorfer 2010). Examples of this type of research include age and growth assessment in elasmobranchs, which requires the removal of vertebral sections to analyse banded calcium deposits within the centra, reproductive studies that typically involve macroscopic inspection of internal organs, combined with tissue harvest for histological analysis and some dietary studies that require dissection of the gut for analysis of contents. Alternatives to lethal sampling in elasmobranchs do exist, such as stomach lavaging (Barnett et al. 2010) or stable isotope analysis (Speed et al. 2011) instead of stomach dissection, the use of caudal thorns (where present) for ageing instead of vertebral extraction (Matta and Gunderson 2007) and ultrasonography for the assessment of maturity in oviparous species (Whittamore et al. 2010). While these methods are valid, ethical and welfare-based, they are not always appropriate or even possible for some species. This leaves researchers little choice but to use lethal sampling for collection of some ecological data.

In tropical Australia, stingrays (Dasyatidae) have been traditionally obtained from the bycatch of commercial fishers, primarily from the Northern Prawn Trawl Fishery. However, since the early 2000's, the use of bycatch reduction and turtle exclusion devices (BRDs & TEDs) has been mandatory, significantly reducing the incidental capture of elasmobranchs (Brewer et al.

2006). In order for certain research on rays to continue, alternative collection methods must be adopted. Given the size stingrays can attain (> 200 cm disc width W_D) and the potential for harm associated with the large and toxic barbs present on their tails, such collections must be undertaken with great care to maintain a safe environment for researchers. Equal consideration must also be given to the ethical treatment of the target animals and minimization of the pain and suffering that they may experience during collection.

The overall objective of this study was to conduct a lethal sampling program in order to collect a large suite of demographic (age, growth and population structure) and ecological (diet, reproduction, genetics) data on stingrays in a coral reef environment. Here, we detail methods of our lethal sampling program used to collect 170 individual stingrays at Ningaloo Reef, Western Australia while ensuring both animal welfare and the safety of researchers.

Methods

Study location and species

This study was part of a collaborative research effort assessing the ecological role of stingrays within the Ningaloo Reef Marine Park (NRMP) in Western Australia (Figure 1). The NRMP is the largest fringing reef in Australia and has a geo-morphologically complex coastline, creating a diverse range of habitats, supporting a high diversity of flora and fauna, particularly elasmobranchs (Last and Stevens 2009). Sampling took place at 18 locations within the Marine Park (Table I) between November 2009 and November 2011. We sampled 170 rays of five sympatric species including the blue-spotted mask *Neotrygon kuhlii* (Müller and Henle 1841), (n=36), cowtail *Pastinachus atrus* (Macleay 1883) (n=43), blue-spotted fantail *Taeniura lymma* (Forsskål 1775), (n=54) porcupine *Urogymnus asperrimus*, (Bloch and Schneider 1801), (n=13) rays and the reticulate whipray *Himantura uarnak* (Forsskål 1775), (n=24) (Table II).

Sampling Design

All rays were captured and euthanased *in situ*, from inshore and offshore sites. Inshore sites were accessed from beaches and typically consisted of water ≤ 2 metres deep. Offshore sites were always within the lagoon and accessed from a research vessel. Maximum water depth never exceeded 8 metres. Of the five species sampled, two are smaller (*T. lymma* and *N. kuhlii*), reaching maximum sizes of 35 cm and 47 cm W_D respectively (Last and Stevens 2009), while three species attain much larger sizes (*Pastinachus atrus*, 200 cm; *Himanutra uarnak*, 140 cm and *Urogymnus asperrimus*, 115 cm W_D) (Last and Stevens 2009). Separate sampling methods were designed based on size and sites of capture. At inshore sites, rays were generally smaller (≤ 100 cm W_D), so beach seines and hand nets were used for capture. Large rays (≥100 cm W_D) and those caught offshore were euthanased with spear guns (Undersee woodie MKII 1700, MKII 1400 and Mares Cyrano 700) while free diving.

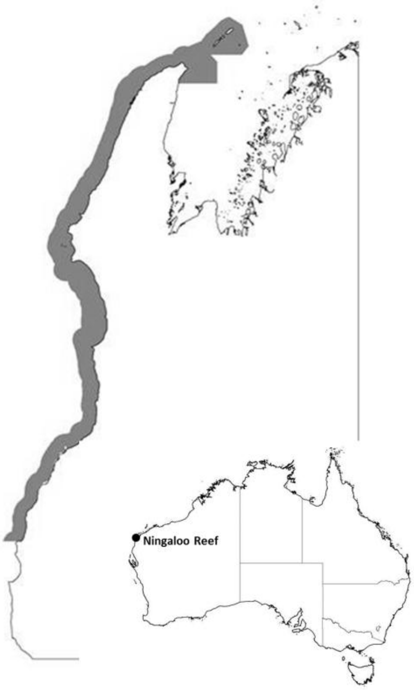


Figure 1: Map of the Ningaloo Reef Marine Park, highlighting the three broad areas targeted for sampling

Site	Latitude and Longitude
Tantabiddi South	-21.9386, 113.9664
Mangrove Bay	-21.9762, 113.9598
Ranger Bay	-21.9403,113.9674
Ranger Bay back reef	-21.9397, 113.9707
Point Look	-21.9968, 113.9252
Point Look back reef	-21.9987, 113.9140
Winderabandi Point	-22.4960, 113.7042
North Lefroy Bay	-22.5155, 113.7070
South Lefroy Bay	-22.5337, 113.6791
Point Edgar	-22.5803, 113.6519
Stanley Pool	-22.9455, 113.7857
Point Maud	-23.1184, 113.7630
The Maze	-23.1218, 113.7514
Skeleton Bay north	-23.1255, 113.7694
Skeleton Bay south	-23.1335,113.7703
Five Fingers Reef	-23.1748, 113.7592
Monk’s Head	-23.2135, 113.7651
South Passage Coral Bay	-23.2204, 113.7695

Table I: Sampling sites and associated waypoints within the Ningaloo Reef Marine Park

	N	Male	Female	Disc Width (cm) range	Weight range (kg)
H. uarnak	24	14	10	37 – 145.5	1 – 68.5
N. kuhlii	36	25	11	12.5 - 47	0.8 – 3.7
P. atrus	43	21	22	27 – 177	0.7 – 136.4
T. lymma	54	29	25	14 – 32.5	0.8 – 3.2
U. asperrimus	13	8	5	93 – 118.5	38.5 – 90.4

Table II: Morphometric and demographic data of rays sampled

Considerations

The rays used as part of this research were selected because they are numerically abundant within the NRMP (Last and Stevens 2009) and have an important ecological role and impact within these environments. It was also considered essential to spread our sampling effort among 18 sites within 3 broader locations – northern, mid and southern sections of the Marine Park to minimise impact on any single population. In order to ensure personal safety, teams consisted of two free-divers, with a spotter on a research vessel boat close by and a skipper. The first diver would descend to the ray and position themselves before firing the spear. The second diver at the surface would observe and if required also free-dive to take a second shot or assist the first diver if any issues arose. Ten metre float lines were attached to the loading butt of each speargun so after the spear was fired, the diver could simply let go of the speargun to avoid potential entanglement or dislodgment of the spear if the animal bolted. Surface floats also allowed divers to return to the boat without losing sight of the ray. Rays were then lifted to the boat using the float lines before the tail was secured to avoid injury, either accidentally or through a post-mortem muscle spasm. Once the tail was secured, the spears were removed and for very large rays, a rope was passed through the spiracles in order to lift it into the boat.

Lethal sampling techniques

The cartilaginous brain casings of rays are penetrated easily by spears at close range ensuring a fast death with minimal suffering. This was deemed the most efficient, direct and safest way to euthanase rays while operating in deeper water. For smaller rays at inshore sites, one ray at a time was caught by actively herding them into the net, rather than passive trapping. Once caught, they were bought into the shallows in a hand net and killed by directly destroying the brain and/or severing the spinal cord immediately behind the head. This was done using a reinforced steel commercial diver's knife. An assessment of corneal reflex was used to confirm death, which involved touching the eye. This would retract if brain function still existed.

Rays are relatively sedentary animals and during offshore sampling were encountered either feeding or resting. This enabled divers to get close enough to allow very accurate shots when firing spears. The three species of larger ray all exhibited different behaviours when first encountered. *Himantura uarnak* were generally buried and inactive in soft sediments within close proximity of the coral reef, seemingly favouring an edge habitat. This species made no attempt to evade the boat or divers. *Urogymnus asperrimus* was detectable due to the large sediment plumes arising from vigorous feeding that was typical of this species when encountered. They also made no effort to move and for this reason, these two species were generally killed instantly with little or no suffering (as perceived by the divers). The third species, *Pastinachus atrus* tended to act more unpredictably and being the largest species of the five, was treated with more caution. Individual *P. atrus* were either feeding or resting in sandy patches within the lagoonal reef complex and would generally flee when the boat approached. Due to excellent camouflage they are harder to see than other large species however, evasive behaviour in response to the boat's presence would expose previously unnoticed rays. Avoidance behaviour exhibited by this species consisted of individuals swimming to nearby coral heads or fragmented reef structure and burying themselves in the sand immediately adjacent to the reef. They are very large and conspicuous animals when swimming and because of the good water clarity at Ningaloo most of the year, they were easily followed, precluding the need to chase them in a way that might have caused undue stress.

Once located, unambiguous identification of the ray was made from the surface to ensure non-targeted species were not collected. Divers entered the water up-current from the ray and approached slowly. It was essential to not alarm the rays so they did not react defensively or initiate a flight response. Rays were approached from the front at an angle of not less than 45º from a line running in the direction in which the ray was facing (Figure 2). This ensured the ray was aware of diver's presence as well as having a space away from the diver in which to flee so it was not 'cornered'. This also enabled divers to return to the surface to observe at a safe distance from the dying, or fleeing animal. In every case, rays (whether speared or not) rapidly swam away from the diver across the benthos.

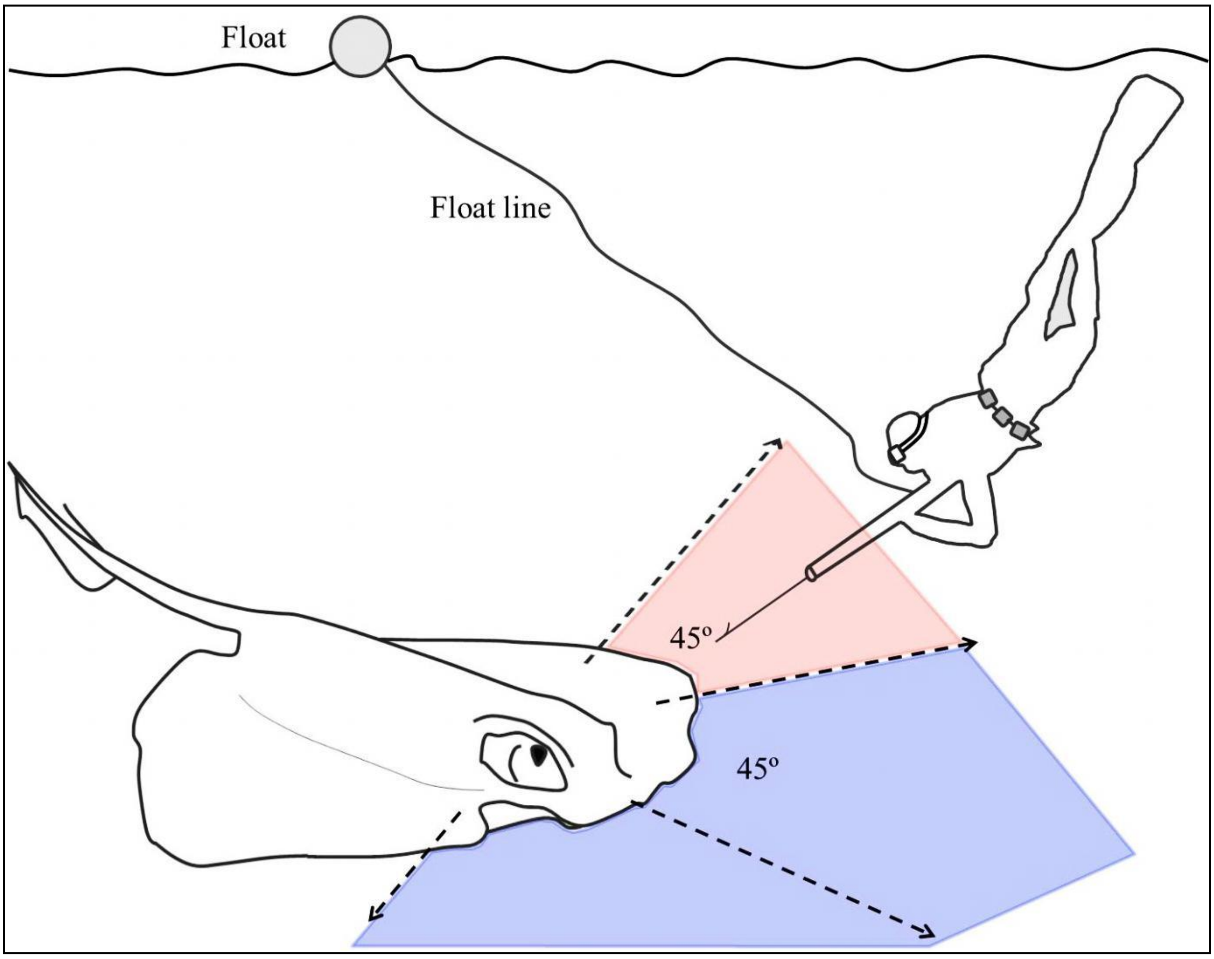


Figure 2: Diagram demonstrating the optimum approach by a diver to a ray while free diving. The arrows represent the directions in which rays will typically swim based on behaviours observed during this research. The blue shaded area highlights the ideal angle of flight, while the red poses the greatest risk. It is important to note, that both red are blue areas are not mutually exclusive, and whichever angle the diver approaches from will always be 'red'.

Observations were made after the spear had been fired to ensure the animal was killed outright rather than seriously wounded. The spearguns used were 1.7 and 1.4 metres in length, and because they were wooden, remained slightly positively buoyant, which allowed the gun to be extended away from the diver's body, thereby increasing the distance from the diver to the target ray, while still enabling firing at near point blank range. The target area on the ray was the interorbital space and when hit, enabled the spear to penetrate the brain resulting in an immediate death. Post-mortem movements, including beating of the pectoral fins and some erratic swimming were common among all rays for up to several minutes after death. A small number of rays (predominantly *U. asperrimus*) did not move at all after the spear was fired. Several individuals (mainly *P. atrus*) bolted prior to the spear being fired and this was always away from divers and the perceived threat. In these instances, spears were not fired, but rays were easily tracked from above the surface. On 11 occasions rays did flee at the same time a spear was fired, which either resulted in a miss, the spear glancing off the ray, or striking the ray with a non-lethal shot. When the latter occurred, divers returned immediately to the boat, where the ray was tracked from the surface and when it came to rest, a second spear was fired. Every ray from the two smaller species was killed with one direct hit.

Discussion

The use of lethal sampling in ecological research is sometimes unavoidable. However, the development of safe and ethical techniques for procuring data remains at the core of any such sampling program. The methods outlined here were developed to acquire data via lethal sampling, while maintaining the highest safety standards and ensuring ethical standards were also met. The use of spearguns to kill rays was effective when compared to other methods. For example, static sampling using hook and line can be non-selective, time consuming and stressful for any animal caught. Of the 170 rays euthanased for this research, 94% (n=159) were killed within an estimated 10-30 seconds by spearing or insertion of a knife into the brain. Of the remaining 6% (n=11), complications arose when the ray evaded the diver at the moment of firing the speargun, resulting in a non-lethal strike. While the protocol described in this paper was designed to prevent this situation and was largely successful, we failed to kill the target animal outright in 11 cases. However, the use of float lines allowed immediate tracking of the ray and administration of a subsequent lethal shot. In each of these cases, the maximum time between the first and second shot was no longer than approximately five – eight minutes. Throughout the course of this 24-month program, no incidents were reported or injuries sustained to any member of the research team.

Pain perception in elasmobranchs

Most of our knowledge of pain perception in animals comes from higher vertebrates, such as mammals and birds (Braithwaite and Boulcott 2007). This subject is a relatively new area of study for fish and has resulted in conflicting views (Sneddon 2003) but see (Sneddon et al. 2003a). Nociceptors are part of the neural apparatus associated with pain perception in most animals, including fish. These are capable of detecting noxious or potentially damaging stimuli (Braithwaite and Boulcott 2007). Nociceptive pathways comprise either A-delta nerve fibres or C fibres with the latter being the predominant nerve fibre type in higher vertebrates involved in pain perception (Sneddon et al. 2003a). Some of the few studies conducted on elasmobranchs found the presence of A-delta fibres in some species (the Atlantic stingray, *Dasyatis sabina* and the pink whipray, *Himantura fai*), but C fibres were either absent or found in very low numbers suggesting pain perception may be reduced less than those species where C fibres are present (Coggeshall et al. 1978, Leonard 1985, Snow et al. 1993, Snow et al. 1996, Braithwaite and Boulcott 2007). This also appears to be the case for the spotted eagle ray, *Aetobatus narinari* (n=1), cownose ray, *Rhinoptera bonasus* (n=2) (Coggeshall et al. 1978), black tip reef shark, *Carcharhinus melanopterus* (n=3), shovelnose ray, (*Glaucostegus typus*) (n=4) and a member of the whipray genus, *Himantura* sp. (n=3) (Snow et al. 1993). Given that some authors have concluded that C fibres are essential for the sensation of pain, it may well be that pain perception in those species that lack them might have little relevance to survival.

Outcomes of this research

The lethal sampling undertaken in our research program has led to a better understanding of the key ecological functions of stingrays in tropical reef environments and can be used to formulate better management and conservation strategies. While our study is the first comprehensive assessment of the ecology and biology of stingrays within the NRMP, it has implications in a broader Indo-Pacific context where rays are harvested for meat, leather and gill filaments (White et al. 2006). Our lethal sampling allowed the evaluation of dietary preferences, contrasted the feeding habits of five sympatric species of ray and assessed the potential for resource partitioning, which can be used to further understand the importance of these species as mesopredators (O’shea et al. under review). Assessment of the age and growth of these rays allows insights into population structure and biological traits of rays within the NRMP (O’shea et al. under review). Further work has described the use of DNA barcoding as a tool for identifying cryptic species, aiding field identification and highlighting species complexes (Cerutti-Pereyra et al. 2012).

Macroscopic analyses of reproductive organs in combination with age and growth data have allowed information about size at maturity to be introduced into fisheries management strategies (O’Shea et al. in preparation). An unexpected outcome from this research was the description of a new locality record for a parasitic leech and two new host relationships, not previously recorded along the west coast of Australia (O'Shea 2010). Finally, collection of these rays has allowed a study of the ecological and phylogenetic factors influencing the distribution and number of electroreceptor sensory organs (Kempster et al. 2011). There are six other research papers detailing vision, electro-sensory morphology, neurone populations and cranial nerve counts that are currently in preparation, directly resulting from this lethal sampling program.

Conclusions

Using the methods described here we have successfully completed a lethal sampling program for dasyatid rays. Our approach factored in ethical considerations, researcher safety and the potential for quality data to be collected. Most animals targeted as part of this research were euthanased quickly, efficiently and potentially without experiencing pain. While the notion of lethal sampling for research will continue to be the subject of debate among all sections of the community, it is hoped that the careful and complete analysis of the samples collected by our study will remove the need for the collection of such types of data in the future.

Acknowledgments:

We thank the Department of Environment and Conservation (WA); Murdoch University Animals Ethics Committee, particularly Moira Desport and Erich von Dietze. Field volunteers, A. Turco, T. Hill, S. Ridley, H. Bowers, T. Schenk, M. Drevander, G. Vianna, D. Ilich, C. Speed, C. Egeberg, K. Brooks, J. Melvin, C. Wood, R. Kempster, E. Garza-Gisholt and especially T. Espeland. This study was funded by the Australian Institute of Marine Science and all animals were collected under WA fisheries exemption permit # RS457/98-05 and Murdoch University animal ethics licence #U6/2010-2011 and Murdoch University ethics permit #R2275/09.

References:

Barnett, A., K. S. Redd, S. D. Frusher, J. D. Stevens, and J. M. Semmens. 2010. Non-lethal method to obtain stomach samples from a large marine predator and the use of DNA analysis to improve dietary information. *Journal of Experimental Marine Biology and Ecology* **393**:188-192.

Braithwaite, V. and P. Boulcott. 2007. Pain perception, aversion and fear in fish. *Diseases of aquatic organisms* **75**:131.

Brander, K. 1981. Disappearance of common skate *Raia batis* from Irish Sea. *Nature* **290**:48-49.

Brewer, D., D. Heales, D. Milton, Q. Dell, G. Fry, B. Venables, and P. Jones. 2006. The impact of turtle excluder devices and bycatch reduction devices on diverse tropical marine communities in Australia's northern prawn trawl fishery. *Fisheries Research* **81**:176-188.

Cerutti-Pereyra, F., M. G. Meekan, N. W. V. Wei, O. O'Shea, C. J. A. Bradshaw, and C. M. Austin. 2012. Identification of Rays through DNA Barcoding: An Application for Ecologists. *PloS one* **7**:e36479.

Coggeshall, R., R. Leonard, M. Applebaum, and W. Willis. 1978. Organization of peripheral nerves and spinal roots of the Atlantic stingray, Dasyatis sabina. *Journal of neurophysiology* **41**:97-107.

Dulvy, N. K. and J. D. Reynolds. 2002. Predicting extinction vulnerability in skates. *Conservation Biology* **16**:440-450.

Gilliam, D. and K. Sullivan. 1993. Diet and feeding habits of the southern stingray Dasyatis americana in the central Bahamas. *Bulletin of Marine Science* **52**:1007-1013.

Heupel, M. and C. Simpfendorfer. 2010. Science or slaughter: need for lethal sampling of sharks. *Conservation Biology* **24**:1212-1218.

Kempster, R., I. McCarthy, and S. Collin. 2011. Phylogenetic and ecological factors influencing the number and distribution of electroreceptors in elasmobranchs. *Journal of Fish Biology*.

Last, P. R. and J. D. Stevens. 2009. *Sharks and rays of Australia.* Second edition. CSIRO Publishing, Melbourne.

Leonard, R. B. 1985. Primary afferent receptive field properties and neurotransmitter candidates in a vertebrate lacking unmyelinated fibers. *Progress in clinical and biological research* **176**:135.

Matta, M. E. and D. R. Gunderson. 2007. Age, growth, maturity, and mortality of the Alaska skate, Bathyraja parmifera, in the eastern Bering Sea. *Environmental biology of fishes* **80**:309-323.

Musick, J. A. and R. Bonfil. 2005. Management techniques for elasmobranch fisheries. FAO.

O'Shea, O. R. 2010. New locality record for the parasitic leech *Pterobdella amara*, and two new host stingrays at Ningaloo Reef, Western Australia. *Marine Biodiversity Records* **3**.

O'Shea, O. R., M. Thums, M. van Keulen, and M. Meekan. 2012. Bioturbation by stingrays at Ningaloo Reef, Western Australia. *Marine and freshwater research* **63**:189-197.

Sneddon, L. U. 2003. The evidence for pain in fish: the use of morphine as an analgesic. *Applied Animal Behaviour Science* **83**:153-162.

Sneddon, L. U. 2009. Pain perception in fish: Indicators and endpoints. ILAR J **50**:338-342.

Sneddon, L. U., V. A. Braithwaite, and M. J. Gentle. 2003a. Do fishes have nociceptors? Evidence for the evolution of a vertebrate sensory system. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **270**:1115-1121.

Snelson Jr, F. F., S. E. Williams-Hooper, and T. H. Schmid. 1988. Reproduction and Ecology of the Atlantic Stingray, Dasyatis sabina, in Florida Coastal Lagoons. *Copeia*:729-739.

Snow, P. J., M. B. Plenderleith, and L. L. Wright. 1993. Quantitative study of primary sensory neurone populations of three species of elasmobranch fish. *The Journal of comparative neurology* **334**:97-103.

Snow, P. J., G. Renshaw, and K. E. Hamlin. 1996. Localization of enkephalin immunoreactivity in the spinal cord of the long-tailed ray Himantura fai. *The Journal of comparative neurology* **367**:264-273.

Speed, C., M. Meekan, I. Field, C. McMahon, K. Abrantes, and C. J. A. Bradshaw. 2011. Trophic ecology of reef sharks determined using stable isotopes and telemetry. *Coral Reefs*:1-11.

White, W. T., P. R. Last, J. D. Stevens, and G. K. Yearsley. 2006. *Economically important sharks & rays of Indonesia*. Australian Centre for International Agricultural Research (ACIAR).

Whittamore, J. M., C. Bloomer, G. M. Hanna, and I. D. McCarthy. 2010. Evaluating ultrasonography as a non-lethal method for the assessment of maturity in oviparous elasmobranchs. *Marine Biology* **157**:2613-2624.

This article was originally published in:
J. CETACEAN RES. MANAGE. 12(1): 137–144, 2012

Euthanasia of beached humpback whales using explosives

Douglas K. Coughran*, Ian Stiles[+] and Peter R. Mawson*
Department of Environment and Conservation

Abstract:

A method for the safe and effective euthanasia of large beached humpback whales using explosives is described. Five recent case studies involving live stranded humpback whales measuring 9.1–12.7m are described to show how the method was applied, and the capacity of the method to deal with the varying conditions encountered when dealing with large baleen whales. Issues relating to the wider application of this method to other species of baleen whale and large odontocete species are discussed along with key safety implications for the safe use of this method.

KEYWORDS: HUMPBACK WHALE; EUTHANASIA; ANIMAL WELFARE

INTRODUCTION

The live-beaching of a great whale presents a complex problem for wildlife managers and local government officials. It raises issues of animal welfare, public safety and the personal safety of the public officials involved. In some parts of the world, it is also often the subject of intense outpouring of public opinion and sentiment and can result in extensive media scrutiny during and after the event. As with many complex problems confronting government agencies, this one can be effectively managed only through cooperation as there are invariably multiple jurisdictions involved with multiple pieces of legislation in play.

With the protection of humpback whales (*Megaptera novaeangliae*) in 1963 and southern right whales (*Eubalaena australis*) since 1935 (Tønnessen and Johnsen, 1982), there have been encouraging increases in the number of both species visiting coastal Australian waters (e.g. Bannister, 2008; IWC, 2011). With the recovery in the numbers of these species, there is an increased likelihood of these animals coming ashore due to natural and human induced causes (Bannister *et al.*, 1996; Coughran and Gales, 2010). Kemper *et al.* (2005) reported more than 20 species of cetaceans as live-beaching in South Australia, including three species of great whale (sperm *Physeter macrocephalus*, Bryde's *B. edeni* and fin *B. physalus*). In Western Australia during the period 1981–2010 inclusive, several species (humpback, Bryde's, southern right, fin, blue *B. m. musculus*, pygmy blue *B. m. brevicauda*, Antarctic minke *B. acutorostrata* and sperm whales) have been recorded live-beaching (Department of Environment and Conservation (DEC) unpublished data).

Relocating live large whales weighing in the tens of thousands of kilograms is difficult and dangerous even under calm sea conditions. During inclement weather, the task can become extremely hazardous especially if the whales are beached on rocky substrates. If the risks are too great to allow a rescue team to work, or the logistics of moving the animal are unviable, then serious welfare issues arise. In circumstances where the whale faces a lingering death, euthanasia becomes a valid option (IWC, 2010).

Euthanasia of small cetaceans has been achieved using a range of techniques, including barbiturate overdose (intravenous or intra-cardiac injection), lancing of major heart blood vessels and shooting (brain or heart shot) using large calibre centre-fire firearms (Needham, 1993). While these methods are useful for smaller species (<6m; see Øen and Knudsen, 2007), they are inappropriate or unfeasible for the euthanasia of larger species such as baleen whales (Blackmore *et al.*, 1997). Data presented to the International Whaling Commission via workshops on whale killing methods (e.g. IWC, 2003) suggest that the use of firearms cannot guarantee a quick or humane death in all circumstances, but can have emergency application in some cases (IWC, 2010). Whales of a number of species are shot with large calibre bullets (7.62mm, 9.3mm, 30.06, .375 or .458 inch) in a number of whaling operations and for euthanasia (IWC, 2003).

The use of explosive charges such as penthrite (pentaerythritol tetranitrate or PETN) in the hunting of whales is well documented. Typically 30g charges are delivered into a whale's body via 50 or 60mm boat-mounted harpoon guns, which fire harpoons weighing between 12– 18kg (Øen, 1995a; 1995c; 1999). Harpoons are aimed at the thorax of the whales and can result in up to nearly 80% of the target animals dying instantaneously (Øen, 2002). Death usually results from blast-induced trauma to the vital organs, the central nervous system or the brain (Knudsen and Øen, 2003). The use of penthrite grenades on larger whales, such as bowhead whales taken during indigenous hunting, has resulted in times to death ranging from instantaneous up to a median time of 15 minutes (Øen, 1995b). Reference has been made in the published literature to the use of a range of methods for euthanasing large (>6m) whales (e.g. Dierauff, 1990; Hyman, 1990). The few publications that mention the use of explosives for the euthanasia of whales either provide no working details on specifics of the method, only mention the existence of field research (e.g. Needham, 1993), or largely dismiss the method for reasons not related to the capacity of the method to deliver a quick and humane death (e.g. Greer *et al.*, 2001).

This paper documents a highly effective and safe method of euthanasing humpback whales using explosives and the process that needs to be undertaken to safely apply it. Five case studies are presented to demonstrate the likely range of issues that can be expected in the field and some of the problems that have been encountered during the refinement of this methodology. This method was developed and refined over a 20 year period to the point where an instantaneous death can be delivered with minimal risk to the public and the wildlife management staff involved. The research was conducted by the Department of Environment and Conservation (DEC) on the lower west and south coast of Western Australia between 1990 and 2010.

MATERIALS AND METHODS

In Western Australia, the DEC is responsible for the administration of the Wildlife Conservation Act 1950 and managing fauna issues, including whales. In this capacity, the DEC has adopted the Australian Inter-Service Incident Management System, which provides a total systems approach to all incident management involving risk[1]. The state police department is responsible for the critical issues of public safety that emanate from public proximity to powerful animals and from the use of explosives, while local government authorities are responsible for public health issues associated with the management of each whale beaching incident.

The process that leads to a decision to euthanase a great whale is relatively straightforward and arrived at following a clinical assessment of each whale (Gales *et al.*, 2008), based on 'Behaviour Criteria' (alert, weakly responsive, nonresponsive) and 'General Condition Criteria' (behaviour in water, respiration, heart rate, body temperature and reflexes) of each whale. While there can be difficulty in interpreting every one of the categories during each assessment,

---

[1] *http://knowledgeweb.afac.com.au/training/aiims* accessed 15 March 2010

the wide array of parameters observed offers the best clinical assessment to determine the prognosis for each whale. Where there is doubt over interpretation, time is allowed in order to ascertain trends in condition. A whale may be in good physical condition but impossible to save. Under these conditions euthanasia is also important. The basic pathways to managers are straightforward and should not be complicated by public expectations and media influences that have no scientific basis.

In all cases reported here, every opportunity was taken to obtain independent veterinary advice either following on-site assessment or telephone discussions. During case 1, DEC staff consulted with a senior veterinary officer from the Western Australian Department of Agriculture and Food on site. For cases 2–5 inclusive, DEC staff on site consulted the senior veterinary officer at the Perth Zoo by telephone with regard to the prognosis and palliative care of the whales. Death of each whale was confirmed using the criteria described in case 1 and in case 5 a local veterinarian who was able to attend the site for the purposes of learning from the exercise was also able to confirm that an instantaneous death had been achieved from the detonation of explosives in that case.

Over the 20-year development period, some of the materials (type of explosive, detonator system) used have changed as technology has advanced. The most up-to-date materials being used are reported here, but the authors (DKC) can be contacted for details of the earlier types of materials used should that information be required.

RESULTS FROM CASE STUDIES

Case 1

On 9 October 1990, a yearling male humpback whale live-beached at 1630h, 200m south of ‘The Cut’ at Koombana Bay (33°18’S, 115°31’E) Bunbury, Western Australia. An unfavourable prognosis from the attending veterinarian, deteriorating weather conditions and the size of the whale (length 9.11m, weight *ca* 10t) precluded any rescue attempt. A decision was made to euthanase the whale. On the evening of 10 October, an explosive charge was detonated over the area dorsal to the cranium and immediately to the rear of the blow-hole (Fig. 1). Six sticks of AN60 (0.2m long × 25mm diameter) explosive were used in this controlled detonation AN60 explosive has now been replaced by more advanced products such as Powergel Magnum® explosive (Orica Ltd). Detonation occurred as planned, resulting in a neat circular hole, approximately 300mm in diameter that completely removed the underlying skin, blubber, skeletal muscle and the top of the cranium. The brain showed evidence of severe trauma, indicating that the whale had most likely died instantly. Death was determined on the basis of a lack of corneal reflex, the relaxation of the jaw muscles, an absence of response to tactile stimulus of the tongue, an absence of visible signs of respiration and visual confirmation of significant damage to the brain. It was noted that the lower cranium was still intact indicating the appropriate amount of charge to achieve the desired result had been used. The force of the blast had been contained and directed downward and into the brain and apart from the blast wound there was no other physical damage to the whale.

Case 2

On the afternoon of 24 September 2008, a 10.5m, *ca* 15t sub-adult female humpback whale live-beached in shallow water 1km south of Jurien Bay (30°18’S, 115°02’E), Western Australia.
The whale beached in shallow inshore waters after being washed in over a limestone reef and sustaining superficial injuries during this process. It came to rest in the shallows of a sandy bay in a weak and debilitated condition. Following an assessment of the animal’s condition, it was determined that the whale was too weak to move and as it had not made any attempt to dislodge

itself from the shallows, it was unlikely to survive any rescue attempt. Due to the size and weight of the animal and the fact that it was lying on the bottom, it would probably sustain additional physical injury and expose staff to a high workplace risk if attempts were made to tow or move the animal back out into deeper waters.

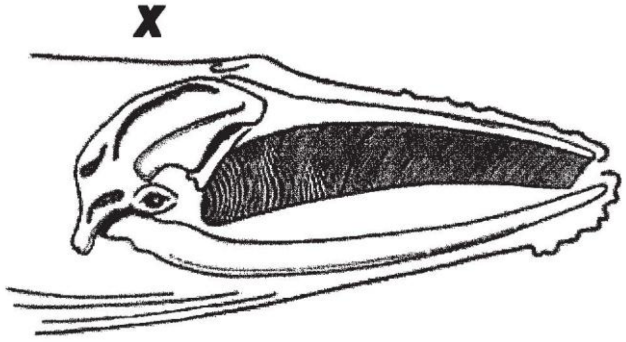


Fig. 1. Profile of humpback whale's head; X shows placement of charge.

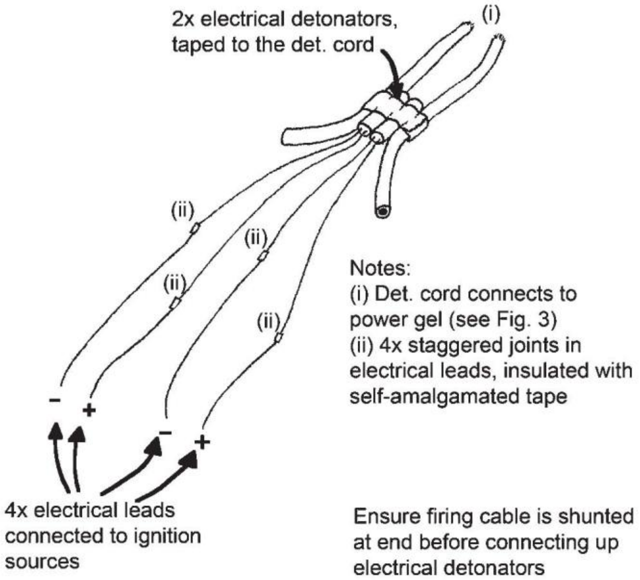


Fig. 2. Arrangement of wiring harness and electronic detonators and detonation chord.

As the stranding was a natural event and there were no immediate public safety concerns, the initial decision was made to allow nature to take its course. DEC officers were on site to ensure that there was minimal disturbance to the whale and to re-assess the situation as needed. A media statement was released by DEC on the morning of 25 September 2008 informing the media of the incident and the management strategy in place. Whilst media response to the strategy was mostly positive, there were some calls from the public, including some international calls, wanting to know why the DEC was not taking more direct action to either 'rescue' the whale or to 'put it down' to prevent it suffering[2].

---

[2] *http://latimesblogs.latimes.com/outposts/2008/10/hard-times-for.html* (accessed 15 March 2010).

DEC chose to maintain the palliative care strategy, and to re-assess the position and consider other options in the coming days. On the morning of 30 September 2008, following a re-assessment of the whale, a decision was made to euthanase it using explosives.

Five sticks of 125g Powergel Magnum® explosive with two electric detonators connected to two electric firing cables were used in this detonation (Figs 2–6). The initial detonation on the afternoon of 30 September 2008 made a crater approximately 200mm in diameter in the whale's head. The whale was only stunned; no externally visible damage had occurred to the cranium or brain, and a short time later it became active. A 0.300 inch Winchester Magnum rifle was used to place five rounds into the area to the rear of the blowhole aimed in the direction of the brain. This had no visual effect other than to cause a significant amount of arterial bleeding. A second explosive charge, double the size of the first, was quickly prepared and detonated in the same area as the first charge. The second charge caused an approximately 500mm diameter hole in the whale's head removing all blubber and tissue dorsal to the cranium along with the dorsal part of the cranium and causing severe trauma to the brain, apparently killing the whale instantly. Death was confirmed using the criteria described in case 1.

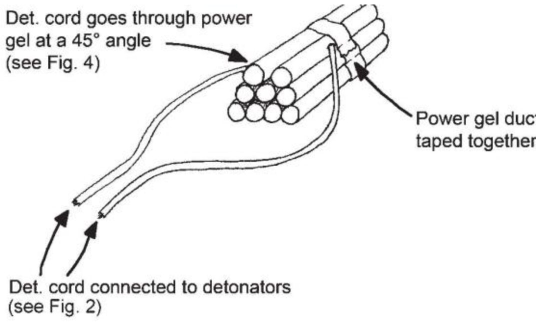
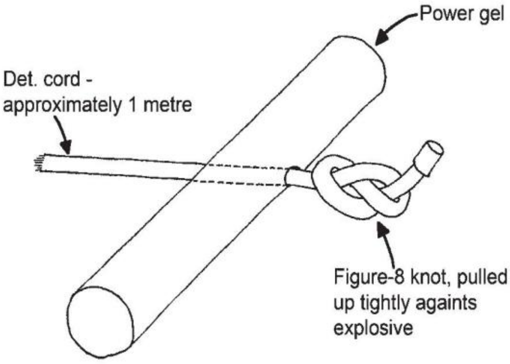
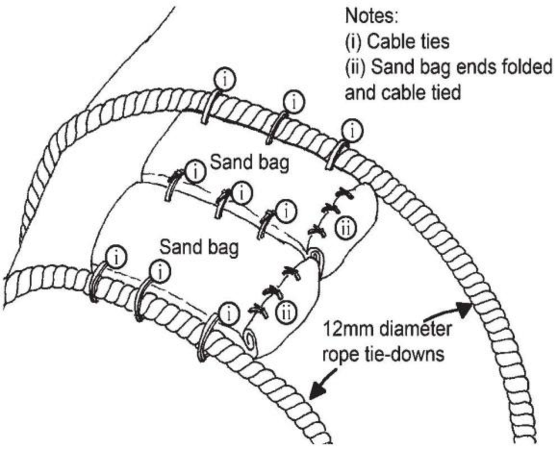


Fig. 3. Configuration of Powergel and detonation chord.



Skewer 2 sticks of power gel with a wooden skewer at a 45° angle to the centre, run the det. cord through and put a figure-8 knot in the end. One for each stick.


Notes:
(i) Cable ties
(ii) Sand bag ends folded and cable tied

Case 3

On the morning of 20 October 2009, a 9.8m, *ca* 15t, sub-adult female humpback whale live-beached in shallow water 500m east of Windy Harbour (34°50'S, 116°02'E), Western Australia. The whale beached in shallow inshore waters after being washed in and sustaining superficial injuries during this process. It came to rest in the shallows of a sandy bay in a weak and debilitated condition. Following an assessment of the whale's condition it was determined that the whale was too weak to move and as it had not made any attempt to dislodge itself from the shallows, it was unlikely to survive any rescue attempt. Due to the size and weight of the animal and the fact that it was lying flat on the sand and almost high and dry on a low tide, it would likely sustain additional physical injury and expose staff to a high workplace risk if attempts to move the animal back out into deeper waters.

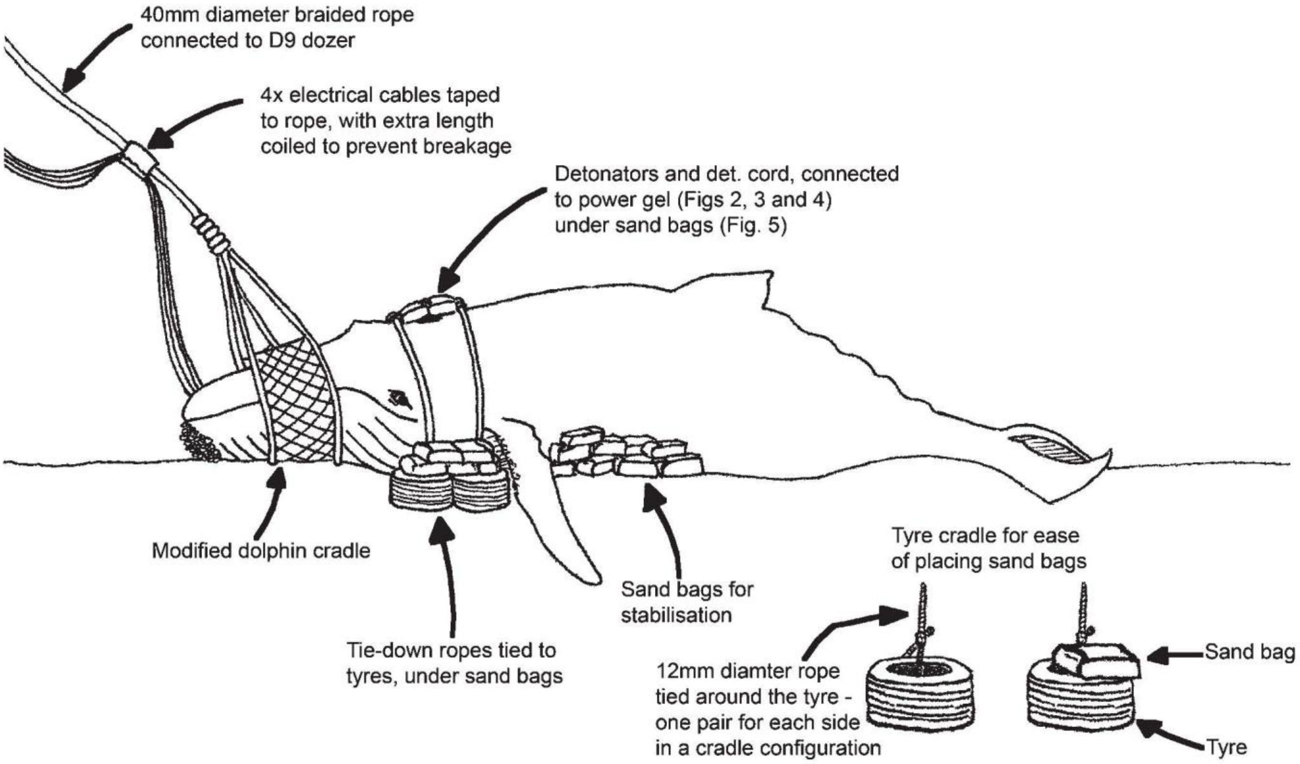


Fig. 6. Overall plan of materials used to secure whale, support wiring harness and placement of charge.

As the stranding was a natural event and there appeared to be no immediate public safety concerns, the initial decision was made to allow nature to take its course. DEC officers were on site to provide palliative care (covering the animal with wet cloth to protect it from the sun) and to ensure that there was minimal disturbance to the whale and to re-assess the situation as needed.

The whale was constantly monitored by DEC staff and veterinary assessments were carried out. The whale's general condition and prognosis was deemed very poor and a decision was made on 21 October 2009 to euthanase the whale using explosives on the morning 23 October 2009 if the animal was still alive at that time. Fourteen sticks of 125g Powergel Magnum explosive were used in this detonation. Detonation occurred as planned, resulting in a neat circular hole approximately 300mm in diameter that completely removed the skin, blubber, skeletal muscle and the top of the cranium (Fig. 7). The brain suffered severe trauma caused by the blast along with fragments of the upper cranium, apparently killing the whale instantly. Death was confirmed using the criteria described in case 1, above.

Case 4
On the evening of 12 January 2010, a 12.7m male humpback whale beached at Kennedys Beach (33°54'S, 122°51'E), Western Australia. It was assessed late that night and was still alive by the morning of 13 January 2010. Its body condition was very poor and the post-cranial depression was such that a pronounced hump was visible posterior to the blowholes. A significant depression was visible along the lateral flanks and a significant sub-dermal protrusion of the scapulae was visible. By 14 January more than 30% of its dorsal body surface had blistered from exposure to the sun. By late on 14 January 2010 it was obvious this animal was terminal and with high temperatures (>40°C) forecast over the ensuing days the decision was made to euthanase the whale using explosives. The challenge with this case was the fact that this animal would be the largest animal the technique had been applied to. With increased size and body mass there was an expectation that the dorsal bone structure of the cranium would be more substantial and that a larger explosive charge would be required. The charge consisted of 22 sticks of 125g Powergel Magnum, assisted by two 50g boosters. At 1610 hours on 15 January 2010, the charge was detonated, instantly killing the whale. The blast penetrated the upper cranium, causing severe trauma to the brain but did not sever the head from the body, leaving the bottom half of the skull intact. Death was confirmed using the criteria described in case 1, above.

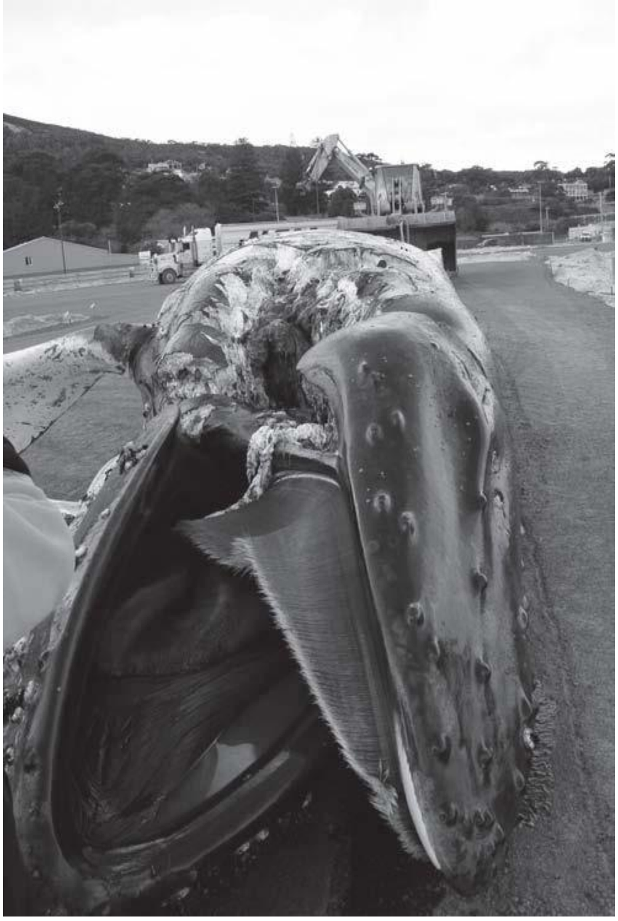
Case 5
On 19 August 2010, a 9.5m, 15t (weight post death) humpback whale beached on a sandbar within the port of Albany (35°03'S, 117°53'E) on the south coast of Western Australia. This whale was in a debilitated condition but still quite active. On high tide this whale could have swum into deep water but never attempted to do so. Its condition was slowly deteriorating, but the site and the activity of the whale did not allow for safe management for palliative care or early euthanasia. This whale was monitored daily by DEC staff until the tide, weather conditions and activity levels of the whale were deemed manageable. On 1 September 2010, the decision was made to euthanase the whale using explosives.

The whale was on a sand bar approx 1.2km from the nearest shoreline and it was noted to be lying on its left side. The right pectoral fin was in less than 0.5m of water whilst the left was in approximately 1m. The whale's blow-holes were submerged which meant it had to raise its head to breathe. The whale's breathing rate increased when first approached but settled down to a slower rate after a short period.

There were several factors associated with this case that had not been encountered in previous cases, necessitating minor modifications to the standard procedure. As the whale was resting on its side, in a left leaning aspect, it was not possible to place the charge to the rear of the blow-holes above the cranium as in cases 1 to 4, above. The whale was raising its head to breathe and there was some concern that this movement may dislodge the charge and sand bag tamping.

As the whale was so far from a beach it was difficult to stabilise the whale's head. An attempt was made to position sand bags under the whale's jaw to support it, without success. The whale would not leave its head up long enough to allow the sand bags to be safely positioned beneath the mandible. Truck tyres and a number of sand bags were positioned on the left side of the whale to stabilise the animal. It was decided to try putting a sand bag on the whale in the position of the charge to see if the sand bag would move when the whale lifted its head. The sand bag did not move in response to this activity, so more sand bags were positioned on the right side of the whale's head, in a line between the eye and to the rear of the blow-holes. These sand bags did not move so it was decided to go ahead with the placement of the explosive charge and detonation on 2 September.

Little information was available on the likely thickness of the lateral part of the skull that was presenting in the dorsal aspect, or the precise distance from the skin to the cranium from the position. Accordingly, three extra sticks of Powergel were used in the charge. The total charge consisted of 15 sticks of 125 gram Powergel explosive. The sticks were taped together forming a pyramid. These were initiated by two lines of detonation chord running through the stick at the apex.



Due to the fact that no heavy machinery could be located close to the whale, no bulldozer blade was available to use as a blast shield. Initiation of the charge by a timed safety fuse was considered, however this would have required leaving a burning detonation chord for two minutes with the possibility of the whale smelling the black powder smoke and becoming agitated and dislodging the charge. A decision was made to detonate the charge electrically from behind a dinghy 50m away. The tamping sand bags were checked to ensure that only wet sand had been used for filling and that there was no chance of 'fly' from the charge. The wet sand in the bags was used to further assist in containing the explosive force to the target area.

Two electric detonators were connected to the firing cable in parallel and then taped to the two lines of detonation chord. The area was checked to ensure no unauthorised people had entered the exclusion area and that it was safe to fire the charge, an air horn was sounded and the charge fired. Upon examination of the whale it was found that the charge had been successful with a 1.0m × 1.5m elliptical hole punched through the blubber and right dorso-lateral section of the skull, causing severe trauma to the cranium and brain (Fig. 8).

OVERVIEW OF THE PROCESS

Circumstances at each site where whales beach vary and as such the range of equipment used, in particular heavy and light vehicles, differ slightly[3].

---

[3] The recommended equipment list to successfully and safely euthanase whales is available from the principal author (DKC) on request.

Public safety and information

In cases where whales have beached in close proximity to populated areas, DEC routinely requests the local police (assisted by State Emergency Service (SES) personnel) to secure and control the site before any operations begin on the whale. The presence of uniformed officers provides a distinct advantage in obtaining crowd compliance with requests to keep a required distance from operations involving heavy and light machinery, potentially inclement sea conditions, firearms and explosives. In remote areas where access to police and SES personnel is not always possible, the DEC incident controller delegates crowd control responsibilities to authorised DEC staff. Authorised DEC staff have powers under state legislation to compel members of the public to comply with given directions. The public are excluded to ensure safety rather than prevent them from gaining an appreciation of the events that are to take place.

Prior to any work related to the preparation or placement of the explosive charge, a briefing is provided to all essential personnel, members of the public (if present) and any media representatives. The briefing covers issues such as the species of whale involved, the conservation status of the whale, the animal welfare issues at hand (including any independent veterinary advice available), why the whale cannot be saved or returned to the sea, what course of action will be taken to end the whale’s suffering and what will be asked of the public/media in order to ensure the safe operation of the euthanasia protocol.

The process

It is important to shape the explosive charge into a triangular pyramid (see Fig. 3) to ensure maximum explosive force is directed downward onto the smallest area of the whale’s head, directly above the cranium. For very large whales such as the one described in Case 4, it is recommended that two 50g boosters be added on top of the charge to ensure optimal detonation of the explosive charge and to direct the blast downwards. The boosters are installed with two lines of detonating cord and detonate before the primary charge. The electrical firing cables should be shorted out to discharge any static current within the wiring system and the charge watched closely to ensure it is not dislodged from the main explosive charge, and that the charge does not move from its central position over the mid-line of the whale’s head (Fig. 5). The electrical firing cables are laid out back to the bulldozer or protective sand dune (Fig. 6). Two electric detonators are connected to two electrical firing cables using self-amalgamating tape. The electric detonators are then taped to the detonating cord using plastic electrical tape.

Heavy machinery (e.g. D9 or D65EX bulldozers) is used to achieve four important functions. The first is to assist in manoeuvring the whale into a position on the beach where it can be stabilised. The second is to provide a secure point of attachment for the wiring harness to keep it clear of rocky substrates, surging wave action and personnel. The third function is to provide protection to the shot-firing team from the effects of the blast, and the final function is to remove the whale carcase from the beach, if necessary.

All non-essential persons are moved 500m back from the detonation site prior to the explosive charge being prepared or placed on the whale. All essential personnel take cover behind the heavy machinery (if available) or the first line of sand dunes present on the beach, prior to the trigger mechanism being connected to the wiring harness. A transmission on the universal emergency and calling marine radio frequency (marine VHF channel 16) is made once all non-essential personnel are moved 500m back from the site and prior to the commencement of the preparation of the explosive charge.

After this point in the process, no electronic communication devices, including mobile telephones, are used or left on to ensure that the explosive charge is not detonated prematurely. It is important to note that electronic communications from aircraft over-flying the site could present a real risk of premature detonation. Military aircraft (or base installations) typically generate much stronger electronic transmissions than commercial or private aircraft and may make the use of electrical detonating systems impractical under some circumstances. Under such circumstances the charge should be detonated using a nonelectric system.

Once the charge has been prepared and secured on the whale the shot-firer then provides a visual signal to the police/SES (if present) to activate their flashing emergency lights and siren. The shot-firer then takes cover behind the heavy machinery or sand dune, arms the system and detonates the explosives. No personnel are permitted to approach the whale carcase until the shot-firer has determined the site safe.

DISCUSSION

Current use of explosives in killing whales at sea is limited to penthrite grenades (typically 30g charges) that are attached to whale harpoons. The harpoons are fired into the body of the whale and typically penetrate 600–700mm before the delayed fuse mechanism detonates the explosive (Knudsen and Øen, 2003). Explosives work by the virtual instantaneous conversion (detonation) of a mixture of chemical compounds into gas and heat. This detonation of the explosive is achieved by sending a shock or detonation wave through the explosive compound. A detonator is used to initiate the detonation wave which once started will propagate through the explosive at speeds of up to 8,000ms^{-1}. The gas volume produced by a 30g penthrite charge is between 768–790L. The more gas produced by the explosive the greater the destructive power of the explosion. Military bombs confine the gas produced by the explosive detonation in iron cylinders allowing it to build up. In civilian utilisation of explosives such as mining, the gases are contained by placing the explosive in a bore hole and positioning ‘tamping’ over it. The greater the pressure build-up, the more productive the blast (i.e. the more rock that will be fractured and dislodged). If the blast is not contained or directed in some manner the gases will take the least line of resistance, dissipating into the atmosphere mainly as heat and noise with little blast effect.

Powergel is a more stable explosive and is less expensive than penthrite. Powergel has a reasonably high velocity of detonation of 6,337 ms^{-1} compared with penthrite’s 7,400– 8,300 ms^{-1} (dependent on the density of the penthrite). When euthanasing stranded whales it is not possible to contain the explosive charge inside the animal and neither can the explosive charge be placed in a metal container in the manner of traditional military style bomb, which when shattered would cause dangerous fragments that could be propelled for quite some distance (1000m). The dying whales do not always choose to beach themselves in places that allow a 1,000m safety envelope for wildlife authority staff to operate with. The combination of layered sand bags containing wet sand as tamping to ‘contain’ the explosive gases produced, along with the larger amount of explosive (compared to the small amount of penthtrite) and the careful shaping of the charge, addresses the issue that the majority of the explosive gases will escape when used in the manner described here. The sand from the disintegrating sandbags, with its low mass and very small particle size will not be propelled by the explosion any more than 30m from the blast site.

In Western Australia a shot-firer’s licence, issued by the Department of Mines and Petroleum under the provisions of the Explosives and Dangerous Goods Act 1961, is required to handle and use explosives. The safe and efficient use of explosives requires considerable expertise, for which DEC relies heavily on outside personnel and agencies, including the military. Matching legislation will most likely need to be complied with in other jurisdictions. Most members of the police or military who have experience with explosives have learnt to use these materials on inanimate structures such as concrete, metal and the like. The physical properties of these inanimate materials respond very differently to the biological materials of blubber, muscle and bone. It is our experience that there is a strong tendency to underestimate the amount of explosive charge necessary to achieve a humane death of a living great whale.

The potential clearly exists to use this implosion technique on a range of large whale species. There is a wide range in head shape and the volume of tissue mass dorsal to the cranial anatomy within different whale species (and possibly even within species and between the sexes). The example provided in case 5 demonstrates that this method has application when the explosive charge needs to be placed on a section of the head other than directly above the cranium and posterior to the blow holes. Beached whales are encountered in a wide range of physical conditions, and this can greatly influence the amount of explosive required to ensure destruction of the cranium and brain. Further field trials involving already deceased animals are strongly recommended. This is particularly important if the technique is to be applied to odontocete whale species such as the sperm whale. The results of any such field trials, whether successful or not, should then be communicated to the wider scientific community either through publicly available fora such as the International Whaling Commission workshops on whale killing methods and/or through peer-reviewed journals.

During Case 2, a media helicopter presented a serious safety breach by over flying the site as the charge was being set on top of the whale’s cranium as electrical detonators were at that time in place within the charge. Presumably the pilot was unaware of the risk of premature detonation caused by electronic devices such as aircraft electronic transmitters and radios. Clearly serious thought needs to be given to how to manage any aircraft movement in close proximity to field operations involving the use of electrical detonators. There may also be situations where the safe use of explosives, especially when combined with electrical detonators, will not be possible and alternative euthanasia methods will need to be considered or nature allowed to run its course.

Management of cases such as these would benefit from professional advice from suitably qualified veterinarians. In many parts of Western Australia where these types of stranding events occur, it is not possible to access the services of a veterinarian, other than by telephone or radio. Added to this is the problem that few veterinarians have any practical experience in the treatment or palliative care of cetaceans, and in particular baleen whales. It is our experience that being able to receive any advice available provides reassurance but an inability to access quality advice from a veterinarian should not be considered an impediment to applying this technique.

The management of beached whales evokes strong public emotions. It is important that public perceptions and lack of appreciation for the facts surrounding beaching events do not prevent responsible wildlife agencies from making science-based decisions about the welfare of beached whales. There is ample opportunity to apply palliative care actions such as covering whales with damp cloths to prevent blistering from exposure to the sun. However, just because a whale is larger than most animals that the public has experience with does not in any way mean that it should be treated any differently. Large animal euthanasia involves issues dictated by physics, and euthanasia by explosives is a feasible and safe response to the issue. The data presented here clearly demonstrate that euthanasia of large humpback whales (and potentially other species) can be achieved safely and humanely with modern commercial explosives. The broader application of this method should be investigated whenever opportunities present, ideally via field trials on already deceased animals.

ACKNOWLEDGEMENTS

We wish to acknowledge the contribution of P. Fuller and C. Ostle, without whose assistance these operations could not have been possible, N. Gales for his encouragement, S. Vitali, P. Sides, J. Steele, M. Meyer, C. Main, D. Mell, K. Morrison, K. Hockey, J. Edwards, C. Broadbent and other DEC staff who assisted during these procedures. A.A. Burbidge and I. Parker provided valuable comment on drafts of this paper. S. Dunstan prepared Figure 1 and M. Steele prepared Figures 2 to 6. This research work was conducted under DEC Animal Ethics Committee approvals DEC AEC 2005/01 and 2008/08 and Scientific Purposes Licences SF000007, SC000619, SC000941 and SC001255.

References

Bannister, J.L. 2008. Population trend in right whales off southern Australia 1993–2007. Paper SC/60/BRG14 presented to the IWC Scientific

Committee, June 2008, Santiago, Chile (unpublished). 13pp. [Paper available from the Office of this Journal]. Bannister, J.L., Kemper, C.M. and Warneke, R.M. 1996. *The Action Plan for Australian Cetaceans*. Australian Nature Conservation Agency, Canberra. 242pp. [Available from: *www.environment.gov.au/library/pubs*].

Blackmore, D.K., Nutman, A., Barnes, G.R.G., Madie, P., Davies, A.S., Bowling, M.C., Donoghue, M. and Kirk, E.J. 1997. Preliminary investigations of techniques for killing whales. *In*: Hindell, M. and Kemper, C. (eds). *Marine mammal research in the Southern Hemisphere. Vol. 1. Status, ecology and medicine*. Surrey Beatty and Sons, Chipping Norton.

Coughran, D. and Gales, N. 2010. An unusual peak in recorded mortalities of humpback whales in Western Australia: normal stochastic variability or a regional indication of carrying capacity? Paper SC/62/SH24 presented to the IWC Scientific Committee, June 2010, Agadir, Morocco (unpublished). 7pp. [Paper available from the Office of this Journal].

Dierauff, L. 1990. Disposition of marine mammals. pp.267–75. *In*: Dierauff, L. (eds). *Handbook of marine mammal medicine: health, disease and rehabilitation*. CRC Press, Boca Raton, Florida. 735pp.

Gales, N., Woods, R. and Vogelnest, L. 2008. Marine mammal strandings and the role of the veterinarian. pp.39–54. *In*: Vogelnest, L. and Woods, R. (eds). *Medicine of Australian Mammals*. CSIRO Publishing, Victoria.

Greer, L.L.O., Whaley, J. and Rowles, T.K. 2001. Euthanasia. pp.729–37. *In*: Dierauf, L. and Gulland, F.M.D. (eds). *CRC Handbook of Marine Mammal Medicine*. CRC Press.

Hyman, J. 1990. Euthanasia in marine mammals. pp.265–66. *In*: Dierauf, L. (eds). *Handbook of marine mammal medicine: health, disease and rehabilitation*. CRC Press, Boca Raton, Florida. 735pp.

International Whaling Commission. 2003. Chair's Report of the Fifty-Fourth Annual Meeting. Annex D. Report of the working group on whale killing methods and associated welfare issues. *Ann. Rep. Int. Whaling Comm.* 2002:76–82.

International Whaling Commission. 2010. Report of the workshop on welfare issues associated with the entanglement of large whales. Paper IWC/62/15 presented to the meeting of the International

Whaling Commission, June 2010, Agadir, Morocco [To be published in: *Journal of Cetacean Research and Management* Supplement 13].

International Whaling Commission. 2011. Report of the Workshop on the Comprehensive Assessment of Southern Hemisphere humpback whales, 4–7 April 2006, Hobart, Tasmania. *Journal of Cetacean Research and Management (special issue 3)*:5–50.

Kemper, C.M., Flaherty, A., Gibbs, S.E., Hill, M., Long, M. and Byard, R.W. 2005. Cetacean captures, strandings and mortalities in South Australia 1881–2000, with special reference to human interactions. *Aust. Mammal.* 27: 37–47.

Knudsen, S.K. and Øen, E.O. 2003. Blast-induced neuarotrauma in whales. *Neurosci. Res.* 46: 377–86.

Needham, D.J. 1993. Cetacean strandings. pp.415–25. *In*: Fowler, M.E. and Miller, R.E. (eds). *Zoo and wildlife medicine: current therapy*. W.B. Saunders Company.

Øen, E.O. 1995a. Killing methods for minke and bowhead whales. PhD thesis, Norwegian College of Veterinary Medicine, Oslo, Norway. 141pp.

Øen, E.O. 1995b. A new penthrite grenade compared to the traditional black powder grenade: Effectiveness in the Alaskan eskimos' hunt for bowhead whales. *Arctic* 48(2): 177–85.

Øen, E.O. 1995c. A Norwegian penthrite granade for minke whales: hunting trials with prototypes and results from the hunt in 1984, 1985 and 1986. *Acta Vet. Scand.* 36(1): 111–21.

Øen, E.O. 1999. Improvements in hunting and killing methods for minke whales in Norway. Paper IWC/51/WK11 presented to the International Whaling Commission Workshop on Whale Killing Methods, 17–19 May 1999, Grenada (unpublished). 8pp. [Paper available from the Office of this Journal].

Øen, E.O. 2002. Norwegian whaling in 2001. Paper IWC/54/WKM&AWI6 presented to the International Whaling Commission Working Group on Whale Killing Methods and Associated Welfare Issues, May 2002, Shimonoseki, Japan (unpublished). 1p. [Paper available from the Office of this Journal].

Øen, E.O. and Knudsen, S.K. 2007. Euthanasia of whales: the effect of .375 & .458 calibre round nosed, full metal-jacketed rifle bullets on the central nervous system of common minke whales. *J. Cetacean Res. Manage.* 9:81–88.

Tønnessen, J.N. and Johnsen, A.O. 1982. *The History of Modern Whaling*. C. Hurst and Co., London. i–xx+798pp.

Date received: July 2010 Date accepted: June 2011
J. CETACEAN RES. MANAGE. 12(1): 137–144, 2012

Reptile Euthanasia – No Easy Solution?

Dr Kristin Warren
Conservation Medicine Program, Murdoch University

Abstract:

Reptiles are commonly the subjects of biological or ecological research projects, and veterinarians or wildlife researchers may be required to euthanase a reptile if it sustains a severe injury associated with the research. When conducting euthanasia of any animal it is critical to confirm death. Whilst in mammals and birds euthanasia and confirmation of death can easily be accomplished, in reptiles these are not straight forward processes due to reptilian poikilothermic biology and physiology. Many traditional methods of reptile euthanasia are controversial and recommended methods of acceptable euthanasia vary amongst the different reptilian orders. Physical methods of euthanasia involving hypothermia or decapitation alone are considered inhumane and are not acceptable methods of euthanasia. Injectable pentobarbitone sodium is considered an acceptable method of euthanasia for all reptiles, except large crocodiles and other large reptile species where carcass removal in the wild may be problematic e.g. sea turtles. However, pentobarbitone sodium is a Scheduled 4 drug with requirements for storage in a locked environment and users other than registered veterinarians must apply for authorisation to administer scheduled drugs. Stunning and destruction of the brain is considered acceptable with reservations in some species of snakes and lizards. Humane euthanasia in reptiles is not easily accomplished and, whilst recognising limitations in accessing veterinary anaesthetic and euthanasia drugs, it can best be assured by using a two-stage euthanasia process – whereby the reptile is initially anaesthetised, and then euthanased by administration of pentobarbitone sodium or decapitation and brain destruction following anaesthesia.

Reptiles are commonly the subjects of biological or ecological research projects and veterinarians or wildlife researchers may be required to euthanase a reptile if it sustains a severe injury associated with the research. Euthanasia should be conducted in a humane manner and by definition it should result in painless death. Following euthanasia of any animal it is critical to confirm death. Whilst in mammals and birds euthanasia and confirmation of death can easily be accomplished, in reptiles these are not straight forward processes due to reptilian poikilothermic biology and physiology. As poikilothermic animals, reptilian body temperature and metabolic processes are determined by the external temperature. Reptilian metabolism and respiration is significantly different to homeothermic animals and this results in differences with regards to tolerance of cerebral hypoxia, which has implications for acceptable methods of euthanasia, for example decapitation alone in reptiles does not produce rapid unconsciousness as it would in homeothermic animals. Reptilian anatomy and physiology also makes it difficult to confirm death following euthanasia. Reptiles have a coelomic cavity and do not have a diaphragm, which means that the heart beat cannot be detected using a stethoscope. Mader (2006) alludes to the fact that heart rates can change rapidly from ‘beats per minute to minutes per beat’ if the reptile is anaesthetised or exposed to cold environmental temperatures. The low metabolic rate and oxygen requirements of reptiles enables many species to breath hold for considerable periods of time, during which there is no evidence of respiration. Reptiles are not as reactive to stimuli, including painful stimuli, compared to homeothermic animals, therefore testing standard reflex responses does not result in comparable responses and can cause difficulties in interpretation of levels of consciousness. Additionally, the corneal reflex which is commonly used to determine level of consciousness in animals, cannot be used in many reptilian species which do not have eyelids. To complicate matters even further, there are numerous stories by veterinarians who have proceeded to conduct a necropsy examination immediately after euthanasia of a reptile, only to find that the heart was still beating. The fact that there can be persistence of cardiac activity and somatic responses, such as body movements, even after brain destruction in reptiles is due to the fact that reptiles have an increased level of somatic responses at the spinal cord level, rather than the brain, and due to the increased tolerance of the spinal cord, peripheral nerves and muscles to hypoxia.

Many traditional methods of reptile euthanasia are controversial and recommended methods of acceptable euthanasia vary amongst the different reptilian orders. Physical methods of euthanasia involving hypothermia or decapitation alone are considered inhumane and are therefore not acceptable. Whilst in the past, cooling and then freezing reptiles was advocated as an easy method of euthanasia for reptiles, this technique has been deemed unacceptable due to pain associated with ice crystal formation in the skin and viscera. Decapitation alone does not result in rapid unconsciousness in the severed heads of reptiles and it has been stated that unconsciousness is only likely to be rendered if decapitation is followed by pithing, to destroy the brain, or double-pithing, to destroy the brain and spinal cord. It is therefore recommended that decapitation should only be used if the reptile has been rendered unconscious in a humane manner. Given this, stunning and destruction of the brain is considered acceptable, with reservations in some small species of snakes and lizards.

Injectable pentobarbitone sodium is considered an acceptable method of euthanasia for all reptiles, except large crocodiles and other large reptile species where carcass removal in the wild may be problematic e.g. sea turtles. However, pentobarbitone sodium is a Scheduled 4 drug with requirements for storage in a locked environment and users other than registered veterinarians must apply for authorisation to administer scheduled drugs. Depending on State regulations in Australia, researchers who are not veterinarians may be able to access Scheduled drugs through application for a competency licence to the health department, or to the Veterinary Surgeons' Board in the state where they work.

Pentobarbitone sodium should be administered intravenously, however it can be difficult, and sometimes potentially dangerous, to physically restrain certain species of reptiles to obtain venous access. Extra-vascular injection of pentobarbitone sodium can be highly irritating and painful, so ideally euthanasia solution should not be administered intra-coelomically unless the reptile has been anaesthetised. If pentabarbitone sodium is used for euthanasia, then researchers need to ensure that the animal's body is disposed of according to regulations to prevent health risks to humans or other animals. The bodies of animals euthanased with pentabarbitone sodium must be wrapped and secured in a plastic bag and then must be preferably incinerated or buried at a depth greater than 1 metre.

Given the difficulties associated with euthanasia and confirmation of death, due to reptilian anatomy and physiology, two-stage euthanasia is recommended as the most humane approach to follow in reptiles. Two-stage euthanasia involves anaesthesia of the reptile, followed by euthanasia. Anaesthesia of reptiles with inhalational anaesthetic agents can be problematic due to the tendency for many reptilian species to breath hold and the delays in anaesthetic induction associated with this, so the use of injectable anaesthetic agents which can be administered intra-muscularly is recommended. It is important to maintain a reptile at its preferred body temperature when administering injectable agents, as optimal reptilian body temperature will ensure optimal metabolism, which will allow for greater efficiency in drug uptake and effect. Whilst the specific anaesthetic agent would need to be determined based on the reptilian species and circumstances, Zoletil® (Virbac, Milperra, Australia) is an injectable anaesthetic agent that can be effectively used in a wide range of reptiles. Following anaesthesia, euthanasia can then be undertaken using pentabarbitone sodium or by physical means depending on the situation. For example, a researcher undertaking a study on reptiles with access to a research station should be able to anaesthetise the reptile and then euthanase it using pentobarbitone sodium, providing disposal of the body can be undertaken as required. However, euthanasia of a sea turtle in a remote part of Western Australia would need to consider the size of the animal, logistics and difficulties associated with carcass disposal, therefore in such a case anaesthesia of the sea turtle followed by a physical means of euthanasia (e.g. blunt trauma, shooting, decapitation, or exsanguination) may be more appropriate.

Humane euthanasia in reptiles is not easily accomplished and whilst recognising limitations associated with access to veterinary anaesthetic and euthanasia drugs, it can best be assured by using a two-stage euthanasia process – whereby the reptile is initially anaesthetised and then euthanased by administration of pentobarbitone sodium or by use of physical means, such as decapitation or brain destruction.

References:

American Association of Zoo Veterinarians (AAZV), 2006, *Guidelines for Euthanasia of Nondomestic Animals.*

Mader DR, 2006, Euthanasia, In Mader DR (ed) *Reptile Medicine and Surgery*, Elsevier, 564-568.

Reilly JS (ed), *Euthanasia of Animals Used for Scientific Purposes*, ANZCCART, Second Edition, 2001.

Euthanasia of large animals for humane reasons

Simone Vitali
Perth Zoo

Abstract:

Perth Zoo's purpose is to inspire and act for wildlife conservation. Aligned with this purpose is a commitment to building knowledge through research and education. These are major considerations in our approach to all aspects of zoo animal management, including euthanasia.

The euthanasia of "megafauna" is a complex issue for veterinarians and other staff working in zoos. Megafauna are characterised by their complex social needs, high level of intelligence and longevity. In consequence, their management is subject to high professional standards and public scrutiny.

Species such as elephants, giraffes and bears are very popular with the visiting public, and individuals of these species are often considered to be well-recognised "celebrities", commanding a high level of public recognition, affection and interest. This high public profile, coupled with their large size, means that euthanasia represents a unique challenge in terms of animal welfare, logistics and human emotional investment.

This presentation explores the euthanasia of very large animals in a zoo setting.

No Paper Received

Animals adapting to change: Can our management of animals help with adaptation processes for the good of research, production and animal welfare?

Samantha Bickell[1] and Dean Revell[2]
[1] School of Animal Biology, The University of Western Australia, Nedlands WA 6009;
[2] CSIRO Animal, Food and Health Sciences, Centre for Environment and Life Science, Floreat WA 6014

Abstract:

Grazing animals face considerable variability in the supply of nutrients over time. Fluctuations occur between years, between seasons, between paddocks, and between patches within a paddock. By focusing on ruminant livestock, we will present examples of how animals aim to meet their nutrient requirements in the face of uncertainty in nutrient supply. Key points that will be discussed are: (i) the importance of prior events in determining diet selection, feed intake and other behaviours; (ii) how animals learn to modify their feeding behaviours (from the womb and from peers) and (iii) interactions between stress and 'nutritional wisdom'. These points will be discussed in the broader context of our role in managing animals to allow them to adapt to change and express individual behaviours.

Introduction

For life on this planet, change isn't the exception to the rule, it's the only rule. However, in a research environment change is often unwanted, or at least tightly controlled. Researchers are trained to ensure the research environment and everything in it, are kept as consistent and unchanging as possible, including the animals in our care. Statistical analysis aims to partition the variability in the trait of interest to known factors with the variability associated with unknown factors often described as 'experimental error'. However, variability and change is not 'error' but a reflection of adapting to the fluctuating circumstances (e.g. responding to a change in temperature, location or housing conditions, food availability, food type, or the imposition of experimental treatments. Therefore, adaptation is a dynamic, physiological process that is always occurring. So whilst we cannot stop or always predict change, we can understand how animals react and adapt to it and by doing so, we can help animals adapt more quickly or more thoroughly to a change imposed on them.

This paper will begin with an overview of the physiological process of how animals respond to change. We will then discuss the importance of acclimatisation and adaptation of animals to the research environment and present some preliminary results assessing the acclimatisation of sheep to methane respiration chambers. We will conclude with some general statements to highlight opportunities for researchers to thoroughly consider animal adaptation and acclimatisation.

Physiological response to change

The first response to change is the detection of that change by the central nervous system (CNS) and a 'decision' on a whether a response is required or not. Once this change is perceived as something the animal must respond to, the hypothalamus sends a signal to activate the sympathetic nervous system (SNS) (Figure 1). This perception is subconscious but is influenced by factors such as genotype and past experiences (Romeyer and Bouissou, 1992, Hutson, 1985). Therefore, due to these differences in perception, animals will differ in their degree of responses to the perceived change, with some showing large or rapid responses and others not responding at all.

The SNS is responsible for the responses associated with the 'fight-or-flight' response. Activation of the SNS releases catecholamines (Figure 1), specifically epinephrine (adrenaline) and norepinephrine (noradrenaline) at various neural synapses (Palme et al., 2005). The release of these catecholamines prepares the body for rapid metabolic change and movement by the acceleration of heart rate, increases in myocardial contraction, vasodilation of the arteries of working muscles and vasoconstriction of the arteries of non-working muscles, increased ventilation, reduced digestive activity and several other functions that prepare the body to fight or flee (Moberg, 2000). Activation of the SNS is a neural response and consequently the response is immediate, but the effects of the catecholamines are very short, lasting only second (Sapolsky et al., 2000).

While the activation of the SNS may enable the animal to make an immediate response, the body has several 'backup' responses which complement the SNS response. These are endocrine in nature and thus have longer-lasting effects on the body's metabolic reactions.

One of these 'backup' responses is initiated by the posterior hypothalamus, which, through its direct neural pathway to the adrenal gland located on top of the kidneys, stimulates the adrenal medulla to secrete the catecholamines epinephrine and norepinephrine (Sapolsky et al., 2000). The effects of the catecholamines on the body's metabolic process are similar to those described above for the SNS but, because they are released into the bloodstream, this endocrine response is of longer duration than the neural response of the SNS and can last for up to two hours (Sapolsky et al., 2000).

Another 'backup' response is initiated by the release of corticotropin-releasing factor from the anterior hypothalamus which activates the pituitary gland to release ACTH. ACTH then travels via the bloodstream to stimulate the adrenal cortex to release corticosteroids, specifically glucocorticoids like cortisol and cortisone (Moberg, 2000) (Figure 1). The functions of glucocorticoids are to help generate glucose for use as an energy source for the CNS and skeletal muscles. Again, due to their endocrine nature, these hormones can have prolonged effects on metabolic functions, lasting from minutes to hours.

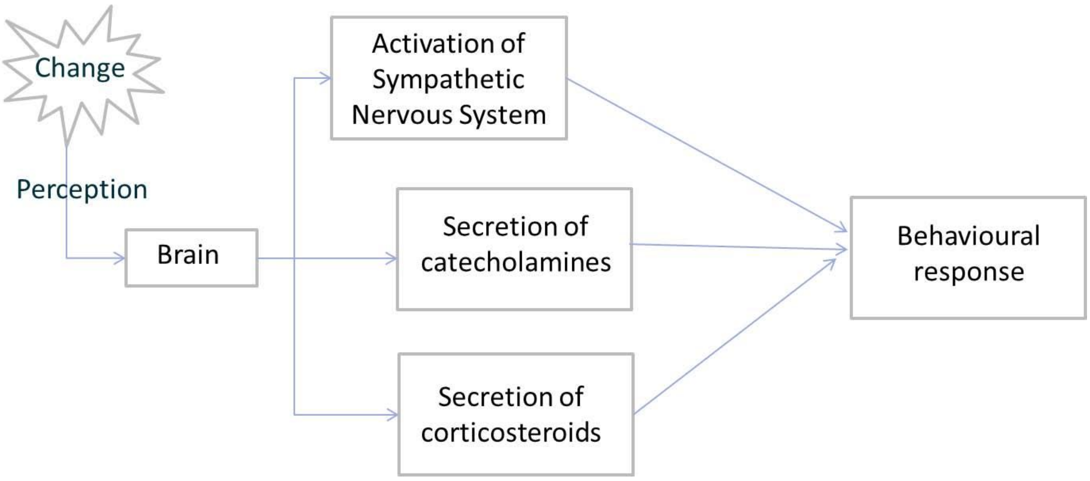


Figure 1: The chain of events involved in perceiving a threatening change which can lead to a behavioural response to cope with the change.

The purpose of these neural and endocrine responses is to prime the animal to make a behavioural response to the perceived change to ensure well-being and ultimately, survival. Examples of typical behavioural response include moving to the shade when ambient conditions threaten the maintenance of body temperature, or running away to escape a threat (or predator). If an animal cannot make a sufficient behavioural response to adapt to the change, then the regulatory mechanisms in the body lose their ability to maintain physiological homeostasis. When the body is unable to return to a state of homeostasis, the animal enters a state of chronic stress that can have significant negative effects on the cardiovascular, digestive, musculoskeletal and immune systems.

Therefore as carers and users of animals in research, it is important for animal welfare and the quality of our scientific results, to understand these physiological responses to change so that we can assist animals in their adaptation processes and help ensure that animals are adapting adequately so that chronic stress does not occur.

Acclimatisation and adaptation

Ensuring animals are adequately adapting to change is also a guideline in the *Australian code of practice for the care and use of animals for scientific purposes*. Section 4.3.2 in the code states: '*Animals should be acclimatised to the holding facility and personnel before their use in a project and those that do not adapt satisfactorily should not be kept*'. However, there is a deficiency in research on the acclimatisation of animals to the research environment, as well as in assessing acclimatisation (i.e. how best to adequately determine if an animal has sufficiently adapted or not).

In a search of the literature in an area in which we have current research activity, measuring methane production from ruminant animals, we found few studies had investigated or outlined the procedures to acclimatise animals or assess the extent of acclimatisation of animals to research environments (Done-Currie et al., 1984). Generally studies list 'prior experience', 'familiarisation with humans' and 'time' indices of acclimation to the research facility or procedure (Hargreaves and Hutson, 1990, Goopy et al., 2011, Klein and Wright, 2006). Likewise, there were few studies on the methodology to assess acclimatisation and, where it was reported, the assessment criteria was non-quantative; e.g. 'animals settled quickly and appeared to behave normally' (Lockyer, 1997) or 'animals consumed 91% of their ration which indicated that they behaved normally' (Klein and Wright, 2006), so that it lacked meaning.

The other criteria by which studies assessed acclimatisation, lack of reaction to humans and human handling, should be interpreted with caution. Such assessments may indicate that animals were adapted to humans, but not necessarily to the research environment or experimental procedures (Miller et al., 1991). Obtaining a baseline concentration of plasma cortisol from hand-reared sheep showed that there was an effect of order of sampling on the cortisol concentrations (Miller et al., 1991). Sheep that were sampled later in the procedure had higher levels of cortisol than sheep that were sampled earlier (Figure 2). These results suggest that although the sheep were adapted to humans and handling, they were not adapted to the procedure of blood sampling. In such a situation, parameters measured in blood may have been affected by the stress experienced during the procedure. Overcoming this can be problematic, as repeated exposure to the blood sampling procedure to allow animals to acclimatise to it also increases the imposition placed on the animals used in the research activity. Therefore, the onus is to develop and use methods that place as little stress as possible on the animals.

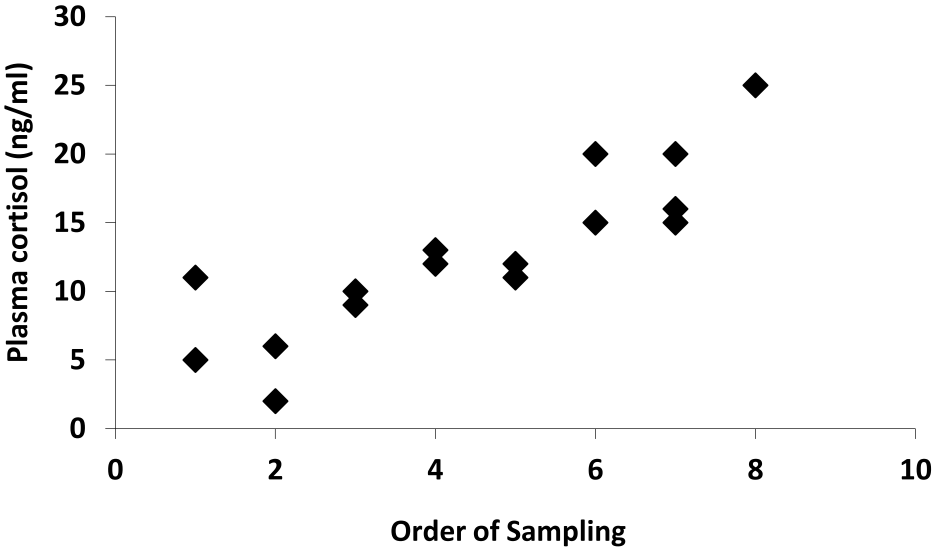


Figure 2: Baseline plasma cortisol concentrations of 8 hand-raised Rocky Mountain bighorn sheep. (Data adapted from Miller *et al.* 1991)

Should researchers do more to assess acclimatisation?

An accepted method of acclimatising sheep to respiration chambers for measuring daily methane production is to place the sheep in the respiration chambers for short periods of time over a period of a few days, increasing the time spent in the chamber with each successive day (Klein and Wright, 2006). Animals are deemed adapted once they show no signs of behavioural distress, in particular, absence of vocalisations, attempts to escape, general agitation and good food consumption. However, these assessment criteria are not quantitative and thus are reliant on the interpretation of the animal handler or researcher.

In an attempt to obtain quantitative data on the acclimatisation of sheep to methane respiration chamber, we have recently measured feed intake, heart rate and behavioural activity from eight sheep in respiration chambers and compared these data with the same parameters obtained from before the sheep were placed in respiration chambers. Preliminary results indicate that over the three days before sheep were placed in the respiration chambers, their feed intake (Figure 3). Likewise, the feed intake of these sheep did not differ over the three days in respiration chambers, but it was reduced by about one-third. These results highlight the importance of having a baseline with which to compare the results obtained in the respiration chamber with since the lower feed intake while sheep were in the respiration chambers may suggest that they were not satisfactorily adapted to the respiration chambers.

Preliminary heart rate data from one sheep from the first day in the respiration chamber and from the third day in the respiration chambers reveals some interesting results (Figure 4). The heart rate of this sheep nearly doubled when it was being handled and put into the respiration chamber on both the first and third day, although the increase on the third day was not as high as the first day. After the sheep was in the respiration chamber, its heart rate quickly declined to an average of 80-90 beats per minute (bpm), which was maintained throughout the remaining time in the respiration chamber. The time for the heart rate to decline to the average 90 bmp was quicker on the third day compared with the first. Based on the heart rate of this particular sheep remaining at an average of 80-90 bmp, it appears that this individual was adequately adapted to the respiration chamber. However, comparisons with a baseline heart rate would need to be made to validate this assumption. Interestingly, it seems that this particular sheep still required adaptation to human handling since handling elicited a large, albeit temporary, increase in heart rate (Figure 4). This is despite deliberate efforts of trained staff to use best practice animal handling and stockmanship.

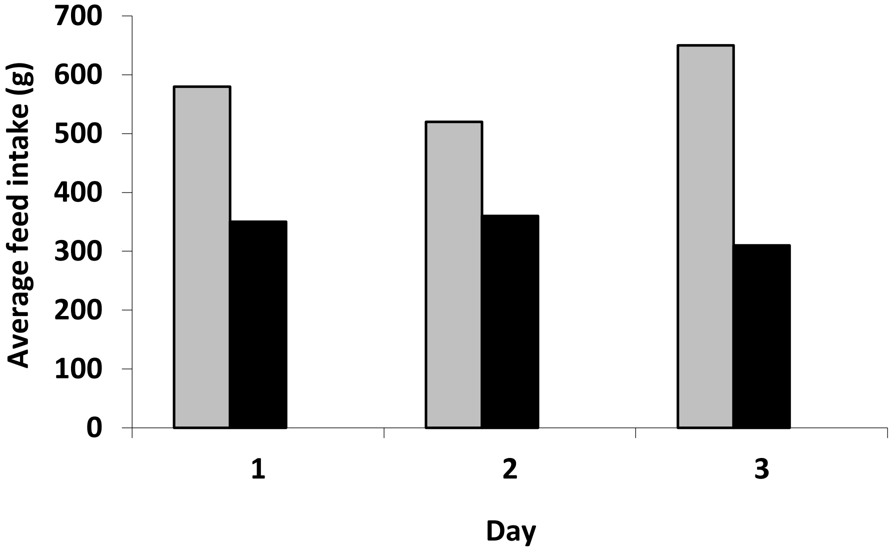


Figure 3: Average feed intake of eight sheep, over three days, before (grey bars) and then during (black bars) methane measurement in respiration chambers. Feed intake was measured over a three-hour period between 09.00 and 12.00 h each day.

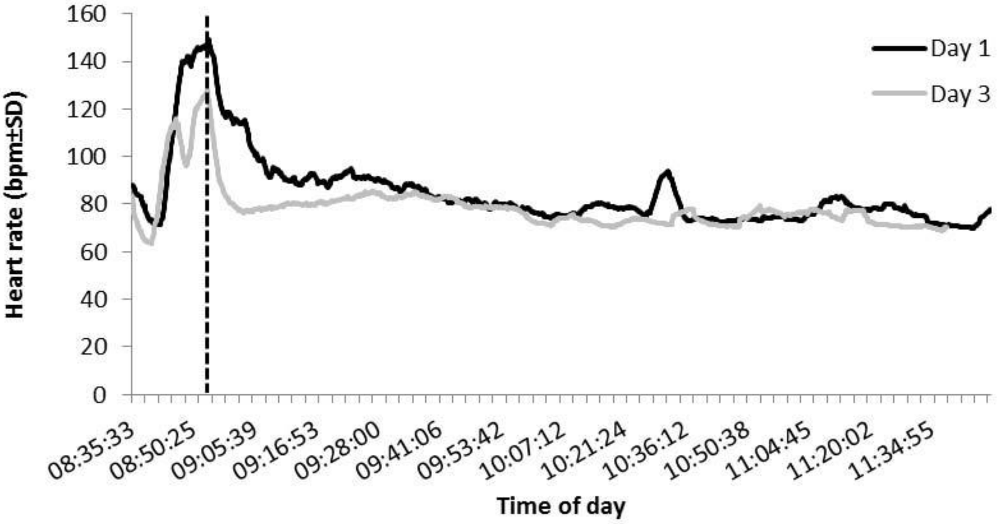


Figure 4: Heart rate of one sheep on the first (black line) and third (grey line) day in a methane respiration chamber. Dashed line indicates when the sheep was put into the respiration chamber.

Concluding remarks

1. Responding to change is a necessary adaptation that all animals face (ourselves included).
2. A complex array of neural and endocrine responses provide the means for animals to develop a behavioural and, if necessary, physiological response to a challenge.
3. The 'challenge' could be a major life-threatening event, but could also be more subtle, such as a change in ambient temperature, housing conditions or diet composition.
4. In the research setting, we must allow animals to adapt to change, especially to changes imposed by us as part of the research environment.
5. Allowing animals to adapt, or acclimatise to their particular conditions and circumstances is necessary for good animal welfare outcomes, to meet a guideline in the *Australian code of practice for the care and use of animals for scientific purposes*, and to obtain meaningful data from research.
6. Animals acclimatised to one factor, such as their housing, may not necessarily show adaptation to another factor, such as a particular experimental procedure (e.g. blood sampling). Care should be taken to manage the adaptation responses to all of the factors encountered by the animals.
7. In general, researchers should be encouraged to think carefully about the processes used to acclimatise animals in their care, and to consider validating their methods with quantitative data rather than relying on overt behavioural signs to assess acclimatisation.

References

Done-Currie, J. R., Heckler, J. F. & Wodzicka-Tomaszewska, M. 1984. Behaviour of sheep transferred from pasture to an animal house. *Applied Animal Behaviour Science,* 12**,** 121-130.

Goopy, J. P., Woodgate, R., Donaldson, A., Robinson, D. L. & Hegarty, R. S. 2011. Validation of a short-term methane measurement using portable static chambers to estimate daily methane production in sheep. *Animal Feed Science and Technology,* 166**,** 219-226.

Hargreaves, A. L. & Hutson, G. D. 1990. Some effects of repeated handling on stress responses in sheep. *Applied Animal Behaviour Science,* 26**,** 253-265.

Hutson, G. D. 1985. The influence of barley food rewards on sheep involvement through a handling system. *Appl. Anim. Behav. Sci.,* 14**,** 263-273.

Klein, L. & Wright, A. D. G. 2006. Construction and operation of open-circuit methane chambers for small ruminants. *Australian Journal of Experimental Agriculture,* 46**,** 1257-1262.

Lockyer, D. R. 1997. Methane emissions from grazing sheep and calves. *Agriculture, Ecosystems & Environment,* 66**,** 11-18.

Miller, M. W., Hobbs, N. T. & Sousa, M. C. 1991. Detecting stress responses in Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*): reliability of cortisol concentrations in urine and feces. *Canadian Journal of Zoology,* 69**,** 15-24.

Moberg, G. P. 2000. Biological response to stress: Implications for animal welfare. *In:* Moberg, G. P. & Mench, J. A. (eds.) *The biology of animal stress.* New York, USA: CAB International.

Palme, R., Rettenbacher, S., Touma, C., El-Bahr, S. M. & Mostl, E. 2005. Stress hormones in mammals and birds. Comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. *Ann. N.Y. Acad. Sci.,* 1040**,** 162-171.

Romeyer, A. & Bouissou, M. F. 1992. Assessment of fear reactions in domestic sheep, and influence of breed and rearing conditions. *Applied Animal Behaviour Science,* 34**,** 93-119.

Sapolsky, R. M., Romero, R. M. & Munck, A. U. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine reviews,* 21**,** 55-89.

Positive Reinforcement Training in Pigs

MJ. Lindeman, Thomas S., Frey B., Bourke DA.
University of Western Australia

Environmental enrichment and habituation to novel housing and handling are increasingly being used by the research community as a key mechanism for improving animal welfare. Is it possible to think 'out of the square' and look at new ways to help research animals adjust and if possible 'enjoy' their new environment? Within the general community, positive reinforcement training is now well recognised as a way of training and interacting with pet companions.

Using food rewards, we demonstrated that laboratory pigs can be trained to accept routine handling for research studies as an alternative to physical and chemical restraint. Regular positive interaction associated with this training helped habituation to people and the research environment and also formed part of an enrichment programme for this very social animal.

Interactive training and habituation can provide a way of improving animal welfare in many research situations, including:

a) Environmental enrichment for those animals held for long periods
b) Improved tractability and tolerance (and therefore improved welfare outcome) of low impost examinations, for example;
    i. Examination of eyes, ears, mouth and feet.
    ii. Body weight recording.
    iii. Minor procedures (e.g. measurement of rectal temperature).
c) Reduced requirement for physical and chemical restraint for pre- and post-operative monitoring and treatments.

Prior to commencing our pig training programme, there was extensive consultation with veterinary and non-veterinary colleagues and animal care staff. Full consideration was given to applying the "3 Rs". There was no replacement possible, but it was considered that the positive reinforcement programme could lead to a reduction in the number of animals used **IF** the welfare impost of repeated examination of individual animals could be reduced and therefore fewer animals were required to obtain information at all the necessary time points. It was agreed that the programme would be a refinement to the current routine practices and would reduce the welfare impost on the individual animals involved.

The programme we devised, **PAWES* Low Stress Pig Handling Programme** (outlined below), is not without cost. More resources (both financial and time) from researchers, animal care and other staff are required. In addition, increasing personal interaction with the animals may lead to an additional emotional attachment cost to the personnel involved - a cost that needs to be considered.

PAWES* Low Stress Pig Handling Programme

This is a three part programme which includes training researchers and animal care staff as well as the pigs themselves. In brief, the programme components are:

1) **Training the humans**
    - ProHand® Pigs Stockperson Training Programme for animal users
    - Piggery visit for hands-on practise of handling methods
    - 'How animals learn' workshop
    - Develop research project specific '*Habituation and training plan'*.

2) **Habituation of pigs to their new environment & the presence of people**

Habituation begins with regular short, non or minimally interactive visits to the pigs (as described in the '*Habituation and training plan').* These continue until the pigs are no longer demonstrating anxiety responses and are actively seeking to engage with humans.

3) **Positive reinforcement training with the pigs**

Follow on regular, short training sessions (as agreed and described in the *Habituation and training plan)* are organised. The training sessions include general handling and training for project specific procedures.

PART 1: TRAINING THE HUMANS

Low Stress Handling for animal users

The **ProHand®** multimedia commercial training package introduces and discusses (in an interactive format) pig behaviour in a production animal context. It provides good, basic knowledge that enables participants to observe and interpret pig behaviour and maximise the welfare of the animals in their care. It includes:

- The link between fear and productivity and welfare.
- The interaction between the handler's beliefs and their own behaviour towards pigs.
- Appropriate pig handling and management in the context of these interactions.
- The link between job satisfaction of the stockperson and animal welfare.

The participants **visit a piggery** – an opportunity to gain confidence and to practice observational, interpretive and handling skills in a practical "real-life" environment with pigs of varying age and size. The mediator guides them through the piggery while discussing and reinforcing the concepts already learnt in this practical setting.

Finally, the participants also learn about **how animals learn** and why suitable environmental enrichment programs help to maximise welfare. They are introduced to a range of training methods. The participants will then work together to **design a comprehensive *Habituation and training plan*** based on what they have learnt and the specific requirements of their own research project.

PART 2 AND 3: HABITUATION AND POSITIVE REINFORCEMENT TRAINING

The participants now have the opportunity to combine their theoretical and practical experience to implement their '*Habituation and training plan'* within their research project, with the ongoing practical support of an animal trainer/mentor.

Motivating your pig to learn.

The first step is identifying what motivates the training subject, both as a species and as an individual. Different pigs will be motivated by different rewards. In general, food rewards are the easiest to work with because they:

- Can be divided into very small portions that allow repeated rewards.
- Tend to be more valued than physical contact. (It is the humans who like physical contact.)

- Can be novel to the animal's normal food. In pigs, we find that raspberries are very attractive but messy.

Clicker training your pig

Clicker training is a positive reinforcement based method of training. This means that desired behaviours are rewarded and are therefore more likely to be repeated. This type of learning is characterised by the animal having a choice. The animal chooses to repeat behaviours because the consequence of repeating the behaviour is a reward.

A clicker is a small device that produces a sharp, unmistakeable sound. This sound is linked with a treat or reward. The click 'marks' the desired behaviour. The association between the sound and the reward must become strong through repeated and consistent reinforcement.

Clicker training is a very good way of generating a common 'language' in which both the trainer and the animal can communicate. This makes training easier and the animal becomes interested in what the trainer has to "say". It also teaches animals to voluntarily 'offer' behaviour that they think will elicit a click (and hence a reward).

In the research setting, a click is linked with a food reward (raspberries). Once the pig is alerting to the click (i.e. looking towards the trainer for the raspberry), the clicker is considered "primed". This means the pig understands that the click means "good" and a reward is coming. Following this, the pigs are trained to touch a "target" on the end of a stick, which is acknowledged by the click marker and food reward. Once the pig consistently touches the target with its nose, it was encouraged to follow the target. This "target training" can then be used to position and lead the pig, for example, into a weigh crate. The pig moves voluntarily and at the same time gets some mental enrichment out of the process.

This programme has now been implemented at UWA and has resulted in successful animal welfare and research project outcomes.

Improving tractability and habituation to humans and the research environment can provide a way of improving animal welfare in many research situations. It reduces or eliminates the requirement for physical restraint, and also provides a novel and stimulating form of environmental enrichment. The UWA *PAWES Low Stress Handling in Pigs* Programme has been designed to educate animal users and to facilitate the implementation of positive reinforcement techniques in research pigs with the aim of improving welfare outcomes.

* **PAWES** Programme in Animal Welfare and Science, University of Western Australia
ProHand® Pigs, Animal Welfare Science Centre, www.animal-welfare.org.au

Special thanks to Dr. Campbell Thomson and Ms. Astrid Armitage for their support in this initiative.

List of Poster Presentations

Amanda Worth (Murdoch University)
Do atypical Australian strains of *Toxoplasma gondii* affect the behaviour of hosts?

Megan Cornelius (Murdoch University)
Worms increase the risk of ewes falling into critical low body condition

Melanie Koinari (Murdoch University)
Identification of gastrointestinal parasites and their prevalence in sheep and goats in Papua New Guinea

Sarah Wickham (Murdoch University)
Qualitative Behavioural Assessment as a measure of animal welfare

Pedro Martinez Perez (Murdoch University)
Health and disease status of the quokka (Setonix brachyurus) – a threatened marsupial and associated implications for its conservation in Western Australia

Thinza Vindevoghel (Murdoch University)
A study of The West Australian community on attitude towards Livestock Welfare

James Macgregor (Murdoch University)
Evaluating the use of instream microchip readers to remotely monitor platypus populations

Nita Harding (Dairy New Zealand)
Improving Stockmanship Skills in the New Zealand Dairy Industry

Post Conference Workshop

Euthanasia Workshop (Hosted by Murdoch University)

Euthanasia Workshop
Friday 27 July 2012
9am – 2.00pm
Murdoch University Vet School

This workshop is designed for researchers or students and wildlife workers. It is also focused strongly on the needs of AECs, including C & D members. The workshop comprise two sessions. The morning session covers different euthanasia techniques, particularly centred on laboratory animals and wildlife and will focus on the theoretical components. The afternoon session (for full day registrants only) provides a specialist practical workshop where hands-on experience can be gained using ethically sourced cadavers.

The workshop addressed a range of topics such as:

- Acceptable versus unacceptable methods of euthanasia
- Criteria for euthanasia
- Comparative anatomy of different species
- Performing euthanasia and confirming death
- Safety and Use of Barbiturates
- How euthanasia agents work and appropriate dose rates
- Routes of administration
- Sedation
- Disposal and Reporting
- Video demonstration of some commonly used techniques for lab animals