2019 ANZCCART Conference Proceedings

"Breaking Down Laboratory Walls"

Tuesday 23rd to Thursday 25th July, 2019

Hobart, Tasmania

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

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2019 ANZCCART Conference Programme

Tuesday 23rd July 2019

Session Chair	*Geoff Dandie, (CEO, ANZCCART)*
9.30am	**Conference Opening** Mrs Nicola Hodgman
10.00am	**Greg Irons –** Small Change – Discussing how we as individuals can help save species
10.30 – 11.00am	**Morning Tea**
11.00 - 12.00 noon	**Workshop 1 - Group Discussions by AEC Category**
12.00 – 12.30pm	**Workshop Reports to Delegates**
12.30 – 1.30pm	**Lunch**
Session Chair	*Mandy Paterson, (ANZCCART Board Member)*
1.30pm	**Annmaree Jackson** – Australian Animal Researcher Competency: Time to Adopt a National Approach
2.00pm	**Scott Godwin** – 'How do you vaccinate a fish?' – Inside the Biosecure Fish Facility at Tasmania's Centre for Aquatic Animal Health and Vaccines
2.30pm	**Clair Alston – Knox & Monica Naughtin** – Sample size in animal studies – Is the justification good enough?
3.00 – 3.30pm	**Afternoon Tea Break**
Session Chair	*Jim Webster (ANZCCART New Zealand Board Member)*
3.30pm	**Bruce Lyons –** Dancing with the devil: immune strategies to combat DFTD
4.00pm	**Megan Verdon –** The importance of animal welfare to research validity and replicability.
4.30pm	**Craig Johnson** – Rehoming experimental animals in New Zealand
4.45pm	**Elyssa Barnaby** – Public Perception
5.30pm – 7.30pm	**Welcome Reception**

Wednesday 24th July

Session Chair *David Mason (ANZCCART Chair)*

9.00am **Gail Anderson** – The development of a State-wide animal welfare and ethics course

9.30am **Craig Johnson** – Pain perception in fish

10.00am **Mark Green** – Using complementary imagery and targeted sampling to address the 3Rs in a marine context

10.30am Morning Tea

Session Chair *Kate Littin (ANZCCART New Zealand Board Member)*

11.00am **Corinne Alberthson** – Breaking down the walls in the animal house space: a novel approach to improving compliance, comprehension and research.

11.30am **John Inns** – The AEC and aseptic surgery

12.00noon **Jodi Salinsky** – Personal openness

12.30 – 1.30pm **Lunch**

Session Chair *Geoff Dandie (CEO, ANZCCART)*

1.30 – 2.30pm **Workshop 2 - Pseudo AEC Discussion Groups**

2.30pm **Workshop Reports to Delegates**

3.00pm **Afternoon Tea**

Session Chair *Pete Hodgson (ANZCCART Board Member / Chair, ANZCCART New Zealand)*

3.30pm **Scott Carver** – How research on Wombat disease is a diverse landscape of social and ethical challenges

4.00pm **Jayson Semmens** – Leaving the void: Tonic immobility, anaesthesia, analgesia and surgical procedures in sharks

4.30pm **Mike King** – Commissioner for animals

5.00 pm End of formal sessions for day 2

6.30pm **Buses Depart Wrest Point for Conference Dinner**

7.00pm **Conference Dinner** – Cascade Brewery Function Centre

Thursday 25th July

Session Chair *Cathy Pitkin (ANZCCART, Board Member)*

9.00am **Ruth Pye -** Wild immunology, natural disease models and Tassie devils

9.30am **Alison Canty** – A stimulating story – Exploring how magnets can influence brain circuitry

10.00am **Annie Philips** – Managing disease in the Orange Bellied Parrot metapopulation.

10.30am **Morning Tea**

Session Chair *Alison Coates (ANZCCART, Board Member)*

11.00am **Jennifer Pelham** – The Bruny Island Cat Management Project

11.30am **Johana Toia** – Meeting the challenges of monitoring remote sites

12.00noon **David Howells** – Better use of tissues from animal experiments

12.30 – 1.30pm **Lunch**

Session Chair *Geoff Dandie (CEO, ANZCCART)*

1.30pm **Malcolm France** – Openness Agreement

2.00pm **James Wynne** – Achieving the 3Rs in Atlantic salmon disease research

2.30pm **Russ Bradford** – Animal ethics in the wildlife space: addressing the 3Rs

2.45pm **2020 ANZCCART Conference announcement**

3.00pm **Conference Close and Afternoon Tea**

Presentations given

on

Tuesday 23rd July

Small Change - discussing how we as individuals can help save species.

Greg Irons

Bonorong Wildlife Sanctuary, 593 Briggs Road, Brighton, TAS, 7030

Most of the injured and orphaned animals that come through our rescue service, and the calls for advice that we receive, could so easily be prevented if individuals were aware that it is our decisions and actions that were the cause of the issue in the first place. At Bonorong, we strive to educate people on the simple things they may not be aware of, that they can so easily change in their day to day lives to help protect our precious wildlife. "Small change" is designed to be a reminder for people of how easily we can affect animals without knowing we're doing it, as well as to educate people on changes they can make that they may not have considered in order to live alongside our wildlife harmoniously.

No manuscript was submitted for this presentation

Australian Animal Researcher Competency: Time to adopt a national approach.

Annmaree Jackson (USQ) and Dr Sarah Wright (USQ)

A major revision to the Australian Code for the care and use of animals for scientific purposes, 8th edition, 2013, was the emphasis for supporting animal wellbeing during their care and management and safeguarding animal wellbeing during the conduct of procedures. This was accomplished through the introduction of Section 3 – Animal Wellbeing, requirements for observance of "current best practice", and the necessity for all procedures to be performed competently. The requirement for competency to perform procedures and for procedures be performed competently is reiterated throughout the Code, through institutional, animal ethics committee, investigator, and animal carer responsibilities. For example, by ensuring access to appropriate educational programs and resources, possession of necessary skills and knowledge, and individuals being deemed competent for the duties they perform.

In the absence of national, state or territory guidelines for implementation of a training and competency framework, institutions have aimed to comply with the Code requirements as best as they are able within their current resourcing capacity. It is suggested that this has resulted in many ad hoc training and competency assessment arrangements being implemented across Australian institutions, with little transferability of "competencies attained" between institutions. Investigators, animal carers, and those responsible for training staff involved with animals for scientific purposes have reported frustrations in identifying, accessing, and maintaining appropriate learning resources that meet both their responsibilities under the Australian Code, and provide an appropriate framework for assessment of 'competence' in a given procedure.

It is therefore proposed that a collaborative national animal ethics training framework be adopted with a view to the development and delivery of open access online training modules. This presentation will outline such a framework, which has been modelled from the education and training framework adopted under Directive 2010/63/EU on Protection of Animals Used for Scientific Purposes within the European Union, contextualised to the Australian legal framework, and mapped against the Code and supporting guidelines. The proposed Australian framework contains a number of mandatory modules for investigators, animal carers, person(s) with ultimate responsibility, veterinarian oversight, and animal ethics committees. A number of species-specific streams (such as laboratory animals, production animals, native animals/wildlife, and aquatic animals) are also anticipated for modules that involve basic and applied biology; animal care, health and management; recognition of pain, suffering, and distress; and humane methods of killing. A benefit of the proposed framework is that it can be expanded to provide for additional "streams" and/or additional modules of training as resources become available to develop and implement these, and/or are required.

By utilising a collaborative arrangement to develop and deliver the module content, compliance with current best practice will be upheld through adoption of teaching and learning theory for adult education and accessing content expertise. Further benefits of adopting and collaborating to an Australian Framework may include provision of immediate access to online module content to collaborating institutions, access to areas of training specialisation that an institution may not currently retain (or for smaller institutions, be in a position to retain), and proving providing a unified approach to measuring competency attainment. This nationalised approach will enhance investigator competencies, promote adoption of current best practice and improve animal welfare outcomes.

No manuscript was received for this presentation

‘How do you vaccinate a fish?’ – Inside the Biosecure Fish Facility at Tasmania’s Centre for Aquatic Animal Health and Vaccines

Godwin, S.[1], Angelucci, C.[1], Carson, J., Cornish, M.C.[1], Giles, C.[1], Hulse, D.[1], La Fauce, K.[1], Spencer, E.E.[1], Morrison, R.N[1].

[1]Centre for Aquatic Animal Health and Vaccines, Biosecurity Tasmania, Department of Primary Industries, Parks, Water and Environment, Mt Pleasant Laboratories, Prospect, Tasmania.

Abstract

The Centre for Aquatic Animal Health and Vaccines (CAAHV) is based at the Department of Primary Industries, Parks, Water and Environment (DPIPWE) Animal Health Laboratory, Launceston. Its purpose is to provide diagnostic and research services to Tasmanian aquaculture industries, particularly the salmonid industry. In the research space, the Centre has a particular focus on the development and testing of veterinary vaccines for use in farmed Atlantic salmon and rainbow trout. A crucial aspect of the work performed by the CAAHV is testing prototype vaccines in live fish. This work is undertaken at the Biosecure Fish Facility (BFF) located adjacent to the Animal Health Laboratory. It is a state of the art physical containment level 2 (PC2) fish facility and a federal Department of Agriculture Biosecurity Containment level 2 (BC2) Approved Arrangement.

A typical vaccine trial conducted in the BFF consists of two phases. The first phase involves vaccinating a small number of fish (typically 20), which are held for 21 days and monitored for signs of adverse effects. The second phase involves the *in vivo* challenge of larger numbers of vaccinated fish with infectious pathogens. Depending on the vaccine in question, the duration of this efficacy testing phase may range from a few weeks to several months.

Specific pathogen free fish for the vaccine trials are supplied by commercial hatcheries and transported to the BFF in a purpose-built 1000 L transporter. All fish are held under maintenance conditions for at least two weeks to allow acclimation to the BFF prior to the commencement of a trial. Throughout the course of each trial, the fish are fed daily, and water quality is monitored to ensure that parameters including temperature, pH, ammonia and nitrite are kept within acceptable limits. In some instances, fish may be transferred from freshwater to seawater to mimic the natural migratory patterns of Atlantic salmon. In addition to routine husbandry and maintenance procedures, a typical vaccine trial may involve the use of several fish handling procedures including anaesthesia, intraperitoneal injection, marking or tagging, blood sampling and euthanasia.

Introduction

According to the United Nations Food and Agriculture Organization (FAO 2019), aquaculture is the fastest growing food producing sector worldwide, and farmed fish now account for 50 percent of the world's fish that are used for food. In Australia, growth in aquaculture has mirrored the overall global trend, with high-value species that provide strong economic returns to growers proving most successful. In terms of both tonnage and dollar value, the Australian aquaculture sector is dominated by production of Atlantic salmon. Commercial production of this species occurs exclusively in Tasmania, where the cool climate is suitable for growth of Atlantic salmon in sea pens. Since its inception in the 1980s, the salmonid farming industry in Tasmania has enjoyed uninterrupted growth and has expanded to become the most valuable primary production industry in the state (AgriGrowth Tasmania, 2018).

A key factor in the success of the salmonid farming industry in Tasmania has been its relative isolation from the other Atlantic salmon growing regions, combined with strict biosecurity and import regulations, which together have provided an effective barrier against the arrival of many of the disease agents that have affected other salmonid aquaculture industries worldwide. As with any intensive livestock production system however, infectious disease has been an ever-present problem. Diseases due to bacterial agents including *Yersinia ruckeri* serotype O1b, atypical *Aeromonas salmoncida* biovar Acheron and *Vibrio anguillarum* serotype O1 were historically managed with antibiotics, but are now largely prevented through the use of vaccines (Costa et al 2011, Lund et al 1991, Munday et al 1992). More recently, vaccines have been introduced to prevent the diseases caused by the Tasmanian *Rickettsia*-like organism (TRLO) (Morrison et al 2016) and the Pilchard orthomyxovirus (POMV).

All of the vaccines used by the Tasmanian salmonid aquaculture industry were originally developed by The Centre for Aquatic Animal Health and Vaccines (CAAHV). The CAAHV is a tri-partite arrangement between the Tasmanian Department of Primary Industries, Parks, Water & Environment (DPIPWE), the Tasmanian Salmonid Growers' Association (TSGA) and the Fisheries Research & Development Corporation (FRDC). The role of the CAAHV is to support the Tasmanian salmonid aquaculture industry through the provision of expertise in matters regarding fish health and infectious disease. This includes coordinating the Tasmanian Salmonid Health Surveillance Program, which has been in operation since 1994 and is designed to ensure early on-farm detection of infectious diseases of fish. The primary research role of the CAAHV however, is to develop vaccines and diagnostics that meet the needs of the industry. A key aspect of this work is *in vivo* testing of prototype vaccines in fish. These tests are performed under controlled conditions at the Biosecure Fish Facility (BFF).

Overview of the Biosecure Fish Facility

The BFF is a purpose built facility for testing and developing fish vaccines. It was constructed in 2014 with funding contributed by the Tasmanian Salmonid Grower's Association, the Tasmanian State Government and the Australian Federal Government through the FRDC. Physically, the BFF is comprised of a single secure building consisting of a central courtyard surrounded by five separate fish rooms (Figure 1.), each of which contains 12 individual 1000 L recirculating aquaculture systems (RAS). To prevent the accidental release of infectious agents or quarantine

material from the facility, each of the five fish rooms has its own anteroom through which personnel must pass to enter the fish room. Each anteroom includes a disinfectant footbath and hand basin. While in the anteroom, and before entering the fish room, personnel don gloves, rubber boots and full length waterproof personal protective equipment (PPE). Any material that is moved out of the fish room such as waste paper, used gloves, fish samples or carcasses is double-contained and transported directly to the neighbouring DPIPWE Animal Health Laboratory, where it is disposed of in accordance with quarantine regulations.

Wastewater from the fish rooms is pumped to an on-site waste treatment plant, where it is treated with chlorine to inactivate pathogenic organisms prior to discharge to the municipal sewerage system. The facility was built to meet the Australia/New Zealand standard for laboratory design and construction (AS/NZS 2982 2010) and complies with the standard for microbiological safety and containment (AS/NZS 2243.3 2010). The BFF is also certified by the federal Department of Agriculture as a Quarantine Approved Arrangement. To ensure that only staff with proper biosecurity training are able to access the facility, entry is restricted to authorised CAAHV staff and is controlled by a swipe card activated lock.

Figure 1. View inside a fish holding room in the Biosecure Fish Facility.

Recirculating aquaculture systems

Each of the 60 RAS in the BFF consists of a 1000 L fish holding tank connected to its own independent filtration system and temperature control unit, which allows the water temperature to

be precisely controlled within a range of 10 – 18˚C. Under normal operating conditions, each RAS is operated as a semi-closed system, in which nitrogenous waste is processed by a biological filter containing nitrifying bacteria, and water exchange is limited to approximately 5-10% of the system volume per day. Bulk solids are removed each day through a central dump valve in the bottom of the fish holding tank, while suspended particulates are removed from the water by a solids filter. Aeration is provided via a reticulated system that is supplied by a single air pump for each fish room. The top of each fish holding tank is enclosed with a soft mesh net to prevent fish from jumping out of the tank.

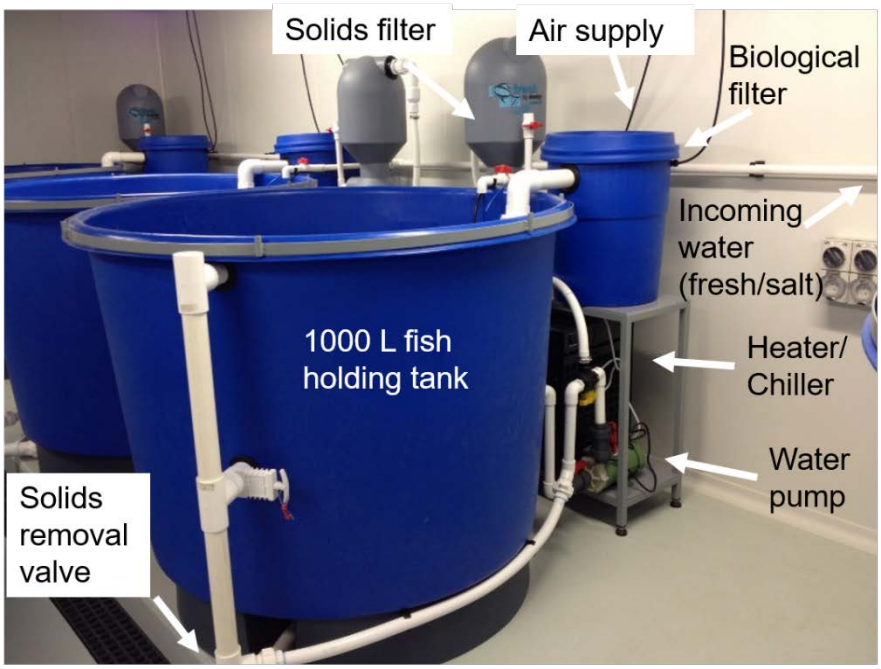

Figure 2. One of the 60 identical recirculating aquaculture systems (RAS) used in the Biosecure Fish Facility, with major components labelled. Note the anti-jump netting was removed for the photo.

Water supply

Each of the fish holding rooms in the BFF may be operated using either freshwater or seawater. Freshwater is supplied by the municipal system and is held in external supply tanks, where it is continuously circulated through activated carbon to remove residual chlorine. The pH of the water is continuously monitored and automatically adjusted by addition of sodium bicarbonate. Seawater is sourced from the Tamar estuary at Beauty Point and delivered to the BFF by truck. It is held in external tanks where it is treated with chlorine to neutralise any potential pathogenic organisms, de-chlorinated with sodium thiosulphate and if necessary, the pH is adjusted with sodium bicarbonate.

Source and transport of fish used in the BFF

All fish used for research trials in the BFF are specific pathogen free fish sourced from commercial salmonid hatcheries within Tasmania. The fish are obtained during the freshwater phase of their life cycle at sizes ranging from <2 g to 350 g, depending on the requirements of the individual trial. The species of fish also varies depending on the requirements of the trial. Atlantic salmon (*Salmo salar*) are used most frequently, but rainbow trout (*Onchrhynchus mykiss*) are used in some trials.

The fish are transported from the hatchery to the BFF in a custom-built fish transport trailer. This trailer includes a 1000 L tank, which allows for up to 80 kg of live fish to be transported. The tank is supplied with oxygen from compressed gas cylinders, which is delivered to the fish transport tank by an automatic computer-controlled system (Oxyguard, Tasmania, Australia) that maintains the dissolved oxygen inside the transport tank at a level between 90 and 110 % saturation. The temperature, pH and oxygen concentration in the transport tank are measured with electronic sensors, and the data is transmitted via a wireless link to a tablet device in the cabin of the vehicle towing the trailer, thus allowing the driver to monitor the conditions inside the transporter in real-time.

Upon arrival at the BFF, fish are distributed into the 1000 L tanks in the fish holding rooms, where they are allowed to acclimate for at least two weeks prior to the start of any experimental manipulations. During this acclimation period, fish are monitored for any signs of disease and depending on the circumstance, the water temperature may be gradually adjusted from that at the hatchery to the temperature that is required for the trial. To minimise stress on the fish, temperature adjustments are limited to 1˚C per day.

Routine fish husbandry in the BFF

Feeding

Fish in the BFF are fed a diet of commercial pellets identical to that used at freshwater salmonid hatcheries and marine farming leases. The brand, size and composition of pellet used when fish first arrive at the BFF is matched to the source hatchery. As the fish grow the size of the pellets are increased according to the feed manufacturer's guidelines. If fish are transferred to seawater, their diet is adjusted to marine-specific pellets.

Fish smaller than 50 g are fed up to two to three times per day, while larger fish are fed once daily. Depending on the requirements of the trial, the fish may be fed to satiation, or they may be fed a fixed maintenance ration, typically 2% body weight per day. Feeding is conducted by hand, and this gives staff an opportunity to observe fish behaviour and assess their general health status. Reduced feed intake is a common early sign of many fish diseases, and may also indicate a deterioration of water quality or other physical parameters.

Water quality monitoring

Basic water quality parameters including temperature and pH are recorded daily for each fish holding tank. Other parameters such as oxygen saturation, salinity, total ammonia nitrogen, nitrite and nitrate concentration are also recorded as required, with the goal of maintaining them within the RSPCA welfare standards for farmed Atlantic salmon (RSPCA 2015). If any parameter is outside the specified range, immediate action is taken to rectify the situation.

Routine fish handling procedures during trials

Capturing and moving fish

Physical handling is kept to a minimum in the BFF to reduce stress and avoid damage to the skin and mucus coat of fish, which can predispose them to infection. When it is necessary to handle or move fish, they are captured using soft, knotless dip nets. Two nets are used simultaneously to avoid chasing fish around the tank. For most trials, the fish are transferred from the 1000 L RAS tanks to temporary holding containers, which vary in size from 50 to 400 L, depending on the fish number and size. These containers are aerated to maintain adequate oxygen levels.

Transfer of fish to seawater

Atlantic salmon are anadromous fish that, under natural circumstances, migrate from freshwater to the sea as juveniles in spring and early summer. Salmon that are ready to move from freshwater to seawater are termed 'smolt', and have undergone a suite of physiological adaptations in preparation for the transition to seawater (Björnsson et al 2001). This adaptive process, termed 'smoltification', is triggered primarily by increased photoperiod. In an aquaculture context, smoltification may be stimulated by exposing fish to a 'winter' with a 12-hour light: 12-hour dark photoperiod, followed by a switch to a 'summer' photoperiod of 24-hour light. In the BFF, Atlantic salmon are prepared for transfer to seawater by maintaining them at 14-15˚C for six weeks under a 12-hour light:12-hour dark lighting regime, followed by 380-400 degree days* under a constant 24 hour lighting regime. At the end of this period, the salinity of the water is gradually increased over a period of seven days by exchanging fresh water for seawater.

{ *[Explanatory Note: Degree days (DD) is a concept used widely in aquaculture. As fish are ectothermic (i.e. cold blooded), the rates of their physiological processes are temperature dependent. Degree days are calculated by simply multiplying the temperature in degrees C by the time in days. i.e 10 days at 15 degrees = 150 degree days.

To give an exaggerated example, the 400 DD referred above could be achieved by keeping the fish at 10 C for 40 days, or at 20 for 20 days. }

Anaesthesia and recovery

Anaesthesia is used during most fish handling procedures including injection, weighing, tagging, fin clipping and blood sampling. The CAAHV uses AQUI-S® for all procedures that involve anaesthesia of salmonid fish. AQUI-S® is a commercially available preparation of iso-eugenol (Ridley Agriproducts, Melbourne), and is registered for use as a salmonid anaesthetic with the Australian Pesticides and Veterinary Medicines Authority (APVMA). AQUI-S® has been shown to reduce plasma cortisol levels in Atlantic salmon during handling procedures (Iversen et al 2003).

To anaesthetise fish, a 0.005% (v/v) solution of AQUI-S® is prepared in water that matches the temperature, salinity and pH of the water in which the fish are housed. Small numbers of fish are then transferred using a net from temporary holding containers into the anaesthetic bath, where they are closely monitored until they reach stage 4 anaesthesia as defined by Iversen et al (2003). This usually occurs within about 5 minutes. At this point, the fish are removed from the anaesthetic bath for the required procedure. Immediately after the required procedures have been performed, anaesthetised fish are placed in a tank of aerated water for recovery. In most cases, this will be the 1000 L RAS tank in which the fish are to be housed during the trial. Atlantic salmon and rainbow trout usually recover from the effects of AQUI-S® within ten minutes (Bowker et al 2002, Iversen et al 2003).

Intraperitoneal injection

Intraperitoneal (IP) injection is frequently used to deliver both vaccines and infectious agents. Fish to be injected are not fed the day before injection to minimise the volume of the gastrointestinal tract, and to reduce the metabolic demand for oxygen when fish are temporarily held at high densities. Immediately before injection, the fish are anaesthetised as described above, then transferred to a work bench with a wet, smooth surface to prevent damage to the skin and mucus coat (Figure 3). Injection is then performed manually using a syringe and a specialised short needle. The specific length of the needle used varies depending on the size of the fish (Table 1), but it must be sufficiently long to penetrate the body wall, while being short enough to avoid injuring internal organs of the fish. The needle is inserted at a perpendicular angle to the ventral midline of the fish, at a point approximately one fin length anterior to the base of the pelvic fins (Figure 3). The volume of vaccine or inoculum delivered by IP injection is usually 100 – 200 µL. After injection, the fish are immediately returned to a recovery tank, where they are monitored until they have recovered from the effect of the anaesthetic. In most cases, fish will resume normal feeding within 24 hours of injection.

Table 1. Recommended needle lengths for intraperitoneal injection of Atlantic salmon (Pharmaq 2019)

Fish Size (grams)	Needle Length (mm)
25 – 35	3.0
35 – 45	4.0
40 – 60	4.5
45 – 80	5.0
70 – 100	6.0
110+	8.0

Figure 3. Delivery of vaccine to an Atlantic salmon by intraperitoneal injection. Injection is performed on a smooth, wet work surface to prevent damage to the skin or mucus coat of the fish.

Blood sampling

Blood specimens may be collected from live fish for a variety of purposes including non-lethal detection of pathogens, measurement of physiological parameters such as packed cell volume or assessment of immune status using antibody assays. The most accessible blood vessel in salmonid fish is the caudal vein, which runs along the underside of the spine and can be accessed by inserting a needle through the caudal peduncle (tail) (Figure 4). For non-lethal blood sampling, fish are first anaesthetised with 0.005% (v/v) AQUI-S® as described above and a maximum of 0.5% of the body weight of the fish is collected (e.g. 0.5 mL for a 100 g fish).

Figure 4. Post-mortem collection of blood from an Atlantic salmon by caudal vein puncture. Non-lethal blood sampling of live fish is also possible using the same method.

Euthanasia

All fish used in the BFF are euthanased at the conclusion of each trial due to the requirements of AS/NZS 2243.3 (2010), which precludes the removal of live animals from the facility. In addition, during trials that involve challenge with infectious pathogens, any fish that becomes moribund is immediately euthanased to minimise distress in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes (NHMRC 2013) and the European Medicines Agency guideline on the design of studies to evaluate the safety and efficacy of fish vaccines (CVMP 2011). Euthanasia is performed by immersing the fish in a bath containing a lethal dose (0.01% v/v) of AQUI-S. Fish are kept in the bath until all movement of the gill opercula, indicative of respiration, has ceased.

Design of typical vaccine trials conducted in the BFF

The vaccine trials conducted in the BFF may be divided into two broad categories. These are: (1) Vaccine safety tests in which a small number of fish are vaccinated to verify that a batch of vaccine does not contain adventitious infectious agents or cause adverse effects in the vaccinated fish and; (2) Vaccine efficacy tests in which a cohort of fish is vaccinated, allowed to develop an immune response to the vaccine and then challenged with an infectious pathogen. The design of these tests are informed by the relevant Australian and European guidelines for the evaluation of safety and efficacy of veterinary vaccines (APVMA 2014, EMA 2011, 2012). Each of the categories of trial is described in more detail below.

Vaccine safety tests

A standardised testing protocol is used to verify the safety of prototype vaccines or individual batches of commercial vaccines (Ph. Eur 5.0 01/2005:1581). Twenty fish are anaesthetised and intraperitoneally injected with the vaccine as described above. To provide an extra margin of safety when the vaccine is deployed in the target population of fish, 200 µL of vaccine is delivered to each fish in the safety test, which is double the standard 100 µL dose. As a negative control, 20 fish from the same cohort are anaesthetised and intraperitoneally injected with 200 µL of sterile phosphate buffered saline (PBS). The fish are allowed to recover and are then monitored daily for a period of 21 days. During this period, any overt signs of adverse reaction to the vaccine such as depressed feed intake or unusual swimming behaviour is recorded. At the end of this period the fish are euthanased with a lethal dose of AQUI-S® as described above, then necropsied and examined for adverse physical effects of the vaccine.

Vaccine efficacy tests

Vaccine efficacy tests aim to evaluate the protective effect of a vaccine by comparing the susceptibility of groups of vaccinated and unvaccinated fish to a standardised challenge with a pathogen. Because the biology of each pathogen is unique, the specific design of the efficacy test varies depending on the vaccine/pathogen being tested. Before any vaccine efficacy trials can be conducted, it is necessary to establish a challenge model for the pathogen of interest that yields repeatable reproduction of disease signs in unvaccinated fish. For some pathogens, this may involve simple direct injection of fish in the freshwater phase of their life cycle with a cultured preparation of the relevant bacterium or virus. Other pathogens may require more complex challenge models that involve transferring the fish to seawater prior to challenge. Methods of exposure to the pathogen also vary, and may involve immersion of fish in a bath containing a specific dose of the cultured bacterium or virus, or the use of unvaccinated 'shedder' fish or 'Trojans' that are directly injected with the pathogen and then housed in the same tank as the vaccinated fish. The latter method is favoured for some viral pathogens, which can produce inconsistent results when fish are exposed to virus produced in laboratory culture. (Bowden et al 2002, Munang'andu et al 2016). It also has the advantage of more closely replicating the natural mode of pathogen transmission from fish to fish.

Regardless of the challenge model employed, the overall principle of all vaccine efficacy tests conducted in the BFF is the same. A cohort of fish is divided into vaccinated and control groups. For most trials, 50 fish are used in each group. This number is based on the guidelines proposed by Amend (1981) which were designed to allow a clinically relevant vaccine effect to be detected, provided that at least 60% of the unvaccinated control group presents demonstrable signs of disease. If previous work with the specific pathogen of interest has shown that the proportion fish that develop disease signs is substantially higher or lower than 60%, the number of fish in each group is determined on the basis of previous trials that were used to establish the challenge model. The results of these trials give an indication of what proportion of unvaccinated fish are expected to develop disease and these data are used to conduct statistical power analyses to determine the sample size necessary to detect a significant protective effect of the vaccine, if one exists.

At the start of a trial, vaccine is delivered to the fish in the vaccine treatment group, usually by IP injection as described above. Fish in the negative control group receive a sham vaccine, which contains the same ingredients as the vaccine, except for the vaccine antigen itself. In most cases, a placebo group is also included, which receives a sterile saline injection containing no active ingredients. This group is included to control for any protective effect that may be observed in the sham vaccine group due to non-specific immune stimulation elicited by the presence of adjuvants or excipients in the vaccine preparation. After vaccination, the fish are returned to their tanks and maintained for a period of 6-12 weeks, during which they develop an immune response to the vaccine. At the end of this period, the fish are exposed to the pathogen of interest using the relevant challenge model. During the challenge, any fish that become moribund or develop overt signs of disease are humanely euthanised using an overdose of anaesthetic as described above.

The duration of the challenge depends on the pathogen and challenge model in question, but in most cases, the challenge progresses until at least two consecutive days have passed during which no fish have presented with any new signs of disease. At this point, all of the remaining fish are humanely euthanised. The disease status of each fish is determined according to predetermined criteria. These criteria vary for each pathogen, but may include morbidity, the presence of specific clinical signs of disease or detection of the pathogen using diagnostic assays. This information is used to calculate the proportion of fish in each vaccine and control group that were affected by disease. The efficacy of the vaccine is expressed as the relative percent survival (RPS) or relative protective effect (RPE).

$$\text{RPS} = 1 - (\text{proportion of vaccinated fish that develop disease}/\text{proportion of unvaccinated control fish that develop disease}) \times 100$$

In practical terms, a vaccine is likely to have a useful clinical effect on farms if the RPS/RPE exceeds 70% (Amend 1981).

Conclusion

The Tasmanian DPIPWE Biosecure Fish Facility is a state of the art fish quarantine facility located at the Centre for Aquatic Animal Health and Vaccines and operated in close partnership with the Tasmanian Salmon Growers' Association and Fisheries Research and Development Corporation. Its design provides for a range of experimental trials to be conducted with live fish and it has been instrumental in the development of vaccines used by the Tasmanian salmonid aquaculture industry.

References

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Sample Size in Animal Studies- Is the justification good enough?

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Abstract

Since the release of the NHMRC Guidelines for Best Practice Methodology in the Use of Animals for Scientific Purposes in 2017, the AEC at Griffith University has become more rigorous in reviewing the statistical justification for sample size in lab-based animal studies. Are justifications such as "experience has shown that a sample size of 5 is sufficient to get statistically significant results" or "the journal requires that I repeat the experiment 3 times" good enough? This led to the realisation that there is a general lack of statistical knowledge among some researchers. We will discuss the limitations of these responses from a statistical perspective and identify the gaps in statistical reasoning. We will discuss the currently available resources for sample size calculations, statistical analysis and study design, and how these are being used by researchers. We have carefully revised our AEC application questions to clearly outline which information the AEC requires to adequately assess the statistical justification provided by researchers. Our presentation will provide an update on how our revised questionnaire and training resources have addressed these issues. We will also discuss how these changes have been received by our researchers, and whether this has led to improved statistical justifications in Ethics applications.

The Griffith University AEC updated their animal ethics application questionnaire regarding the statistical justification of sample size in May 2019. Previously, the application contained just one question relating to sample size justification:

" Provide a justification for the numbers of animals requested, including evidence that the numbers are minimal, but statistically robust to achieve the aims of the research. Where appropriate, information must be provided on Experimental design and statistical considerations"

Why did we decide to change? The typical responses that the Committee would receive often lacked detail or details were not easy to find within the application. Often no input data or calculations were ever provided. A typical answer to this question would often be something similar to the example below:

"previous experience of studies in our lab show than the minimum number to achieve 80% power is 5 mice per group. These calculations were done using an online calculator for sample size or as described in the attached paper "

When an answer like this was provided, using the limit of acceptable power (80 %), concerns were often raised about whether the study would really generate robust and reproducible data.

The NHMRC Best Practice Methodology guidelines were released in 2017. Four major issues were highlighted in these guidelines; Quality of experimental design, quality of experimental statistics, quality of techniques and procedures and reporting. Three out of four of these major issues relate (at least in part) to statistics. This indicated to us that the statistics presented in AEC applications need to be critically assessed.

The reproducibility crisis in animal research

Over the last 10 years, there has been more and more discussion about the "reproducibility crisis" in animal studies. John Ioannidis published a paper in 2005 with the provocative title "Why most published research is false"[1]. In this paper, he discusses the limitations of interpreting results based solely on statistical significance determined by p-values. Animal studies are particularly vulnerable using this approach because sample sizes are often small. From an animal welfare perspective, and as animal studies often represent important precursors prior to clinical trials in humans, this is particularly concerning. Tsilidis and colleagues[2] comment further on this, stating that "Biases in animal experiments may result in biologically inert or even harmful substances being taken forward to clinical trials, thus exposing patients to unnecessary risk and wasting scarce research funds." Apart from the risks to patients, the economic outcomes of irreproducible clinical data are alarming, with the cost estimated to be $28.2 billion in the US alone[3].

In certain fields, meta-analyses of published data have found alarming differences between the estimated and actual power of reported studies. In the field of neuroscience for example, the median statistical power of studies is estimated to be as low as 8-31%[4]. It is clear that small sample sizes lead to fragile data. Data can be so fragile that effects are reported in the wrong direction, and effect sizes can be massively exaggerated[5]. Schoenfeld and Ionnidis[6] presented a paper where cancer-causing effects of certain aliments were compared from numerous publications. Studies showed that everything we eat both causes and prevents cancer. In other words, effects in both directions were reported (cancer-causing and protective effects) for all aliments tested.

To address these reproducibility issues, the New England Medical Journal has gone as far as to recently release new guidelines for statistical reporting in their journal. In an article released on the 18 July 2019, Harrington et al.[7] present the new requirements of the journal with regards to statistical reporting:

"The Methods section of all manuscripts should contain a brief description of sample size and power considerations for the study, as well as a brief description of the methods for primary and secondary analyses."

"Original and final protocols and statistical analysis plans (SAPs) should be submitted along with the manuscript, as well as a table of amendments made to the protocol and SAP indicating the date of the change and its content."

How can the AEC provide statistical guidance?

Our experience is that researchers still prefer the basic hypothesis testing approach (eg. T test) for analysing their data, even when the data is highly variable and the sample size small. There are several reasons for this, one being that the journals themselves insist on applying a p-value during the peer review process. Another is that knowledge about statistics is pretty limited. Most institutions teach basic statistics principals in undergraduate courses, which are suitable for a large class environment. Many smaller institutions do not provide statistical support to researchers, and researchers rely on collaborations to have statistical support for their projects.

We have a statistics advisor on our Animal Ethics Committee, which is a great asset. The AEC now has the capacity to provide thoughtfully considered feedback about sample size and suggest alternative approaches for data analysis. We provide relevant literature to research teams and have even organised workshops with individual research teams. We hope to develop some targeted seminars and other resources over the next year.

The new Animal Ethics review approach and questions

The below figure is a framework that Committee members can use to consider reviewing an application, while thinking "statistically." It was with this framework in mind that we developed a new series of questions for the statistical justification in our AEC application questionnaire. We have gone from one question to a series of seven questions, which are outlined below.

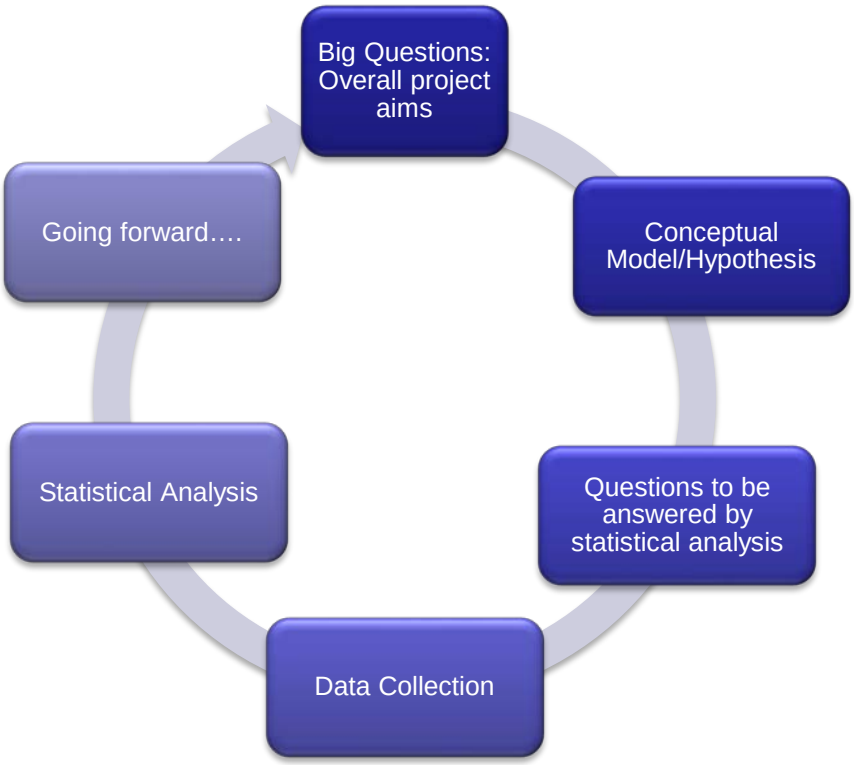


Figure 1. Framework for reviewing animal ethics applications.

New Questions + Fantasy Response:

A. Will you be using a statistical analysis as part of the research?

Yes!

B. Please list (in order of priority) the research questions that you wish to address using a statistical analysis.

Research Question 1: Will sheep of Breed A return to within 5% of their pre-drought lean meat yield within 6 weeks of re-alimentation?

Research Question 2: What is the rate of decline for the 2 drought treatments and do they plateau?

C. What will you be measuring in each experiment (outcome measure) to address each research question? Outcome measure refers to the parameters that you intend to measure quantitatively. Please indicate if they are direct measures from the experiment, or derived from experimental measures. If they are derived, please explain how this measure is calculated.

Initially data will be collected as CT numbers in a series of scans. This is an indirect measure of body composition. These values will range ± 1000, and integer in nature, but will be treated as continuous. These numbers will be used to estimate weight of lean meat in kg (continuous data), which will be used in the statistical analysis to answer RQ1.

D. Please indicate the planned statistical analysis for each outcome measure (E.g. T test, ANOVA).

Stage 1 Analysis: Bayesian Mixture models to estimate percentage of lean meat in carcass. This transforms indirect measure (CT number) into the measure of interest (lean meat yield)

Stage 2 Analysis : Longitudinal growth using mixed effects models will be used to describe lean meat yield and test end points for droughted stock and compare with control. A Bayesian framework will be used

E. Are there independent variables to be included in your analysis?(E.g., sex, breed, treatment). Please list these, and any interactions between these variables that may be required in the analysis (E.g. Do you expect that the treatment response may vary between the sexes?).

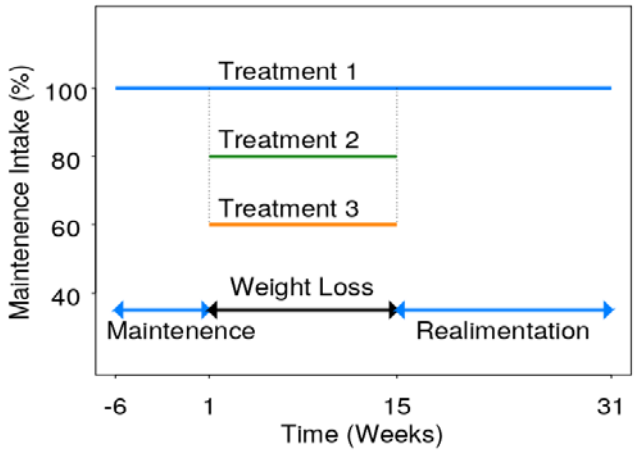
This is a single breed, single sex experiment. Animals will be CT scanned, weighted and condition scored before trial commencement. If variation is more than ± 3kg, sheep will be matched and allocated between treatment, otherwise simple random allocations will be used.

F. Please explain the design and data collection for the analysis, including information about experimental groups, and interventions. Please define this clearly, pictorially would be ideal. Diagrams can be uploaded as supporting documents in the documents tab. The 3Rs experimental design assistant (EDA) online tool can help to build a stepwise visual representation of an experiment.

Data will be collected 1 week prior to droughting commencing.

CT scanning will be performed weekly during the experiment.

(Animals are health scored each day, this is not part of the statistical analysis).

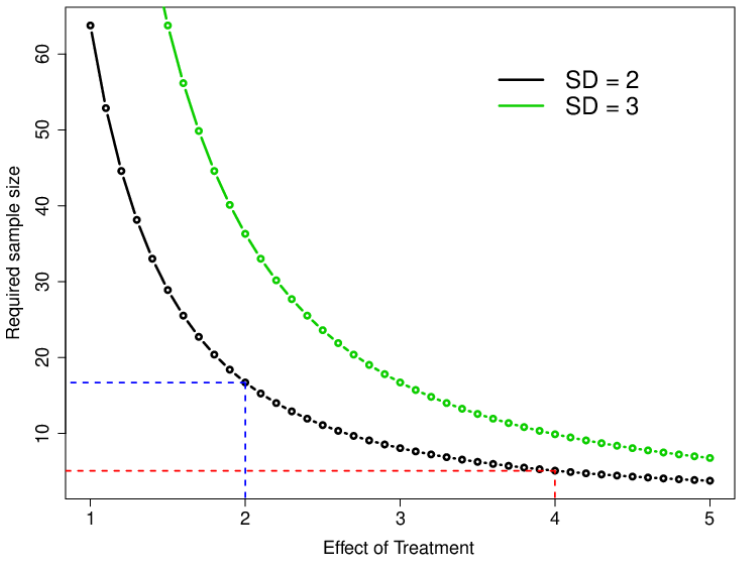


G. In order to ensure that the number of animals requested are minimal but sufficient to achieve statistically robust results (Code, Clauses 1.21-1.27), please justify your sample size. If using a Power analysis, please indicate which sample size calculation you are using. Please note, the committee would like to see all inputs, such as anticipated group means, and standard deviations. Please reference papers and research from which these values are elicited, and if the research is entirely new, please provide the rationale for your assumptions or data from a pilot study. Also, please consider providing a set of power curves under multiple likely scenarios which take into account the potential variability of your results. Please upload a supporting document with your statistical justification.

Sample size calculation was performed using a simulation study.

Estimates of lean meat yield for the study came from Thompson et al (2001), and 2 levels of potential standard deviation were tested.

Power curves indicate 16 animals per group will be adequate.



We conducted a brief survey of AEC members to assess how the new questions have changed the application review process. Six out of eight Committee members who responded said that the new questions had changed the way they review applications. Seven out of eight were more confident about the statistics and sample size justification provided with these new questions. Only half were confident with their own ability to assess whether the statistical justification was appropriate, indicating that some members still prefer to rely on the expertise of others in this area.

In summary, the changes to our AEC questionnaire have assisted greatly in the review process. Applications need to outline the conceptual model used to determine sample size and power considerations. The estimated means and standard deviations underlying the calculations need to be provided and justified. Small sample sizes and inappropriate statistics are fraught with hazards.

A preprint publication announcing the revised ARRIVE Guidelines was released just before ANZCCART, although we only became recently aware of it[8]. These revised guidelines for publishing *in vivo* research are a refinement of the original ARRIVE guidelines published in 2010. The revised ARRIVE guidelines propose an "essential 10 items" which should be reported in *in vivo* publications; study design, sample size, inclusion and exclusion criteria, randomisation, blinding, outcome measures, statistical methods, experimental animals, experimental procedures, and results. This covers exactly what we are asking in our new questions. Investigators must be considering these at the planning stages of the project and that is why including them in the Animal Ethics questionnaire is essential and will ultimately lead to better reporting of *in vivo* studies.

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Dancing with the Devil: Immune strategies to combat Devil Facial Tumour Disease

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Abstract:

Since 1996, a transmissible facial tumour (DFTD) has been estimated to have reduced the population of wild Tasmanian devils by 80-90%. Efforts to preserve the species have included a meta-population across many sites throughout a Australia and around the world, coupled with a captive breeding program. Our group has focussed efforts on determining why this tumour allograft is not targeted by the recipient's own immune system, with the aim of developing a protective vaccine. Our studies indicate that we can induce an immune response against DFTD in the most devils, and parallel immunotherapy studies show it is possible to trigger tumour rejection in a subset of devils. Furthermore, a small percentage of animals in the wild exhibit tumour regressions, which often coincides with detectable immune responses against the DFTD cells. The astonishing discovery of a second, independently arising facial tumour in the channel area of southern Tasmania has challenged the widely held view that transmissible cancers are rare events.

Introduction

Since 1996, a transmissible tumour, devil facial tumour disease (DFTD), has caused a large decline in the population of the Tasmanian devil, the largest extant carnivorous marsupial endemic to the island state of Tasmania (reviewed in Woods et al 2019[1]. Several complementary strategies have been implemented to save the species from potential extinction. The focus of this mini-review will be the immunology of the devil, and its potential for generating either protective immunity, or curative immunotherapeutic approaches. As DFTD is a transmissible clonal cancer derived from a single female devil sometime before 1996, it must have developed strategies to avoid immune rejection across an allogenic barrier. The immunobiology of the tumour and attempts to induce devil immune responses to the tumour will be examined.

DFTD

The first recorded sighting of DFTD was by a wildlife photographer in the NE of the island state of Tasmania, near Wukalina / Mt William. In subsequent years, the prevalence of devils with the disfiguring disease increased, until it was realised that it was affecting population densities in the area and was spreading west and southwards. It became apparent that the tumour was invariably fatal within months of being visible. During this time, several theories abounded regarding the cause of the tumour, including environmental carcinogens or a viral origin. The striking similarity of the tumour morphology and site then led to speculation that the cancer was transmissible, and possibly of viral aetiology. However, no evidence of a viral signature was found. A major advance occurred when karyotypic examination revealed that the complex chromosomal organisation was shared between all tumours, was of female origin, and different to the normal hosts, leading to the

allograft transmission theory[2]. This model has been consistently supported by data, including the finding where a devil with a somatic chromosomal rearrangement did not share this with its tumour, and later studies using microsatellite typing showing that tumours could not have arisen from host cells reviewed in Woods et al 2019[1, 3]).

Marsupial immunity

Transmission as a cancerous allograft means that DFTD avoids recognition by the host's immune system. Initially, it was suggested that limited genetic diversity, particularly at MHC loci, prevented rejection from occurring[4], or that marsupial immunity was somehow weaker than placental mammals, resulting in graft acceptance. However, even though genetic diversity is somewhat limited, skin grafting experiments demonstrated that there was sufficient alloresponsiveness for graft rejection to occur[5]. Furthermore, other investigations demonstrated that devil immunity was robust[6-8], countering the dogma that marsupial immunity was somehow inferior to that of eutherian mammals[9].

Immune evasion strategies of DFT

A major advance came with the demonstration that DFTD cells fail to express MHC-I molecules on their surface[10], which would prevent them from becoming targets for allo-specific CD8 T cells. This raises the question of NK cell activity in devils. In eutherian mammals, NK cells would normally delete cells not expressing MHC, however their existence in devils had not been formally demonstrated. Studies showed that a NK-like activity in devil PBMNC's could be demonstrated[11], and genes associated with NK cells were be identified[12]. The reason that this mechanism was not effective against MHC non-expressing DFTD cells was suggested to perhaps be due to the expression of non-classical MHC, which may negatively regulate NK activity, and not be targeted by CD8 T cells[13]. Studies also focussed on the ability of DFTD cells to produce immunosuppressive cytokines or other immune modifying products. Whilst not sufficient to fully explain non-rejection, production of TGF-□ was noted in some cases[14, 15] (Howson, unpublished observations), as well as upregulation of immune checkpoint molecules under inflammatory conditions[16].

Early attempts to generate anti-DFTD responses

The first attempts to generate immunity used irradiated cultured DFTD cells in order to prevent any chance of inadvertent transmission. In combination with a proprietary oil emulsion adjuvant, immunisation produced no detectable antibody response[11]. Later, adjuvants included montanide and CpG oligonucleotides which stimulate via Toll Like Receptor 9 resulted in modest antibody and/or cytotoxic responses[17]. Challenge experiments with live DFTD showed this was not protective but may have delayed tumour engraftment[17].

Rational vaccine design – Upregulation of MHC-I and TLR agonists

A major advance came with the discovery that IFN-□ could upregulate expression of MHC-I on the surface of DFTD cells[10], and the demonstration that devils expressed and were responsive to the full range of TLR agonists[18]. Investigations using KLH as a model antigen allowed design of an adjuvant combination using Iscomatrix (a proprietary adjuvant from CSL Ltd, Melbourne) Hiltonol® (an RNase resistant poly I:C activating TLR-3) and imiquimod (TLR-7 agonist)[19]. Combined with IFN-□ treated DFTD cells, this candidate vaccine theoretically would stimulate the two main pathways of antigen presenting cell activation and thus boost both humoral and cell mediated immunity.

Immunotherapeutic approaches

After immunising devils with the candidate vaccine described above, challenge experiments were performed which initially appeared effective, but animals developed tumours albeit at a slower rate. However, it was demonstrated in 3 out of 5 of animals, the injection of live, IFN-□ treated DFTD cells with upregulated MHC-I adjacent to the tumour resulted in a dramatic regression of the tumour, with infiltration of immune cells into the tumour noted[20], suggesting an alloresponse was triggered. This was the first demonstration that the immune system of the devil could be harnessed to combat DFTD.

Wild recovery program – release of immunised devils

With the success of the breeding program of disease-free devils on Maria Island off the east coast of Tasmania, the state government instituted a wild recovery program to reinforce devil populations in areas decimated by DFTD in order to restore ecological balance. This release also allowed the testing of candidate vaccine in larger cohorts of devils. Since 2015 there have been 4 releases totalling over 110 immunised devils into 4 sites around mainland Tasmania. Over 95% of immunised devils responded with a robust antibody response to DFTD[21]. Although some animals subsequently developed DFTD, there are several animals which have persisted in the wild without getting the tumour. Re-trapping rates preclude statistical analysis, but monitoring is ongoing and may help in understanding what effect immunisation may be having in the long term.

Immune responses to DFTD in wild devils

In the last 5 years, particularly in the NW of the state, there have been several reports of spontaneous regressions in wild devils that have been sequentially trapped (R Hamede, personal communication). A study of 52 wild devils trapped in the West Pencil Pine area in the NW of the state revealed that 6 animals had spontaneously developed antibody responses against DFTD cells, with 4 of these animals exhibiting tumour regression[22]. In a small number of additional cases, tumour regression again correlated with development of the antibody responses. Where regressing tumours have been biopsied, immune cell infiltration was also seen. This suggests that a small proportion of devils in the wild may have the capability to reject DFTD, but it is unknown if this represents the development of fully protective immunity, as there have been instances where animals present with additional tumours after regression (R Hamede, personal communication).

The wild card of DFT2

A surprising finding in 2014 was the identification of a second transmissible tumour in the channel area in SE Tasmania[23]. Unlike the original tumour DFT1, which arose in a female devil, the second tumour DFT2 arose in a male devil. Surprisingly, the tissue origin of DFT2 was found to be very similar to that of DFT1, which was of Schwann cell derivation[24]. The impact of this DFT is currently under investigation, with 23 devils identified with this tumour (R. Hamede, personal communication). Whether it will remain confined to its current range on a peninsula is unknown.

Conclusions and future directions

Recent findings demonstrate that the devil immune system is capable of rejecting DFTD under some circumstances, both experimentally and in the wild. The finding that over 95% of devils can have a specific antibody response induced by immunisation demonstrates that immune recognition is essentially universal. Immunotherapy can 'cure' a proportion of devils with experimentally inoculated tumour, but only those in which an immune response has been previously generated. Together, these studies support the notion that an immune solution to DFTD is possible.

Acknowledgements

Support for these studies was provided by the Australian Research Council and the Save the Tasmanian Devil Appeal. All studies involving devils were approved by the Animal Ethics Committee of the University of Tasmania, and the Tasmanian Government's Department of Primary Industry, Parks, Water and Environment (DPIPWE).

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The importance of animal welfare to research validity and replicability

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Abstract

The use of animals for research is a privilege granted to scientists with the understanding that significant new knowledge will be generated without causing unnecessary harm to the animal (Bailoo et al., 2014). The determination of "unnecessary harm", however, involves subjective judgements about value. Such judgements are influenced by an individual's experiences and exposure to animals, the type of animals being discussed (e.g., companions vs. production animals), culture or religion, socio-economic status and ethical viewpoints (Hemsworth et al., 2015). Thus, animal ethics is what is viewed as an acceptable way for animals to be. Animal welfare, on the other hand, relates to the animal's health and needs.

An animal is in a good state of welfare if it is healthy, comfortable, well nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear, and distress (OIE, 2018). In other words, animal welfare relates to how an animal is coping with the conditions in which it lives. It is not something that is given to the animal, nor is it either the management procedures applied to the animal or the features of its environment, which may affect its welfare (Mellor et al., 2009). If an argument is to be made that a resource, environment or management technique is going to improve animal welfare, there needs to be sufficient evidence that it either improves animal health or it gives the animals what they want. If this can't be shown, provision of that resource cannot be said to improve animal welfare (Dawkins, 2012).

It is the ethical responsibility of researchers to ensure that the health of experimental animals is optimal while in their care, and that the animal's needs are being met. However, researchers should also strive to keep animals in a high state of welfare to protect the integrity of their research. Experimental animals are often reared, housed and managed in unnatural social and physical environments which may induce a state of negative welfare. This paper discusses the effects of a chronic negative welfare state for the physiological function and behaviour of animals, which compromises the credibility, validity and replicability of experimental findings.

The stress response

When an animal is confronted by a real or perceived threat the hypothalamus stimulates a neuroendocrine response, namely the release of hormones called glucocorticoids, through what is known as the hypothalamic-pituitary-adrenal (HPA) axis. At the same time, the sympathetic nervous system triggers the release of cathecholamines (e.g., adrenaline) via direct stimulation of the sympatho-adrenal medullary system (SAM) by the central nervous system (Fig 1; Blache et al., 2011). The acute stress response, comprising reactions from both the HPA and SAM systems, rapidly makes energy available to the animal by breaking down muscle protein into glucose (gluconeogenesis) (Moberg, 2000). Therefore, during this stage, a steady state is achieved in which the increased demand for energy is met by increased metabolic performance. This process is critical to survival and is commonly described as the 'fight or flight' response (Cannon, 1914). Upon removal of the stressor, the physiological state of stress disappears and the animal is generally left experiencing no ill effects, other than a depletion of energy reserves. If the stressor continues, however, the response proceeds to what is described as the chronic stress response which is characterised by continued activation of the HPA axis (Matteri et al., 2000). While in the acute phase the effects of cortisol are potentially beneficial, chronic HPA activation has been associated with decreased metabolic efficiency (Elsasser et al., 2000), impaired immunity (Blecha, 2000) and reduced reproductive performance (Breen and Karsch, 2006). The severity of these costs will depend on how long the animal is required to divert physiological resources to maintain homeostasis.

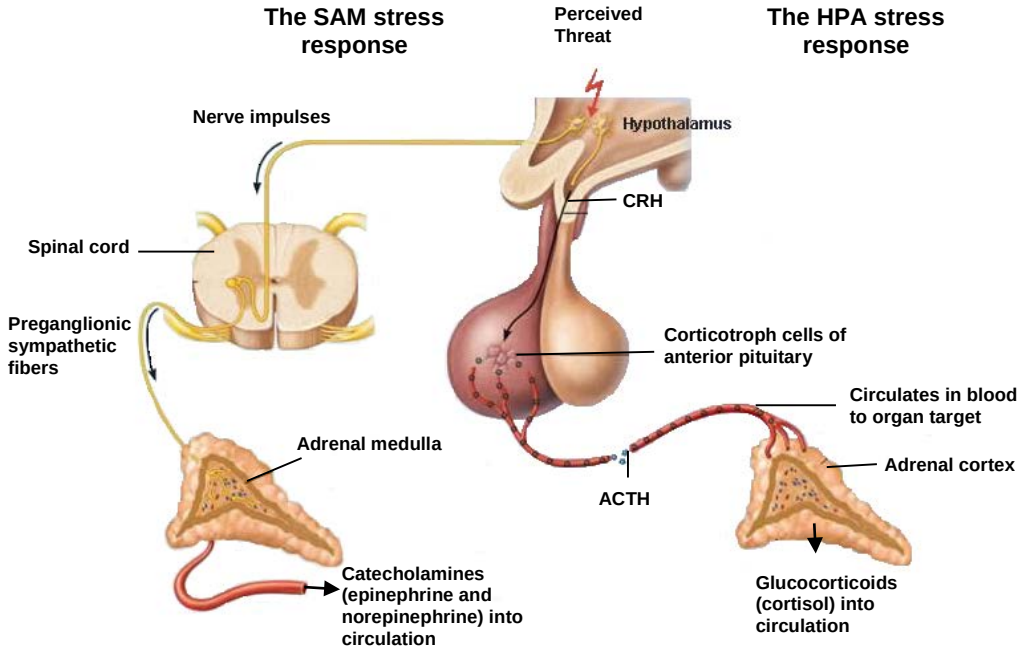

Fig 1 Mechanisms of the physiological stress response (Adapted from Marieb and Hoehn, 2010).

The effect of chronic stress on immune system

The immune system defends the body against invading pathogens, removes old cells and damaged tissue and destroys abnormal or mutant cells. The spleen, thymus and bone marrow are all integral in the production and maturation of immune cells and are all innervated by the autonomic nervous system (ANS) (McEwen et al., 1997). Upon activation of the acute stress response, catecholamines are released from the nerve endings of the ANS directly into the immune tissues. This activation has the potential to affect lymphocytes in these organs as well as compromise their responsiveness (McEwen et al., 1997). Corticosteroids, however, are the most prominent hormonal regulators of immune function (McEwen, 2000). While moderate acute stress can temporarily increase the function of the immune system, chronic activation of the HPA axis suppresses it (McEwen et al., 1997; McEwen, 2000). Physical and psychological stress has been associated with decreased immunocompetance and increased susceptibility to disease (Cockram and Hughes, 2011). Variations in white blood cell populations and reduced lymphocyte activity may lead to a diminished immunological reaction against disease outbreaks, an increase in the risk of infection from injuries and a shift in the cytokine balance resulting in immune dysfunction. Such changes increase susceptibility to inflammation, infection, fever, and hypersomnia and depress social behaviour (Cockram and Hughes, 2011).

The effect of chronic stress on reproduction

The gonadotrophic axis controls reproduction in animals and consists of the hypothalamus, the anterior pituitary gland and the gonads. In females, the hypothalamus produces gonadotropin-releasing hormone (GnRH) which regulates the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary (Matteri et al., 2000). FSH supports maturation of the ovarian follicles and stimulates oestrogen production and a LH surge acts on the ovaries to induce ovulation (Matteri et al., 2000). Although the placenta contains enzymes which are able to inactivate glucocorticoids, particularly high levels of stress hormones may overwhelm the capacity of the enzymatic conversion and prevent buffering of the developing embryo (see Curley and Branchi, 2013). While the exact mechanisms remain unclear, elevated peripheral ACTH and cortisol may impact the hypothalamus, reducing GnRH secretion, and the pituitary gland, effecting LH and FSH secretion (Matteri et al., 2000; Blache et al., 2011). Stress around the period of attachment of embryos to the uterine wall can be particularly detrimental to reproduction (Moberg, 1985).

The effect of chronic stress on animal behaviour

Behavioural responses are key for survival as they enable the animal to locate food, water, and shelter, and to avoid injury, illness, predators and parasites. Furthermore, for many stressors the first and most effective response is a behavioural one, such as physically removing oneself from the presence of the stressor (Moberg, 2000). In captive animals the ability to perform highly motivated natural behaviours can be compromised, or the intended purpose of the behaviour can be un-achievable (Olsson et al., 2011). This results in some animals developing irregular behaviours or redirecting the appropriate behaviour toward inappropriate objects or pen mates (Olsson et al., 2011). Such behaviours are described as being ‘abnormal’ and encompass displacement (normal behaviour performed in an inappropriate situation), re-directed (normal behaviour directed towards an inappropriate target) and stereotypic (repetitive behaviour with no obvious goal or function) behaviour. The development of stereotypies has been associated with a poor physical or social environment (Barnett et al., 2001), boredom (Wemelsfelder, 1990), restraint (Cronin, 1985), frustration (Ödberg, 1978), inadequate nutrition (Barnett et al., 2001) and heightened arousal (Dantzer, 1986), and their presence are generally accepted to be indicative of poor welfare.

Case study 1: Enriched environment for laboratory rodents

The housing of laboratory rodents is based on economics (minimal use of space and resources), ergonomics (ease of handling and visibility of animals), hygiene, and standardization (reduction of variation) (Bailoo et al., 2018). This results in a barren environment that lacks sensory and motor stimulation and restricts the expression of species-typical behaviour (Bailoo et al., 2018). Despite concerns that more complex environmental conditions might increase variation in experimental results, recent research on laboratory rodents suggests that enrichment strengthens the validity and therefore reproducibility of experimental results (Würbel 2007; Bailoo et al., 2014, 2018). For instance, mice and other rodents housed in barren laboratory cages (e.g., see Fig 2A) show impaired brain development, develop stereotypies and exhibit an anxiogenic behavioural profile compared to mice from more enriched environments (e.g., Fig. 2B) (Würbel 2007; Bailoo et al., 2018).

Figure 2. Mice housed in a conventional barren environment with sawdust, food and water (2A) or enriched semi- environment with sawdust, food, water, shelter and rotating enrichment items (2B). Photos from Würbel (2007).

Case study 2: Early life social environment on behaviour development of pigs

Direct experience from social interaction is likely the major means by which immature animals acquire social skills (see Taborsky, 2016). A significant body of research on mice reared in communal nests demonstrates the positive effects of housing that resembles the natural social environment on early development for a range of social behaviours through to adulthood, via profound effects on the neurobehavioural profile (reviewed by Curley and Champagne, 2016). Like communal nests, group lactation housing of sows and their litters (Fig 3, left) offers a multi-

faceted enriched rearing environment to piglets by providing greater opportunities for a wider range of interactions with peers, their dam and other sows, as well as increased space, substrate and larger group sizes. Research has shown the social behaviour is improved in piglets from group lactation compared to those from conventional farrowing crates (e.g., Fig 3, right; Verdon et al., 2016, 2019a,b), as is piglet adaptation to the post-weaning environment (van Nieuwamerongen et al., 2015).

Figure 3. Group lactation housing of sows and litters (left, sourced from R. Morrison, Rivalea Australia) and standard commercial farrowing crates housing (right, sourced from www.stuff.co.nz).

Conclusion

The use of animals for research is a privilege given on the provision that new knowledge will be generated without causing unnecessary harm to animals. It is the ethical responsibility of researchers to ensure that the health of animals is optimal while in their care and that the animals needs are met. Researchers should also strive to keep research animals in a high state of welfare to protect the validity and replicability of experimental findings. Thus, the scientific justification of animal use in experimentational should encompass critical thinking not only of how experimental animals will be housed and managed, but why. Barren or un-naturalistic environments should be avoided unless specifically required by the experimental design.

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Rehoming Experimental Animals in New Zealand

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Many animals used in research are killed at the end of the study with which they are involved. Whilst this is sometimes a requirement of the experimental procedure, it can often be a routine means of disposal of animals. In 2016 more than 250,000 animals were used in Research Testing and Teaching in New Zealand and 25% of these died during or were killed at the end of the studies (Anon 2018).

In July 2018, The Ministry for Primary Industries announced its support for the rehoming of ex-laboratory animals. Following this, the New Zealand Antivivisection Society (NZAVS) and Helping You Help Animals (HUHA) offered to be points of contact for organisations and researchers with animals suitable for rehoming. Massey University contacted NZAVS and HUHA about 50 mice that were being used for teaching ecology students. These mice became the first group of animals to be rehomed.

Enquiry into HUHA's rehoming strategies was undertaken during initial dialogue with the rehoming organisatons. Prior to rehoming, a simple health check (body condition, coat condition, condition of tail and feet, presence/absence of porphyrin, mouse posture and behaviour) was undertaken on each animal. Response to the presence of an unfamiliar hand in the cage and handling were also evaluated to ensure they were likely to successfully be rehomed as pets. The animals' demographics, previous use, husbandry practices, health and behavioural assessment findings and basic instructions for rehoming and housing were provided. Opportunity was provided for Massey personnel to help prepare an agreement outlining minimum standards of care, to be signed by the new owners and HUHA personnel. The mice were transferred to the care of HUHA and the NZAVS and a document signed to relinquish responsibility for any future health care.

New owners were screened and approved and mice transferred to their care. The rehoming process attracted significant media attention, but the vast majority of comments on social media portrayed both the NZAVS and Massey University in a positive light. This collaboration clearly demonstrates that rehoming of many research animals is possible and has benefits for both the animals and the organisations associated with their rehoming.

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No manuscript was submitted for this presentation

Animal Care in Research: An Opportunity to Improve Public Perceptions

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Abstract

In research, veterinarians and animal husbandry staff have a legal obligation to provide best practice animal care. Consistently performing to that standard and showcasing this in the public domain is an opportunity to break down laboratory walls. There are many scenarios in which research animals receive better care on an individual basis than animals on farm. These situations include the use of modern multimodal anaesthetic protocols, evidence based surgical skills, effective and appropriate analgesia or enrichment of animal enclosures/paddocks. Regardless of the 'use' of the animal (for research or the dinner table) individual animals deserve the best care possible and research facilities can lead the way in this. Examples of standard farm practices used in research include artificial insemination, prevention of horn growth (disbudding), environmental design and individual disease treatment/management. The sharing of information in an ideal world should always be evidence based, but a lot of information shared between veterinarians is experience-based, as not all animals follow the textbook and not all species/diseases are fully researched. Despite the lack of scientific rigour, new knowledge can be obtained from these case-studies which can and should be distributed within the veterinary and research community. An example of such a case study which I will present involves a research goat named Jamie, who developed a painful condition in both front feet. A novel approach was used to treat the condition, where on farm she would have likely been culled. This demonstrates the level of care that animals in research benefit from and is a worthy message to go beyond the wall. There may also be aspects to this novel approach, or findings from it, that could trickle down and help inform treatment of hoof problems on farm.

In research, veterinarians and animal husbandry staff have a legal obligation to provide best practice animal care. Consistently performing to that standard and showcasing this in the public domain is an opportunity to break down the laboratory wall. Public perceptions of animals in research over time tend to lean on the negative with minimal understanding of the animal ethics process. Showcasing that animal technicians, research vets and scientists care about the animals they work with whether it is via case studies or just stories, may go a long way in helping to change this perception.

Lameness on dairy goat farms is a widespread and significant welfare issue and no information is currently available on the prevalence of lameness on NZ or Australian goat farms. Data from the UK in 2015 showed that total lameness ranged from 12%-67%! Ulcerative foot granulomas in goats have very little information on their cause, treatment and prevention. Reports from the UK suggest the bacterial genus *Treponemes m*ay be associated with these claw lesions but could not conclude if it was a primary or secondary cause.

A case that was presented at conference in Hobart involved a behaviour research goat named Jamie, who developed ulcerative granulomas on both front feet. A novel approach was used to treat the condition, where on farm she would have likely been culled. The slides below are a summary of this case.

Photo 1. A fleshy lesion as present on two claws, one on each front foot. Not easy to see on these photographs. Jamie was lame on her right forelimb and grazed on her knees.

Photo 2. Radiographs were taken to ensure there wasn't any underlying disease such as laminitis, osteomyelitis or joint infection. This would greatly lower the chance of recovery. None of these conditions were seen on the radiographs.

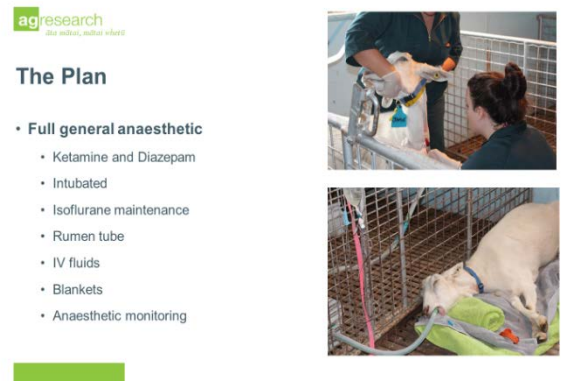


Photo 3. The plan to treat the granuloma involved a general anaesthetic as it was unknown how long this procedure would take, and to ensure no pain was felt during the procedure. Dose rate for induction Ketamine (100mg/ml) 5mg/kg and Diazepam (5mg/ml) 0.25mg/kg.

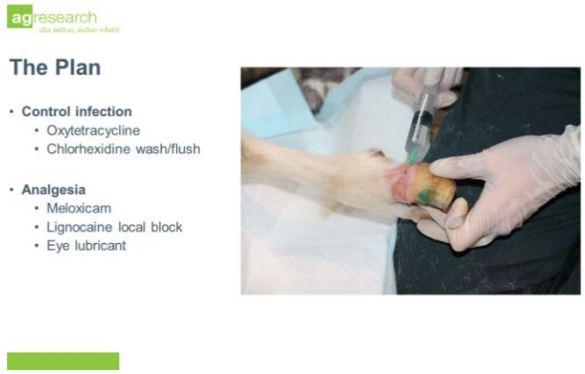


Photo 4. Antibiotics and pain relief were administered. A local nerve block was applied to the claw.

The granuloma was removed with a scalpel. A tourniquet was placed to reduce the amount of bleeding as these lesions are highly vascular.

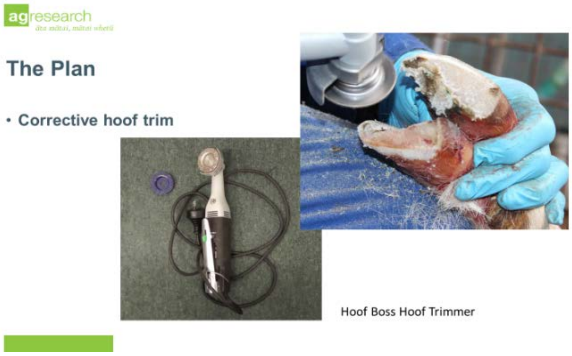


Photo 5. Hoof boss hoof trimmers were bought from the USA to help remove the overgrown wall, sole and heel. It enabled trimming of the difficult to reach areas with minimal risk of damaging the underlying sensitive tissue.

https://mybosstools.com/applications/goat-hoof-trimming/

- Copper sulphate was placed over the area and a bandage placed.
- Bacterial culture of the lesion was taken to ensure correct antibiotic use.
- Histology of the lesion was undertaken by a pathologist at NZVP, notes listed below:
  - Severe ulcerative pododermatitis with exuberant granulation tissue formation
  - Cause not evident
  - Co-infection with *Treponemes* is suspected (PCR not available in NZ)

Photo 6. Pain relief was continued for a week and antibiotics were continued for two weeks. Jamie was kept separate from the herd with a companion in a dry paddock.

Unfortunately, these lesions grew back so a new approach was undertaken to remove them under sedation.

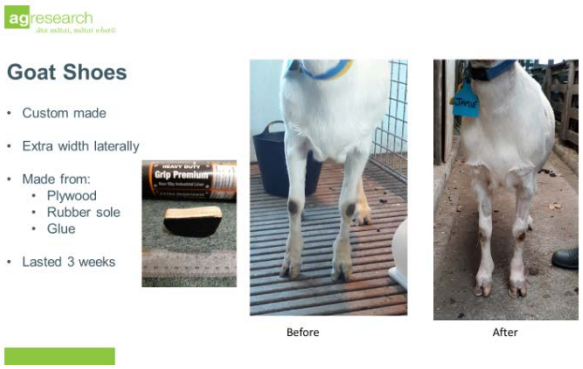
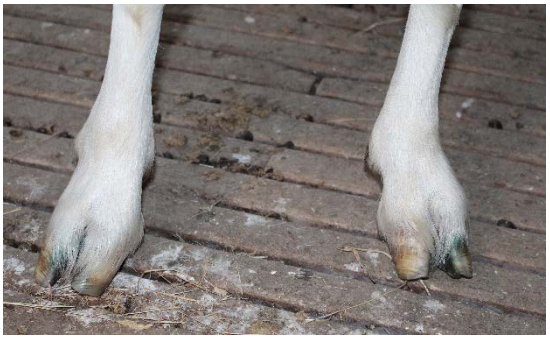
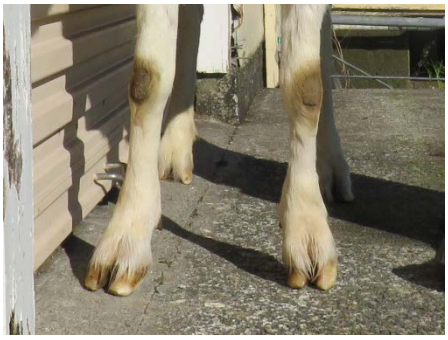


Photo 7. Custom made goat shoes were also placed to prevent weight bearing on the affected claw. This allowed Jamie to stand a lot straighter and walk much more comfortably. These lasted 3 weeks and came off on their own in the paddock.



BEFORE



4 MONTHS LATER

Before and after photos show the improvement corrective trimming has made on the overgrown and thickened hooves and the angles the foot weight bears on. Corrective trimming is a slow process as can only remove a limited amount of hoof wall/sole every 4-6 weeks.

After the 2nd procedure Jamie was sound on all four limbs for 3 months. During winter the lesion on the right foot returned, possibly due to the exposure to wet conditions out in the paddock. Another method of granuloma removal is being attempted, based on further research of other species successes in treating similar lesions. The fact that the lesions returned does not take away the significant effort and time people have put into caring for Jamie. Many hours, working around a small but workable budget has given Jamie a chance to recover and live a long life, where elsewhere this likely would not have happened. This case also gave us a chance to investigate how to treat these lesions more effectively than just with cautery which is what currently occurs on NZ farms. Jamie's quality of life will always be the focus, so if she continues to live a good life, treatment will continue. Since we discovered that this lesion is difficult to treat, it gave us an insight that perhaps the current methods used on farm may be insufficient. Research in this area would be the next step as more information about this condition is required urgently.

This case demonstrates the level of care that animals in research can benefit from and is a worthy message to go beyond the laboratory wall. Research institutions could showcase their animal care and husbandry to the public with the aim of improving their perceptions of animals in research. The next step for AgResearch will involve building a playground for the goats to provide enrichment but also a dry and abrasive surface to help natural wear of their claws. The plan is to also build a new foot trimming station which allows the goats to be fed treats on a raised platform while trimming takes place. The public could be informed of these new builds and it could be made educational regarding goat health, welfare and management. I believe introducing the public to our behaviour goats via the new playground build is a great way to showcase our animal care and will be a good start in breaking down the laboratory wall.

Further information: *Severe Foot Lesions in Dairy Goats Associated with Digital Dermatitis Treponemes. H.E. Crosby-Durrani et al. 2016*

Presentations given

on

Wednesday 24th July

The Development of an Online Animal Welfare and Ethics Course

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Abstract

Background The various animal ethics committees (AECs) in South Australia have almost completely achieved recognition of reciprocity of the ethics review process of proposals for animal use. This means that animal users who are appointed across institutions no longer have to submit proposals for approval to multiple committees. With this more collaborative review system in place, the need for a consistent approach to animal user training in welfare and ethics was seen. A face-to-face training day has traditionally been held across institutions for new users annually but students and staff, who may start at any time of year, require timely training necessitating a more accessible online course. To this end, a course has been developed to replace that originally housed exclusively on the University of Adelaide web site for Uni Adelaide students and staff only.

Course Content The course currently has 15 units; some core and others elective to target more specific areas of animal use e.g. livestock or laboratory animals. The six core areas cover the Code, the legislative framework, applying to an AEC, pain assessment, refinement techniques, and zoonoses. The nine other units are more species, or method, specific. These include Are your animals well?, Aseptic technique, Breeding Colony Considerations, Post mortem examination method, Experimental surgery, Animals in teaching, Livestock in teaching, and the Use of Simulators in teaching. Variability of user needs is thus accommodated by the ability to choose relevant materials from a broader offering.

Assessment Tools Each short unit is presented as a set of slides with an audio track such that the user can progress at their own pace. Each presentation will take about 30 minutes to complete. At the end of the subject matter, there is a short quiz with answers revealed as the student attempts various choices. These are formative to allow the user to assess their level of understanding of the material before progressing to the next topic. At the end of the course a summative quiz is available for those needing proof that they have completed the course for their institution. A certificate of completion can be generated and emailed to the participant as evidence for their AEC that they have successfully completed this training. The details of the core and noncore components in the quiz are currently being sorted.

Accessibility Considerations The original course was accessible only via the University of Adelaide log-in and so-called "a" number, thus limiting access to registered students. This was hosted by the University's learning management system "Canvas". The aim of the new course is to make it accessible without a security log-in and thus much more widely accessible to members of committees and students not associated with the University of Adelaide, although it will still be hosted by Uni Adelaide web server. The University of Adelaide has enabled this by access to the Canvas Catalogue system where the Uni also hosts its MOOCs. Canvas catalogue can accommodate free offerings or offer access on a user pays basis. The aim is to make this a free offering. This effectively means that this course might be available to AEC members or animal users from all over Australia.

Potential Usefulness The aim is really to allow *pro bono* access to this course material via the University of Adelaide for any AEC, teachers and trainees as a means of enhancing training for animal user groups or those currently without access to a similar resource. The presentation will hopefully offer more detail of the content (already developed) and the means of accessing it (a work in progress for the test component).

Introduction:

The various animal ethics committees (AECs) in South Australia have almost completely achieved recognition of reciprocity of the review process of proposals for animal use. This means that animal users who are appointed across institutions no longer have to submit proposals for approval to multiple AE committees. With this more collaborative review system in place, the need for a consistent approach to animal user training in welfare and ethics was seen. A face-to-face training day has been held collaboratively for new users annually for some time but new students and staff may start at any time of year and require timely training necessitating an online course option. To this end, a course has been developed to replace that originally housed exclusively on the University of Adelaide web site for University of Adelaide students and staff only.

Course Structure:

The course has 18 units; some Core and others Elective addressing more specific areas of animal use e.g.:
Laboratory animals,
Teaching with animals,
Wildlife or
Surgery of experimental animals.

The core unit includes six talks entitled:
The Legislation and Regulations relating to Animal Use,
Replacement, Reduction and Refinement,
Applying to an Animal Ethics Committee,
Unexpected Adverse Events,
Training Models and Simulators in Teaching,
Working with Animals and Zoonoses.

These talks are in the form of a Power point slide show with audio recorded for each slide. The sections also include web links to helpful materials as background for each area. These materials are from many sources including NHMRC and the University of Newcastle N3CRs materials to underpin the messages in the talks.

Each talk concludes with several formative multiple-choice questions to prepare the student for the summative test at the end of the core section of the course.

Assessment of Core Content:

After the core section, there is a compulsory assessment task consisting of 40 multiple-choice questions. If the candidate successfully finishes this test, gaining a score of 80 percent or higher, then a certificate of completion is emailed to the candidate. This certificate of completion has the ANZCCART logo on it and as such can be used as proof of training for any institution. This course is open to all who need it, be they animal users or members of the Animal Ethics Committees.

Elective topics:

Elective topic one relates to Animals and Teaching and includes talks on:
The Use of Animals In Teaching,
Livestock Teaching and
The Role of the Animal Welfare Officer.

Elective topic two relates to Laboratory Animal Use.
The Laboratory animal section includes talks entitled:
Are my animals well?
Breeding Colony Considerations, and
How to do a Post-mortem examination.

Elective topic three includes material relevant to surgery and research animals.
Talks include:
Experimental Surgery,
Aseptic Technique and
Pain Assessment and Management.

Elective topic four has material on Wildlife research and has a talk on this topic as well as the zoonosis talk from the core section.

Similarly, there are supportive web links included in these elective sections.

Accessibility Considerations:
The aim of the new course is to make it accessible without a security log-in and thus much more widely accessible to members of committees and students not associated with the University of Adelaide, although it will still be hosted by Uni Adelaide web server. The University of Adelaide has enabled this by access to the Canvas Catalogue system where the University also hosts its MOOCs. Canvas catalogue can accommodate free offerings or offer access on a user pays basis.

To access the course: Go to the University of Adelaide web site research services page, then animal ethics, then training and you will find the course there. Or paste the link into your web browser AWEI 2019 - https://myuni.adelaide.edu.au/courses/45316 or just click on it!

Potential Usefulness:
The is a free offering. This effectively means that this course is available to AEC members or animal users from all over Australia. This course material can be used by any AEC member, teachers and trainees as a means of enhancing training for animal user groups or those currently without access to a similar resource.

Pain Perception in Fish

Craig Johnson
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Abstract

Fish form a major vertebrate subgroup that accounts for more than half of the known vertebrate species. They are characterised by the huge diversity and range of physiological complexity seen in its members. Many (but by no means all) jurisdictions have animal welfare legislation that considers fish to be within the group of animals that possess the ability to suffer and whose welfare must be protected. This inclusion implies that fish are sentient (some legislation is explicit about this) and have the ability to perceive pain. Whilst the majority of the scientific community would agree, this view of fish is not universal and is subject to ongoing debate.

This presentation will explore the features of responses to noxious stimuli that are thought to characterise pain perception rather than be indicative of nociception only. For each feature examples from animals commonly thought to possess that feature will be compared to fish and also to other cases where the concept of pain may be even more challenging.

If it is accepted that pain perception exists in fish, then analgesia becomes important. The presentation will conclude by highlighting some of the important pysiological and pharmacological challenges relating to fish that must be considered when providing pain relief for these animals.

The Definition of an Animal

Animals are described as multicellular eukaryotes of the kingdom metazoa (1). This definition is obviously not helpful for considerations of animal welfare and other definitions more suited to this purpose appear in the welfare literature and particularly in animal welfare legislation. Such ‘welfare definitions’ most often appear as lists of organisms that are included in the category of ‘animal’. They are usually based on an amalgam of political expediencey and the concept of animal suffering that originated with Bentham and the early utilitarians (2). Fish are variously included as animals by some animal welfare acts but excluded by others and the question of whether a fish has the capacity to suffer has an important bearing on the way that legislative definitions of animals develop in the future.

Suffering can take a number of forms, but the suffering caused by pain is best understood in terms of the underlying neuroscience. For this reason, the question of suffering often evolves into a consideration of a particular animal’s ability to perceive pain. This is why the question ‘can fish perceive pain?’ has such an important bearing on our understanding of the ethical implications of interacting with fish in production, recreational and research settings.

Pain and Nociception

The pain that follows physical stimulation by a suitable stimulus, known as a noxious stimulus (3), is the result of two separate processes. It is important to differentiate between these when considering an animal's ability to perceive pain. The first process is nociception. This is the neural encoding of the noxious stimulus together with automatic or reflex behavioural responses such as increases in blood pressure or withdrawal reflexes (4). The second process is the perceptual process by which the animal becomes consciously aware of the pain. It is important to note that the presence of nociception does not imply pain and occurs in animals that are not capable of perceiving pain as well as in those that are.

Features of Pain and Nociception

Elwood (5) noted that when an animal is subjected to a noxious stimulus, it displays a number of reponses. Some of these imply only nociception, whilst others are sufficiently complex that their presence requires conscious awareness and so implies the ability of the animal to perceive pain. Of the following features related to nociception, only those in bold imply the ability of the animal to perceive pain:

Suitable Receptors
Suitable Nervous System
Response to Analgesics Especially Opioids
Physiological Changes
Avoidance Learning
Protective Motor Reactions
Trade-off with Other Activities
Motivational Changes

Evidence of these features in a particular animal would constitute evidence for that animal's ability to perceive pain.

The remainder of this article will briefly introduce research papers which identify the presence of these features in fish.

Suitable Nervous System

Pain is an emotional and sensory experience (6). In order to perceive pain, an animal must have a nervous system that is sufficiently complex to allow it to experience emotions. It is argued by some that pain cannot be perceived by animals that do not possess cerebral cortices (structures unique to mammals) because the cerebral cortex is the location of pain perception and emotion in humans and other mammals. A more common view is that, in order to be able to perceive pain, an animal does not need to possess a cerebral cortex, but only an area of the brain that is functionally analagous to the cerebral cortex of a mammal. In a similar way, cephalopod molluscs can form a visual representation of their environment even though they have no cerebral cortex because they have optic lobes which are functionally analagous to the optic cortex of a mammal.

Response to Analgesics

Lopez-Luna *et al*.. 'Reduction in activity by noxious chemical stimulation is ameliorated by immersion in analgesic drugs in zebrafish' (7). This study investigated the ability of a number of analgesic agents (aspirin, flunixin, lidocaine and morphine) to ameliorate behavioural responses to two noxious chemicals (acetic acid and citric acid) in larval zebrafish. They concluded that the analgesics were able to reduce changes in behaviour caused by noxious stimuli. This amelioration implies that the changes were due to the perception of pain and this in turn implies that fish are capable of pain perception.

Avoidance Learning

Ephrensing and Michell. 'Similar antagonism of morphine analgesia by MIF-1 and naloxone in Carassius auratus' (8). Teleost fish are capable of learning to avoid electric shocks (9). This study demonstrates that they lose this ability if given analgesia prior to the presentation of the shocks. This demonstrates that pain is necessary for the learning to take place (10) and implies that the fish are capable of pain perception.

Trade off with Other Activities

Millsopp and Laming. 'Trade-offs between feeding and shock avoidance in goldfish (Carassius auratus)' (11). Two groups of goldfish were trained to receive food in a particular area of their tank. Once training was complete, the fish were exposed to an electric shock to the flank, delivered in the same area of the tank. In one group the intensity of the shock was varied and in the other, a variable duration of food deprivation was applied prior to the delivery of the shock. The number of feeding attempts and the time spent in the feeding area both decreased after the shock, but decreased more with increased shock intensity and less with increased food deprivation. These data demonstrate that goldfish can balance their need for food against avoidance of an acute noxious stimulus. The stimulus is perceived as unpleasant and the fish make a cognitive decision to avoid it rather than demonstrating a nociceptive withdrawal response. Such a cognitive response implies that the fish are capable of perceiving pain.

Motivational Changes

Alves, Barbarosa Jr and Hoffmann. 'Anti-nociception in piauçu fish induced by exposure to the conspecific alarm substance' (12). Antipredator behaviour can be elicited in ostariophysan fish using a pheromone known as chemical alarm substance. In this study, fish responded to a noxious stimulus in the form of a subcutaneous injection of formalin by increased swimming activity. The increase in swimming was reduced in the presence of chemical alarm substance. This demonstrates that fish are able to modify their behavioural response to a noxious stimulus in the presence of another stimulus with a higher motivational priority. This motivational change is characteristic of pain perception and implies that the fish are able to perceive pain.

Conclusions

The ability of fish to perceive pain is still controversial, though an acceptance of pain perception in fish is rapidly becomming orthodox. This paper discusses the features of responses to noxious stimulation that are requirements for pain perception and goes on to present evidence that each of them occur in fish. The commentary on each study is necessarily simplified due to the requirements for brevity of this format. It is hoped that the material presented will enable interested readers to engage with the articles discussed and develop a more informed view of the possibility that fish can perceive pain.

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Using complimentary imagery and targeted sampling to address 'Reduction, Replacement and Refinement' of scientific animal use: Two examples in a marine context.

Mark Green, Franziska Althaus, Alan Williams and Candice Untiedt,

CSIRO Oceans and Atmosphere.

Abstract

The marine environment presents many challenges for biologists wishing to investigate fauna whilst observing animal welfare, particularly in the remote deep sea. Historically, faunal sampling relied heavily on extractive collecting methods using a variety of nets to bring the fauna to the surface and be killed prior to study. Now, however, photographic methods, supported by other enabling technology to geolocate and target sampling, offer credible non-extractive alternatives. But despite these alternatives, some extractive sampling of marine fauna is still necessary to obtain taxonomic, genetic and biodiversity information, and for broader biogeographical applications. Here we present two case studies that illustrate this balance of needs and show how imagery and targeted sampling both represent effective implementation of the 3 R's for animal welfare: 'Reduction' (numbers of animals used), 'Replacement' (alternatives to using animals), and 'Refinement' (reduced impacts on animals). The first is a deep-sea study that examines deep-sea coral community distribution and recovery from fishing impacts on the Tasmanian Seamounts; the second study is a population assessment of the EPBC-listed spotted handfish in coastal embayments. We provide examples of Reduction, Replacement and Refinement by showing how video/still imagery and other observational techniques have a strong and increasing potential to complement, and in some cases, replace extractive sampling. We also identify why extractive sampling is still important – but show how contemporary net-sampling can be highly targeted using detailed bathymetric mapping, GPS and equipment locating beacons to carefully select and limit extractive tows over specific and small areas of interest. The deep-sea study also draws attention to problems with estimating and reporting the numbers of animals 'used'. Primarily these are: (1) pre-survey estimates are a best guess only; (2) the animals observed (relatively many) and killed (relatively few) clearly have quite different welfare outcomes; and (3) complete counts of observations may not be available from post-survey analysis. These issues bring both a risk of inaccurate and negative perception – for the AEC committee, government compliance regulators and the general public – of marine animal use, even when the three R's are being effectively implemented.

Introduction

Terrestrial environments are typically easily accessible to researchers to conduct complex ecological studies. In contrast, specialised and remote study methods need to be employed in airless marine environments that are increasingly dark and cold with depth, and which are relatively difficult places to conduct ecosystem investigations.

Historically, the only option for marine research investigations of fauna was extraction/collection, using capture methods such as hooks, nets and dredges to remove whatever was there for examination on the surface as an aggregated catch. The topography of the seafloor was typically unknown and, even into the 1970's, depth sounder technology produced very simple representations of sea floor structure. The resulting biological data from collected material was very broad scale, 'clumped' and therefore not appropriate to investigate detailed spatial, abundance or ecological relationships.

For those undertaking marine conservation projects, applying the 3R's (Reduction, Replacement and Refinement as defined by the *Australian code for the care and use of animals for scientific purposes*, referred to as "the Code" in this paper) to marine studies is a natural progression as animal/habitat conservation, animal welfare (AEC) and sampling efficiency (resource) considerations all overlap. In other words, the principle of the 3R's are complimentary to both the need for scientific/professional integrity (defensible data/analysis/results and cost-effective operations) and conservation outcomes.

The marine realm is divided into five main benthic (sea floor) zones, namely- coastal, continental shelf, continental slope, continental rise and abyssal plain (Figure 1). Seamount features rise 100's of metres from the seafloor of both the continental slope (~140–4000 m) and continental rise (~4000–5000 m) zones. Features like canyons on the continental slope and shallower seamounts form localised areas of high biological diversity and abundance that that are attractive (cost-effective) harvest zones for commercial fishing (Figure 2).

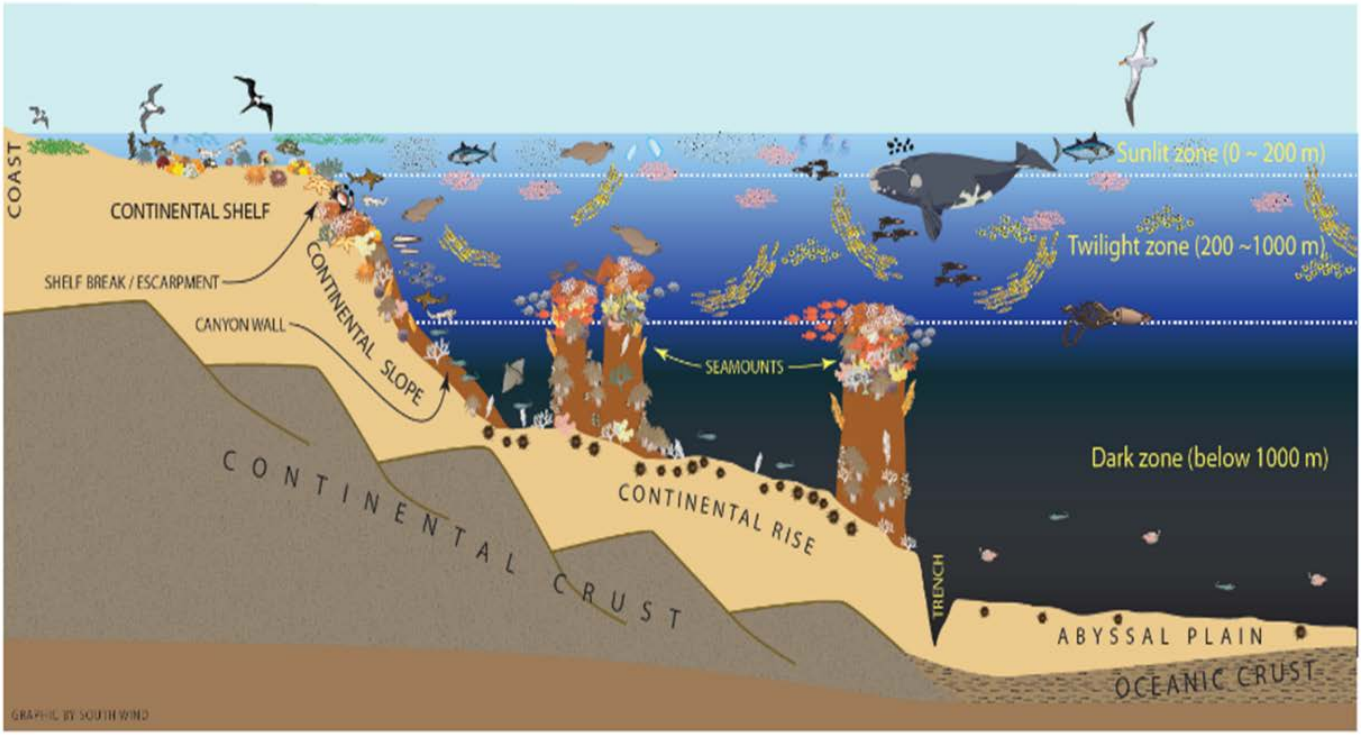

Figure 1. The marine realm. Graphic credit to Peter Boyer – Southwind <http://southwind.com.au/>

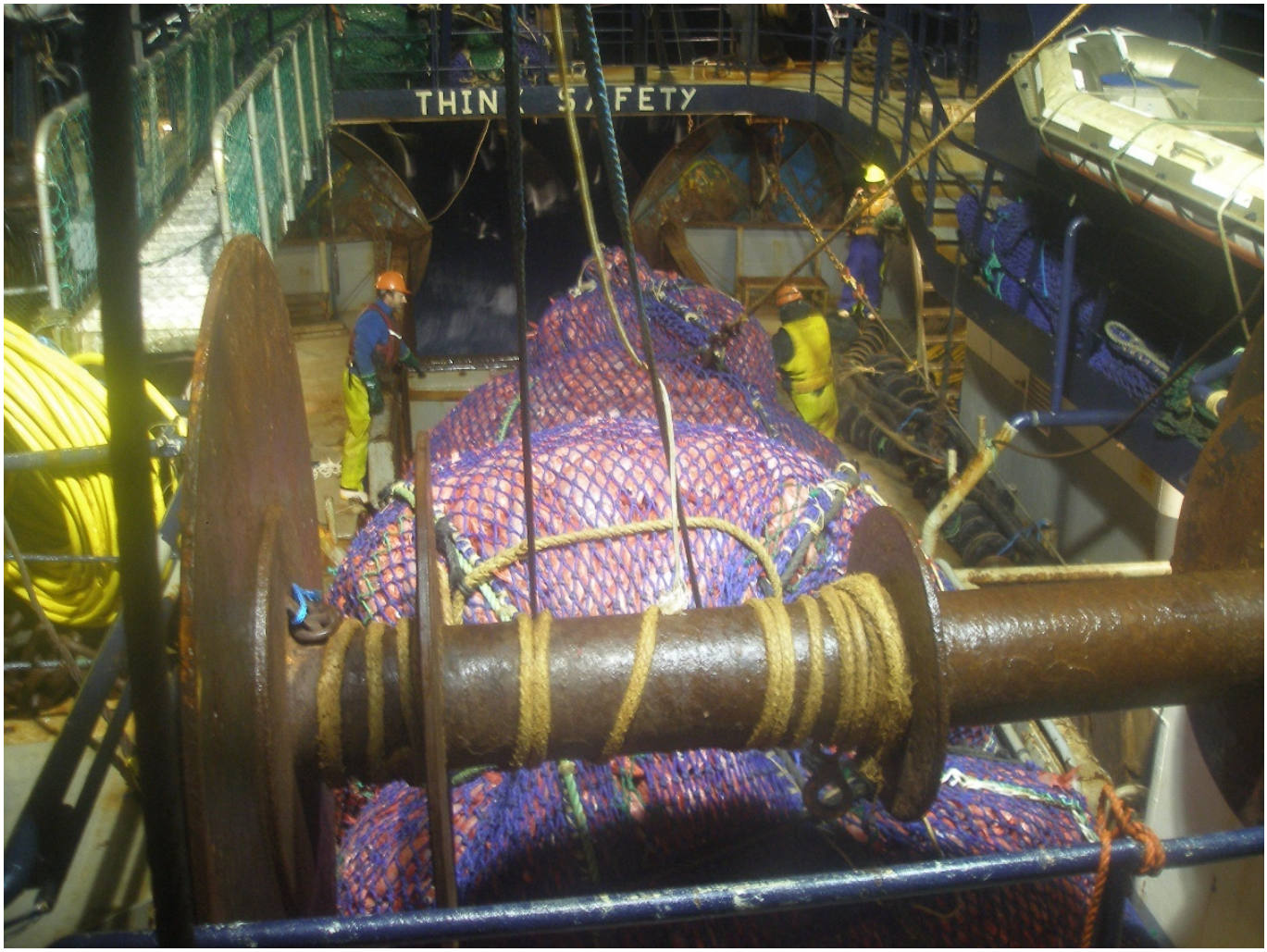

Figure 2. Orange roughy in trawl net of commercial fishing vessel.

The Australian, state and territory governments have established 58 Australian Marine Parks (AMPs) around the country covering 3.3 million square kilometres to protect some of these areas and conserve a portion of Australia's natural marine resources (Parks Australia, 2019) (Figure 3).

Shallow coastal marine environments can be studied directly by scientists using SCUBA equipment (Figure 4), but these studies are limited to depths and times within the physiological capability of humans when exposed to breathing air under pressure (see Daltons, Boyles and Henry's Laws of gas physics). Each ten metres of water depth is one additional atmosphere of pressure and even dives down to just 30 metres can only be several minutes long unless special gas mixtures are used. Clearly, conducting research at 100's or 1000's of metres under the surface is not directly possible unless the humans are protected within expensive submarines. The high cost of using manned submarines, their operational requirements and the speed at which they can do survey work means they are not an efficient option for wide ranging ecosystem surveys. Similarly, deep water capable Remote Operated Vehicles (ROV's) are cost prohibitive for most biological survey work.

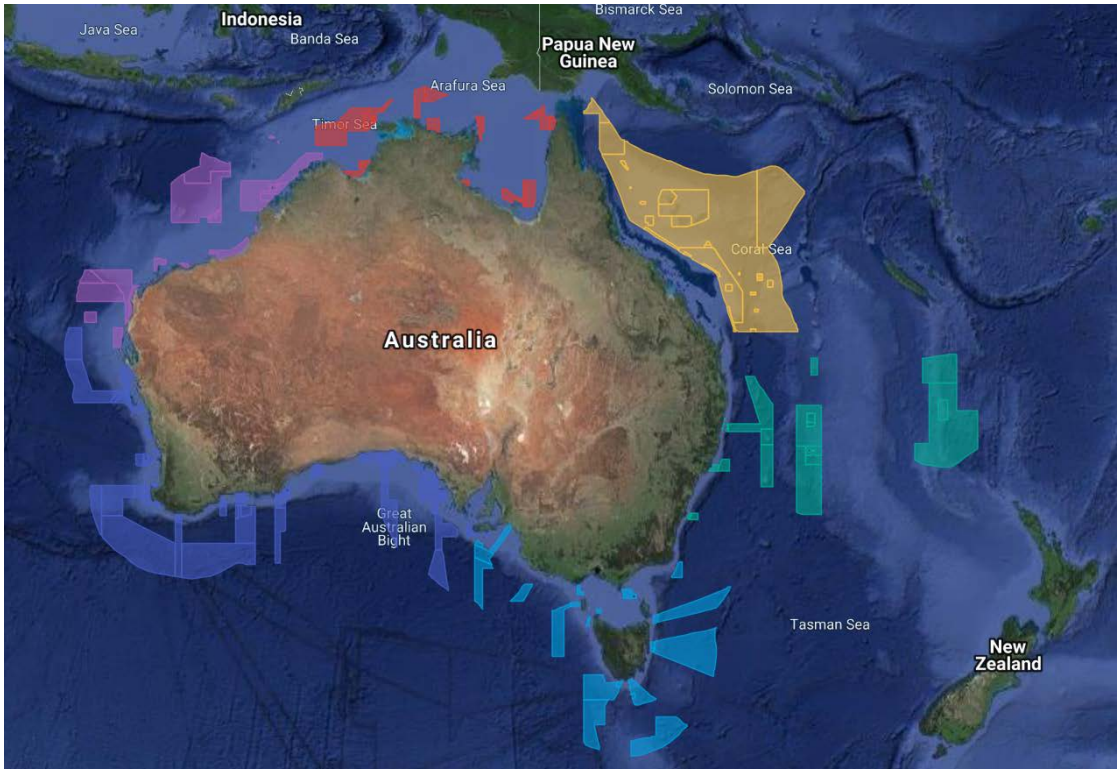

Figure 3. Australia's Marine Parks. The different colours represent the six MPA zones; see https://parksaustralia.gov.au/marine/parks/ for more details.

Figure 4. SCUBA equipment enables low impact, direct observations possible for marine scientists in relatively shallow coastal depths. This image shows a diver measuring a spotted handfish in the Derwent Estuary (Tasmania). Note the stalked ascidians near the divers' hand, which are used by handfish for nesting sites. Both the fish and the critical habitat would be destroyed by dredge sampling.

This paper presents two case studies from contrasting marine environments that demonstrate the application of modern technology to address the desire to ***Reduce*** the number of animals used, ***Replace*** animals used, and ***Refine*** methods to reduce animal impacts during marine ecological surveys.

Case study 1: Benthic community distribution and recovery from fishing impacts of the Tasmanian Seamounts using cameras and targeted sampling.

Species and habitat distribution models are being increasingly used to predict the distributions of potentially vulnerable or high value marine assets such as deep-sea coral. Models are necessary because there are typically insufficient samples to cover the geographical areas of interest. Models also have an important role as a Replacement (*sensu* "the Code") to extractive sampling, but sample data is necessary for constructing the initial model and for subsequently testing the validity of model outputs. A CSIRO survey of deep-sea corals conducted in Nov–Dec 2018 was designed to test modelled predictions of deep-sea coral community distributions prepared before the voyage from local data collected in 2006/07. These initial maps predicted where corals are likely to be present based on whether an area is on or off seamounts, in a trawled or untrawled (commercial fishing) zone, and at three depths zones (500–1100, 1100–1350, 1350–2000 m). The biological and physical data collected on this 2018 survey will be used to refine these models, improve predictions, and provide guidance for Australian and international governments to monitor and manage deep-sea coral reefs and conservation assets. Specifically, the 2018 voyage objectives were to map the deep-sea coral habitats and biodiversity in and out of two AMPs south of Tasmania and on a seamount east of Tasmania (Figure 5). Statistical models were used to develop a spatially balanced sample design addressing the three variables in the predictive distribution model mentioned above, i.e. (1) seamount vs off seamount, (2) fished vs unfished and, (3) the three depth zones. This experimental design promotes a rigorous scientific result and thus will guide robust natural asset (Marine Park) management.

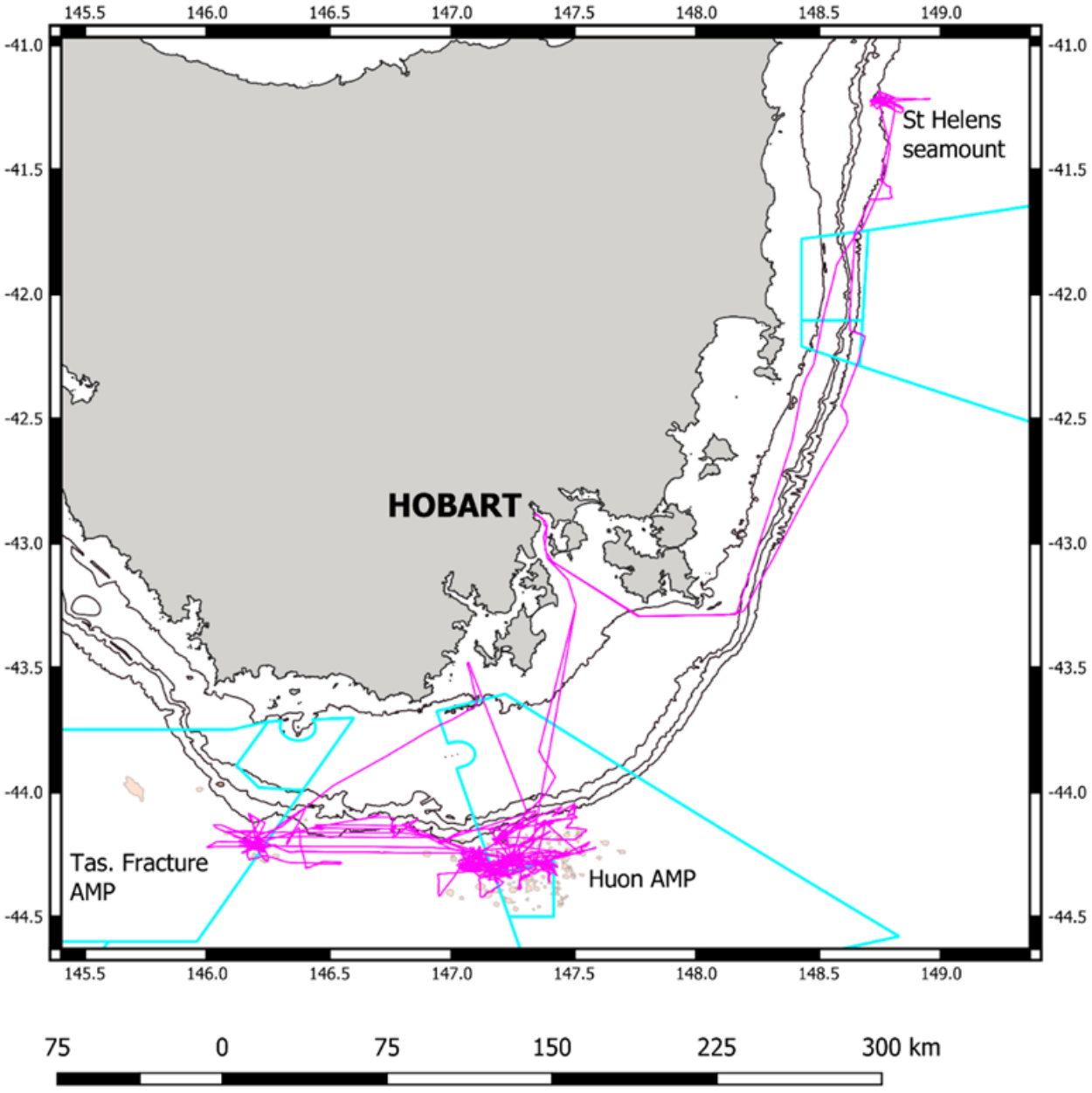

Figure 5. The RV Investigator vessel track (pink line) for the 2018 southern seamount voyage. The blue lines are Australian Marine Parks boundaries.

The two Tasmanian AMPs, Tasman Fracture to the SW and Huon to the SE, contain numerous fished and unfished seamounts (extinct undersea volcanoes) in 700–2000 metres depth, covering all three of the modelled depth zones, with many of the target seamounts now within a Habitat Protection Zone (Figure 6). The St Helens seamount lies off NE Tasmania rising from 1100 to 600 metres depth and has been historically heavily fished by trawl for orange roughy (*Hoplostethus atlanticus*) and remains open to fishing today.

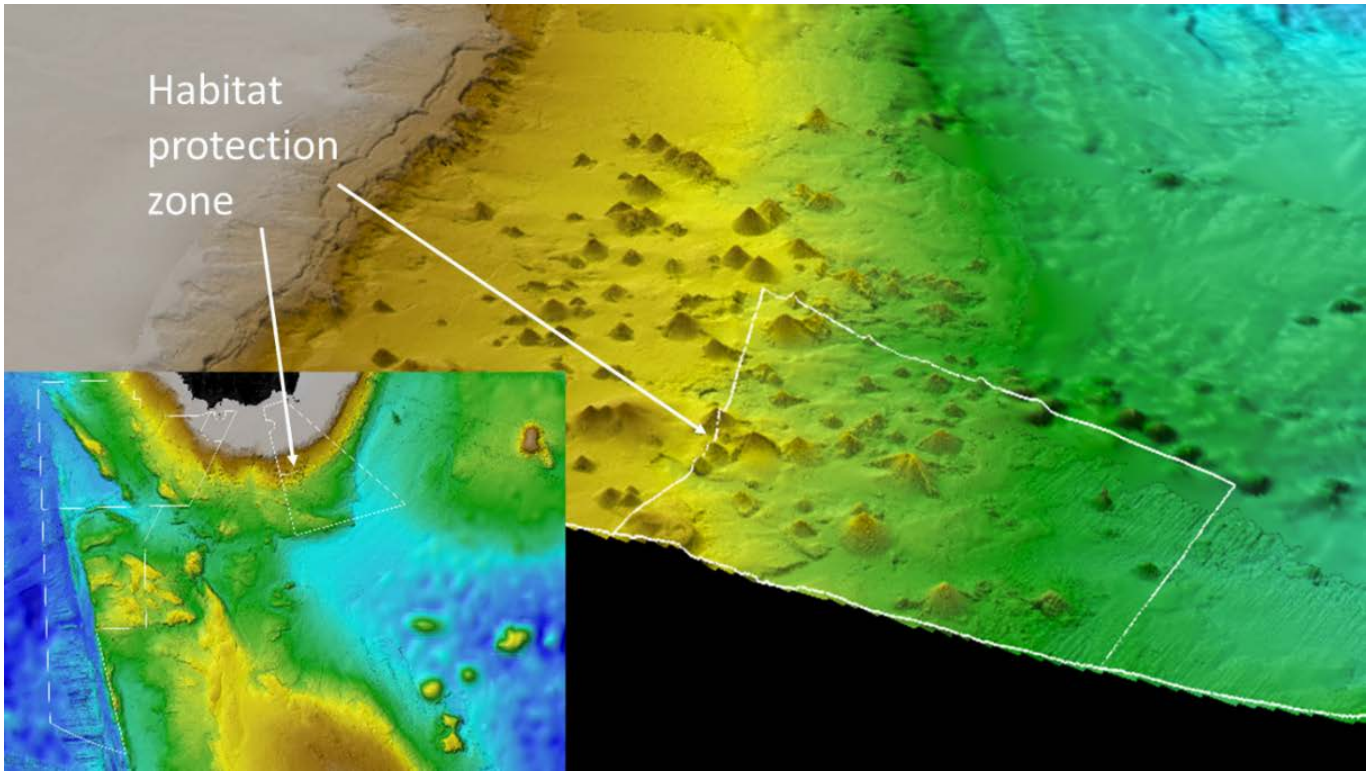

Figure 6. A 3D rendering of the Huon AMP (main image) with the boundary of the Habitat Protection Zone shown as continuous white line. The broken white line in the inset image are the MPA boundaries.

A survey of these Australian conservation assets requires a suitable research platform. For this survey our team had been allocated 27 days of support from Australia's Marine National Facility research vessel, the RV Investigator (Australian National Marine Facility, 2019). This modern research vessel is fitted with a multi-beam echosounder (sonar) that produces a 'swath map' of the sea floor, i.e. a detailed 3D representation. RV Investigator also has responsive equipment winches with real time telemetry via optic fibre, advanced GPS for accurate ship positioning, and acoustic beacon systems to accurately geo-locate sampling equipment that is lowered to the sea floor or towed behind the vessel. A combination of high definition sea floor maps, accurate real time geo-location of sampling equipment and sensitive equipment control enable targeted sampling over specific terrain structures of interest. Employing these technological advancements for marine surveys are Refinements (*sensu* "the Code") that minimise animal interactions because areas of no research interest are avoided.

When surveying within sensitive conservation areas it is desirable to have a low impact on the valuable natural assets. To do this, CSIRO has been developing (refining) image-based data collection from deep seamounts since at least 1993 when a 'drop camera' that used 35 mm film was tested on some seamounts south of Tasmania with just 156 usable images obtained from 3 locations (CSIRO, 1993). During the first dedicated southern seamount survey in 1997 a total of 570 usable drop camera images were taken by drifting over four seamounts, triggering the camera from the surface based on altimeter readings (CSIRO, 1997). These early images of seamount areas were exciting and informative, but the going was slow, the images were only viewable after processing the film and the number of unusable images was proportionally high. CSIRO have been constantly refining efficient image collection in the sea and have transitioned to a more efficient towed camera platform for deep water research. Successively improved instruments have

been built over the last two decades with changes in technology enabling better image data and increased depth capability.

CSIRO's latest towed camera platform, designed and build at the Hobart laboratories for the 2018 survey, is suitable for depths of 2000 metres and uses the latest in digital photography to provide real time imagery via an optic fibre within a 6000-metre-long tow cable. This camera platform is fitted with stereo (paired and synchronised) HD still 'survey' cameras, a HD video 'survey' camera, and a forward-looking video 'collision avoidance' camera. In 2018 this towed camera platform produced ~60,000 pairs of HD survey stills and 300 hours of HD survey video from 8 targeted seamounts and numerous randomly placed 'baseline' locations that sampled an additional 30 seamount and slope sites. These imagery data were obtained with minimal disturbance to the habitat and resident biology within the study area.

The camera platform is fitted with an altimeter to help the pilot keep the cameras at an optimal distance from the sea floor, and an acoustic position beacon that uses through water telemetry to accurately locate the cameras in relation to the ship (Figure 7). This allows navigation of the towed platform towards features of interest and precise geo-location of the imagery. The opportunity for on-board image viewing and processing gives researchers the ability to make quick and precise decisions about where to target extractive sampling of the biota; minimising animal interactions and reducing the number of animals collected by 'blind' sampling tows.

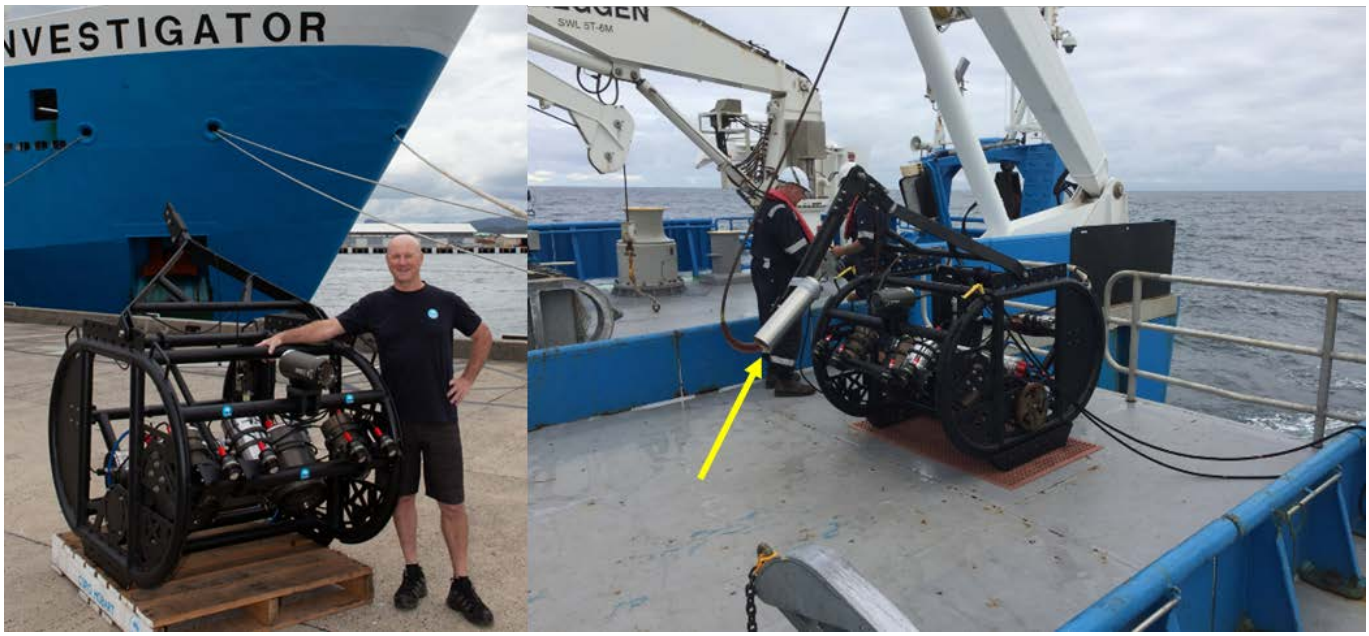

Figure 7. Left image - Voyage Leader Alan Williams stands next to the brand new Deep Towed Camera platform. Right image – the camera platform sitting on the stern of RV Investigator prior to deployment. The yellow arrow indicates the acoustic beacon for accurate positioning of the instrument and thus the observed biota; a critical part of the research which dovetails with addressing the 3R's.

The digital stereo cameras are synchronised and programmed to automatically take pairs of images at intervals that results in a relatively continuous record of the seafloor and the associated biota. Randomly selected image pairs are then processed using software (SeaGIS, 2019) to place quadrats of a known dimension into images selected for annotation. Counts of identifiable organisms within a quadrat can thus be converted to accurate densities (Figure 8).

Just as image data is an important part to the objective of 'ground-truthing' the coral distribution models, the image data subsequently requires 'ground-truthing' with physical collections of the corals to enable their accurate identification (e.g. Durnden et al. 2016). Unidentified or misidentified organisms results in an under estimate of biodiversity (Williams et al. 2015), but some corals look similar in the images when they are actually different species. Some corals in images look unlike anything described before, and living organisms (in a natural habitat) often look different from preserved material (in a jar). As it is important to put a name to biota observed in the images, taxonomic study is necessary. Taxonomy is the key-stone of measuring biodiversity and this requires actual specimens for detailed examination, curation of these samples for later reference and study (e.g. Durnden et al. 2016), the identification of voucher specimens for each species and possibly the eventual published descriptions of new taxa in peer review literature.

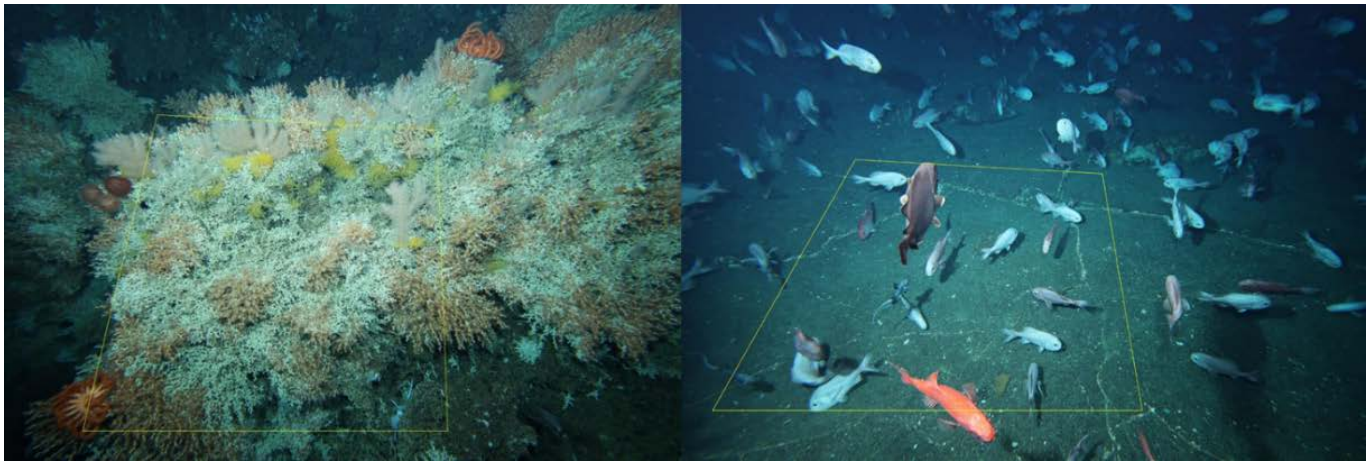

Figure 8. The SeaGIS software places quadrats of a known size onto the still image to enable accurate density estimates of organisms.

The net-samplers towed across the bottom are not selective for any particular species, thus it is not possible to limit the catch to just the target species - in this study we were after corals. However, the 'bycatch' animals collected all (importantly) form a more holistic understanding of the biological communities that live in deep-sea coral habitats. Many small and cryptic animals (too small to see clearly in an image) are hosted by corals, so understanding and identifying these ecological relationships is only possible by collecting organisms.

Most deep-sea creatures on the sea floor are not vertebrates or cephalopods and are thus not within the scope of the Code. Interactions with those that are – specifically teleosts (bony fish), elasmobranchs (cartilaginous fish such as sharks, rays, skates and chimaeras) and the cephalopods (squid, cuttlefish and octopus) – must be anticipated for the purposes of AEC considerations. Some of these animals are unavoidably collected and it is important to apply the 3R's when planning extractive sampling. It is however, not possible to pre-determine how many animals might be captured (by collection or in imagery) on any deployment, particularly for highly mobile animals like fish.

However, using the geo-located image data, detailed sea floor maps and acoustic location beacons fitted to equipment, it is possible to deploy extractive samplers on precise target areas, avoiding those areas where images indicate potentially high fish abundance. Thus, precise targeted sampling results in reducing interactions (*sensu* "the Code") with animals to the least that is practicably possible.

When these animals are captured, particularly from great depths, they usually do not survive the capture process and are dead when they are removed from the net. Those that show any sign of life are humanely killed using accepted protocols adopted from the Museum of Victoria's AEC SOP for using aquatic animals (Museum Victoria AEC, 2010). To first anaesthetise we place fish into a 20 mg/L solution of AQUI-S (a clove oil derivative) and cephalopods into a 5.4% solution of magnesium chloride until they are completely unresponsive. Animals are then humanely killed by placing into solutions of ethanol, formalin or into a -20°C freezer.

The number of animals anticipated and 'used' for both imagery and collection in the 2018 southern seamount voyage are presented in Table 1. We clearly underestimated the number of animals that were eventually 'used' by observation (using cameras), and this illustrates the difficulty of coming up with these numbers. Predicting the relative location and abundance of marine animals - particularly fish schools - is challenging. Conversely, in this case study we clearly captured fewer animals using extractive sampling than we predicted. We estimated catch numbers using previous survey catch data, albeit in a variety of habitats. However, by using the image data immediately available during this survey we managed to **refine** the net-sampling locations and **reduce** mortal animal interactions.

Table 1. Animals used during the 2018 southern seamount voyage. Note that the actual number of animals used with cameras is an estimate as many animals are very small or cryptic, and where there are vast schools of fish it is not possible to accurately determine those that had some interaction with the camera system.

Operation type	Operation objective	Number of Operations	Animal type	Animals used	
				predicted	actual
Towed camera - non extractive)	Coral recovery and mapping image data	153	Teleosts and elasmobranches	4000	Estimated at 8251 but unknowable
			Cephalopods	20	76
Net sampling - extractive	collection	20	Teleosts and elasmobranches	5000	692
			Cephalopods	200	6
Line fishing - extractive	collection	2	Teleosts	20	2

Predicting 'animals used' when applying "the Code" to field studies of wild animals in the marine environment is a process of best judgement by an experienced applicant seeking AEC authorisation, and a huge problem for someone with little or no experience. It is therefore necessary for the Animal Ethics Committee assessing applications/reports for field studies of this nature to understand the particular challenges in quantifying the number of animals that will be

used. The authors feel that a distinction between the kinds of usage (observation vs killing) must be made in the animal usage reports that are sent to government compliance regulators as the animal welfare outcomes are clearly very different. Undifferentiated animal usage data inflated with 'observations' poses a real risk of inaccurate and negative perceptions with compliance regulators and the general public.

Case study 2: Population study of the Environment Protection and Biodiversity Conservation (EPBC) Act listed spotted handfish using geo-located imagery

Spotted handfish (*Brachionichthys hirsutus*) are a Critically Endangered anglerfish (Department of Environment and Energy, 2019), restricted to nine local populations in the Derwent River estuary (Hobart, Tasmania) and potentially two more in adjacent waters of the D'Entracasteaux Channel (Lynch et al. 2016) (Figure 9).

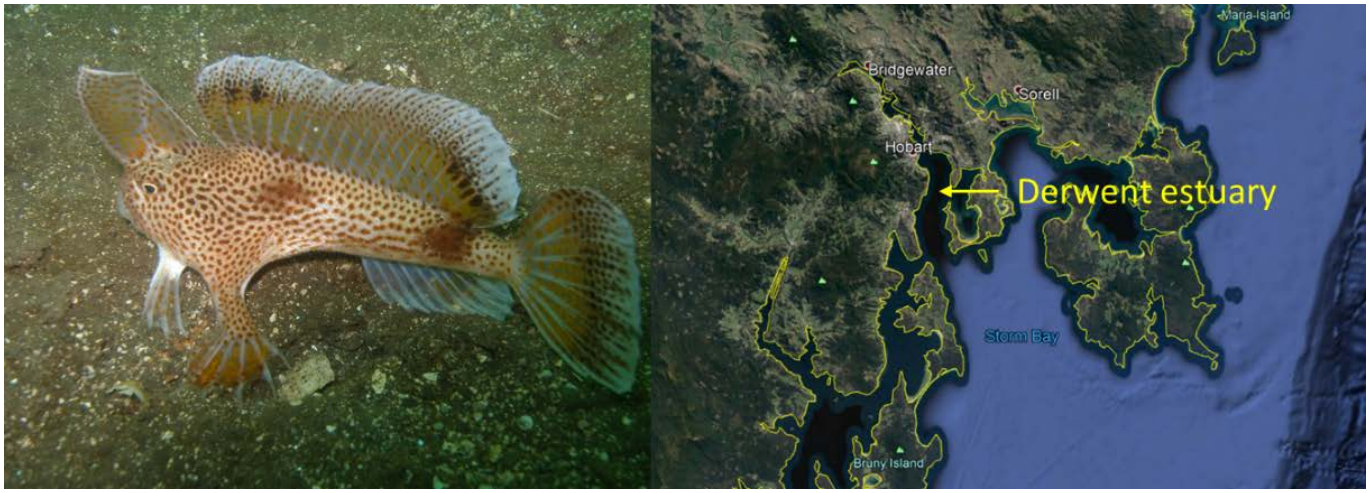

Figure 9. Left - a spotted handfish from the Derwent estuary. Right – the Derwent estuary is the port of Hobart in Tasmania.

The first dedicated handfish survey – immediately prior to EPBC Act listing – used divers to sample 28 locations and, where depth or visibility restricted diving, a 1.9-metre-wide beam trawl to sample a further 10 locations (Barrett et al. 1996). Just six individual handfish were located at five locations, all during the dive searches in depths of 6–10 metres. For a fish that was previously considered abundant in their narrow range (Last et al. 1983), this was considered a shocking result and it quickly followed that Tasmanian state and Commonwealth government agencies gave the fish threatened species status. Commonwealth government funding became available to do a more intense population investigation and develop a Recovery Plan (Bruce et al. 1997, Bruce and Green 1997, Bruce and Green 1998).

The additional fieldwork identified three sites that were occupied by local spotted handfish populations. Traditional fish tagging is not possible for these small animals and fin clipping is invasive, however it was immediately obvious that individual fish could be identified by their unique markings, so photography was used to 'mark' each fish observed for population size calculations and to examine fish movements. At the most studied site 78 individual fish were found, including newly hatched and juvenile specimens (Figure 10) – a very fortunate discovery and indicative of a breeding population.

Figure 10. Left and right images - Adult and juvenile spotted handfish. Inset – newly hatched handfish are fully formed and develop distinctive and unique spot patterns as they age. Note: hatchlings all start off white with a few darker markings, then develop line markings as juveniles that usually but not always break up into unique spot patterns.

Field observations at this site revealed that handfish wrap their eggs in a tight mass around vertical structures on the sea floor. A type of stalked ascidian (*Sycozoa* spp.) appeared to be the most available suitable vertical structures at the study site (Figure 4 and Figure 11) and this observation of a critical spawning habitat was our first hint at a potential threatening process to the spotted handfish population. Counts of these ascidians and direct predatory observations indicated they were steadily being grazed down by an introduced seastar, *Asterias amurensis*. This 'northern Pacific seastar' was first observed in the estuary in 1986 where it has become a dominant invertebrate predator (Grannum et al. 1996).

Figure 11. Left image - spotted handfish female guards eggs attached to stalked ascidian. Right image - spotted handfish next to a stalked ascidian and an introduced seastar.

During this initial study period, it was recognised that sampling using dredges or beam trawl nets was an unacceptable risk to the species and the critical habitat they required for successful breeding. Various methods to do quantitative population surveys were tested and it was shortly determined that the least destructive, most efficient and most effective method was using divers to

do Underwater Visual Census (UVC) surveys along transect lines of a known length. Traditionally UVC surveys used robust transect lines comprised of thin rope with a lead core or regular weights to keep it on the bottom. This sort of line was rejected as it had the potential to scare handfish out of the survey zone (resulting in underestimation of density) and also damage the fragile stalked ascidians; of particular concern if a survey is done during the breeding season when eggs are attached. A long (100 m) fibreglass tape was tried as a transect line but this tended to drift on the bottom in the current, again posing issues with scaring fish away and damaging the critical spawning habitat. Our first Refinement (*sensu* "the Code") to solve this problem was to make super-light transect lines using 8 strand fluorescent nylon 'builders' line, weighted at regular intervals with tiny lead fishing sinkers and anchored at either end with a dive weight and surface float for recovery. These nylon lines were very strong for their weight.

Super-light transect lines were set from the surface using a boat to run out the line from a spool, and then recovered by reversing the process. A GPS on the boat was used to mark the surface floats and the fish positions along the transect line recorded then used to plot fish approximate locations using mapping software. Divers swam either side of the transect line photographing and recording details for each handfish found within 1.5 of the line. Handfish did not appear to be bothered as the line sank gently down over the substrate (Figure 12) and super-light transect lines were successfully used from 1997 until 2014.

Figure 12. Spotted handfish were not disturbed by the super-light transect line

In 2014 we reviewed the use of super-light transect lines. From an efficiency point of view, they required a boat to set/recover, which was time/cost additional to the actual diving. We had also found that when surveying in areas with vessel moorings, the transect lines could become tangled or fouled on the moorings or debris, with breaks happening at wear points. Apart from being inefficient this also meant lines were being dragged over the bottom, potentially scaring handfish out of the survey zone and damaging critical spawning habitat. It was time to come up with a better method.

From February to September of 2014 we tested a towed GPS method reviewed by Schories and Niedzwiedz (2012) to see if it was suitable for surveying handfish and shared the results in Green et al. (2014) and Lynch et al. (2015). Portable GPS units are now cheap, digital cameras record a date/time stamp on each image and free software is available to accurately geo-locate images by synchronising the camera clock with the GPS time. As GPS don't work underwater, we attached a small unit to a surface float that was towed by a diver (Figure 13). The diver tightens the tow line to get the float overhead when a position needed referencing. Transect start and end points were referenced with photos taken on the sea floor, and each handfish located during the dive in a 3-metre-wide swath was similarly geo-located when the images of pattern markings were taken.

The software was used to clip out the ‘off-transect’ GPS track such that the actual transect length is known (via the software calculation), which is necessary to calculate fish density.

Figure 13. Small GPS units are placed into waterproof containers, fitted to a float and towed by a diver

The trail was a success with 49 individual handfish accurately geo-located over the study site. Of these, five were observed twice after periods of 13–102 days and had moved 26–135 metres. Figure 14 shows a map with the recorded positions of the fish that was observed the furthest from its initial location. This second Refinement (*sensu* “the Code”) of using a towed GPS unit rather than setting transects has now been adopted for all handfish surveys as it further reduces any risk of fish or sensitive habitat being negatively impacted.

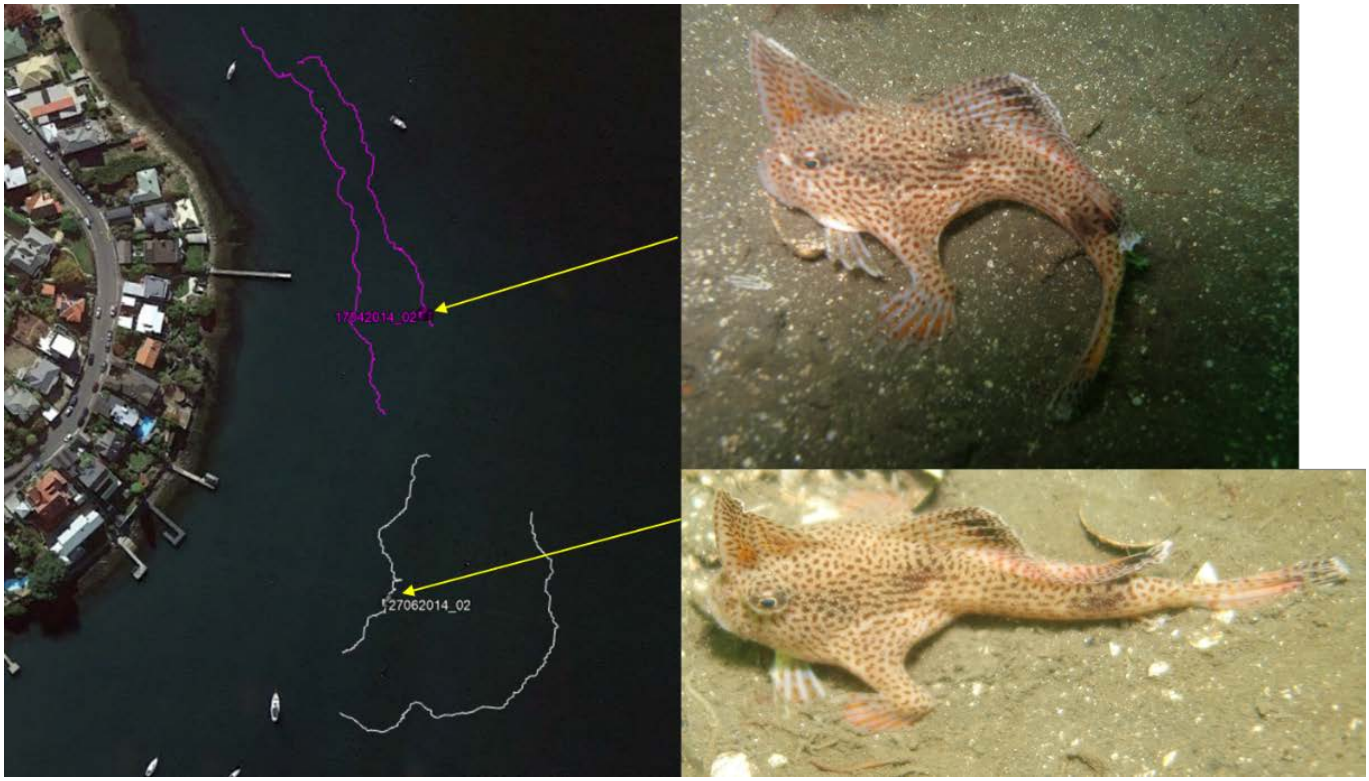

Figure 14. One example of fish movement over the site. This one was found 135 metres from its first position after 71 days. The white lines on the map indicated the towed GPS tracks on 27 June, the purple lines are from 17 April.

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Breaking down walls in the Animal Ethics space: a novel approach to improving compliance, comprehension, communication and research.

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Laboratory Compliance Coordinator, Mater Research

The bureaucratic and practical applications of Animal Ethics requirements for animal-based biomedical research can be overwhelming for many researchers. While the majority of researchers comprehend the importance of the ethical review process and compliance with *The Code*, it can be a complex task to navigate the specific institutional conditions and numerous steps involved in undertaking an animal-based research project.

Mater Research conducts all animal-based research in association with the University of Queensland as a faculty level Institute (MRI-UQ), while primarily utilising Animal Facilities and laboratory spaces in the Translational Research Institute building. This means researchers need to comply with the policies and procedures of essentially 3 large organisations in order to obtain all necessary approvals and assistance, to conduct world-class scientific research. It was identified in 2015 that MRI-UQ researchers were struggling to 'break down the walls' within this multi-organisational arrangement and were not operating efficiently or at the desired 'best practice' standard. Evidence of poor compliance, comprehension and communication with partner groups resulted in up to 6-8 month delays in obtaining necessary AEC approval, training and access to facilities.

A review of animal-based research activities at Mater Research was conducted to assess and appraise compliance with relevant legislation and institutional requirements. All research groups undertaking animal based research activities were interviewed and key areas for compliance activities (document and record management, responsibilities and delegations of activity and authority, training, internal and external reporting, Animal Ethics Committee (AEC) applications, and animal facility use and interactions) were audited and assessed.

Through the process of this project it was found that Mater research groups genuinely care for the animals used for scientific purposes, were committed to the principles of the three R's and eager to comply – but were lacking the necessary support in order to do so. The resultant series of recommendations on how Mater Research could bridge compliance gaps and streamline practices to achieve effective and efficient processes included the appointment of a centralised person to drive and manage institutional oversight for assisting Mater researchers to reach compliance, enable information sharing, promote communication between stakeholders and provide support services surrounding animal-based research activities.

The purpose of this talk is to share the success this role has achieved in changing the institutional culture around animal-based research, improvement in compliance, and streamlining work practices for all parties. It is hoped that by presenting the Mater Research 'experience', other institutions in a comparable position could potentially adopt similar changes to improve the animal ethics process (including, and arguably most importantly, better outcomes for research and animal welfare).

No manuscript was received for this presentation

The AEC and aseptic surgery

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Abstract

Members of an Animal Ethics Committee may have minimal or no experience of the surgical environment. In the absence of a knowledgeable observer at the time of surgery, how can an AEC ensure that surgery is performed using aseptic technique as required by the *Australian code for the care and use of animals for scientific purposes*? Can a Standard Operating Procedure (SOP) or description of the surgical procedure give an idea of whether minimal standards of surgical asepsis are being maintained?

Using knowledge gained in the previous presentation on "What is aseptic surgery?" we will deconstruct a SOP describing a surgical procedure to see whether aseptic technique is likely to be achieved. Simple and inexpensive modifications will be discussed that achieve a higher degree of asepsis with minimal input of time and resources. The minimum equipment and resources required to achieve aseptic technique to an acceptable standard in a research setting are discussed.

At the end of this presentation you will be able to:

- Deconstruct a surgical SOP to determine if aseptic surgery is likely to be achieved.
- Recommend simple modifications to a SOP that will promote surgical asepsis.
- Detail the minimum equipment required to achieve surgical asepsis in a research setting.

Introduction

The *Australian code for the care and use of animals for scientific purposes* (Code) states (3.3.16): *the wellbeing of animals that have undergone surgical procedures must be supported and safeguarded by:*
*(ii) using **aseptic procedures** if the animal is expected to recover from surgery and*
*(iii) ensuring that all procedures conform to **accepted standards in veterinary or medical practice**, as appropriate for the procedure and circumstances*

These requirements not only protect animal welfare from the impact of infection, but also reduce the potential impact of infection on research results.

What are aseptic procedures?

Aseptic procedures are the techniques put into place to minimise the risk of infection following a surgical procedure. They have evolved from the work of Joseph Lister in the 1860's, who

reduced the human mortality rate from infection (sepsis) following limb amputation from 50% to 15%. This was through the adoption of techniques to minimise bacterial contamination at the surgical site, including the use of an antiseptic spray of carbolic acid (phenol) during the surgical procedure.

What are the accepted standards for aseptic surgery in veterinary practice?

There is little documentation available on what constitutes an accepted standard in veterinary practice. Most of the aseptic techniques adopted in veterinary practice have evolved from what is taught at vet school. However, the Veterinary Board of the ACT has recently published the *Veterinary Practice Veterinary Premises Standards 2018*, which incorporates *Minimum Requirements for Veterinary Premises*. These stipulate that, "*All surgical procedures* (are) *carried out under sterile operative conditions – using individual dry, sterile packs and drapes* ..."

What are the accepted standards for survival surgery in a research setting?
The NHMRC *Guidelines to Promote the Wellbeing of Animals for Scientific Purposes* (2008) reinforce the need to perform surgery aseptically by requiring:

- *the conduct of surgical procedures in a designated area that has been disinfected—for large animals, this will usually require a dedicated facility, but for small rodents it may be a designated work area*
- *the preparation of the surgical site to minimise the risk of entry of bacteria into the wound— this will usually involve removal of hair, fur or wool in the immediate vicinity of the intended surgical wound and cleaning and disinfecting that area*
- *the surgeon and others in the vicinity of the operative field wearing protective clothing, masks and head cover*
- *the surgeon and surgical assistants performing a surgical scrub and using sterile surgical gowns and gloves (gloves only may be used in rodent and field surgery)*
- *the surgical site being draped with sterile drapes to create a sterile 'field' around the site…*
- *sterile instruments and packs being used*
- *only sterile instruments, drapes, packs and gloves coming into contact with the surgical site*
- *sterile surfaces kept dry to avoid moisture contaminating the surgical area.*

A research environment differs from that encountered in veterinary practice. It is uncommon to have an area solely dedicated to surgery, a high throughput of surgical procedures is often desired and use of complex equipment e.g. stereotaxic apparatus, can impact on the ability to

maintain aseptic technique. A pragmatic approach needs to be adopted that maintains asepsis with minimal impact on time and cost.

Overview of aseptic technique

The overall goal of aseptic technique is to minimise contamination of the surgical wound with bacteria. Bacteria reside on the body, on instruments and equipment and in the environment. Entry of bacteria into a wound at the time of surgery may lead to the development of infection. This is dependent on the number of bacteria inoculated into the wound and the immune status of the animal – immunodeficient strains and animals treated with cytotoxic drugs are more susceptible to infection. The likelihood of infection is also dependent on the duration of the procedure, the skill of the surgeon and whether foreign materials or objects are introduced into the animal.

Aseptic technique requires consideration of the surgical environment, animal preparation, preparation of instruments, materials and equipment and preparation of the surgeon. Once an aseptic surgical environment is achieved, it is important that it is not subjected to contamination by contact with non-sterile items or personnel.

Surgical environment

- A dedicated surgical area with minimal clutter is ideal for conducting surgery but is not commonly available in a (rodent) research environment.
- In the absence of a dedicated area, the surgical locality should be cleared of unnecessary equipment and cleaned with disinfectant prior to surgery.

Preparation of the animal

- Bacteria are present on the hair, skin surface and within hair follicles.
- It is impossible to sterilise the skin surface (remove all bacteria) - the aim of skin preparation is to minimise the number of bacteria present.
- Removal of hair prior to application of an antiseptic is required. Bacteria are present on hair and incising (making an incision) through hair results in contamination of the surgical wound with pieces of hair. Hair may be removed with clippers (preferred), by shaving or the use of a depilatory (hair removal) chemical. Hair is removed from the site of the surgical incision together with an adjacent area to create a surgical field.
- Once hair has been removed, an antiseptic is applied to the skin to reduce the number of bacteria present. It is important to understand the characteristics of the antiseptic being used:
    - **Alcohol** (ethanol) – alcohol has been used as the sole antiseptic in a research environment and was endorsed by AAALAC in 2001 for this purpose (provided it is in contact with the skin for at least a minute). However, *The Guide for the*

Care and Use of Laboratory Animals (NRC 2011) states that, "Alcohol is neither a sterilant nor a high-level disinfectant." In veterinary practice the use of alcohol as the sole antiseptic would not be considered as meeting an acceptable standard. Its use in combination with chlorhexidine (see below) is acceptable.

- **Iodine based antiseptics** e.g. Betadine, are currently used in veterinary practice in skin preparation. They are effective provided they are in contact with the skin for a minimum of two minutes. They have the disadvantage that they can cause skin reactions and stain clothing very effectively!
- **Chlorhexidine based antiseptics** are the preferred veterinary antiseptic. They have the advantage of a rapid onset of action (within 20 seconds) and ongoing activity as a result of binding to keratin in the skin. A chlorhexidine scrub solution contains detergents to assist with removal of skin surface debris and oils and can be followed with a chlorhexidine in alcohol solution.

- Following hair clipping and antiseptic preparation of the skin, a sterile "drape" is applied to isolate the surgical field from adjacent (unclipped) skin. A drape is essentially a sheet of sterile material e.g. plastic which acts as a barrier, to prevent contamination of the "sterile" surgical field from adjacent non-sterile areas. In a research environment, the equipment associated with the procedure being undertaken e.g. stereotaxic apparatus, may prevent the application of a drape. In such cases great care needs to be taken to avoid inadvertent contamination of the surgical field.

Preparation of instruments, materials and equipment

- Sterile instruments, equipment (that will contact the surgical site) and materials are required for aseptic surgery.
- Most metal instruments can be sterilised in an autoclave. If an autoclave is not being used for instrument sterilisation, it is questionable whether strict aseptic technique is possible (unless disposable, sterile instrument packs are being used).
- Many facilities have heat bead sterilisers that are used to sterilise the tips of metal instruments. However, great care and awareness is required to avoid contamination of the surgical wound. The handles of instruments sterilised in heat beads are not sterile, which results in contamination of the sterile surgical gloves and drape. If sutures (stitches) are used during the surgery, there is a high likelihood they will become contaminated as a result. Heat bead sterilisers are **not used** in veterinary practice.
- Immersion of instruments in alcohol is **not** considered an effective sterilant for instruments, materials and equipment (AAALAC, 2001)
- Instruments and materials must not be placed on a non-sterile surface such as a bench top during surgery because they will become contaminated. If performing rodent surgery, the periphery of a sterile drape serves as a useful area to hold instruments that are not in use.

- Other equipment e.g. reusable telemetry devices require a strict preparation regime as dictated by the manufacturer to ensure sterility.

Surgeon preparation and behaviour

- The surgeon must wear standard Personal Protective Equipment e.g. face mask, protective clothing, head covering (as commonly practised in animal facilities).
- Sterile surgical gloves must be used (NHMRC, 2008).
- The surgeon should be aware of aseptic technique and the basic principles of surgery including the importance of gentle tissue handling, minimal dissection of tissue, appropriate use of instruments and ability to control bleeding.
- Great care must be taken to prevent inadvertent contamination of surgical gloves by the surgeon e.g. adjusting stereotaxic equipment, lights, etc.

Deconstructing a surgical description or Standard Operating Procedure (SOP) to determine if aseptic surgery is being performed.

- Photographs embedded into a surgical description or SOP allow better evaluation of the procedure from the point of view of asepsis. It is also extremely valuable for standardisation of technique and as a resource for other researchers contemplating undertaking the same procedure. The Category A Member (Veterinary Surgeon) of the Animal Ethics Committee (AEC) is ideally placed to evaluate the SOP against current accepted standards in veterinary practice.
- An example of a **checklist to assist in evaluation of a surgical SOP**:
    - Suitability of the proposed location for surgery.
    - Method of hair removal from the surgical site – clipping (preferred), shaving (greater risk of infection) or depilatory product (risk of skin irritation from residue).
    - Antiseptic used in skin preparation – chlorhexidine or chlorhexidine in alcohol preferred, iodine compounds acceptable with prolonged contact, alcohol on its own does not meet the accepted standard of veterinary practice.
    - Use of a drape to isolate the surgical site – usually possible if the animal is not surrounded by research equipment e.g. stereotaxic apparatus.
    - How will instruments, materials and equipment be sterilised?
        - Autoclaving or single use packs for metal instruments.
        - Sterile packs of suture (stitch) material – avoid use of silk (induces an inflammatory response, associated with a higher risk of infection and no longer used in contemporary veterinary practice).

        - Heat bead sterilisers require meticulous self-awareness to prevent contamination of the surgical site (heat bead sterilizers are not used in veterinary practice).
        - Specific sterilisation procedures for more complex materials and equipment.
        - Use of alcohol alone to sterilise instruments is not acceptable (AAALAC, 2001).
    - Sterile surgical gloves must be worn (NHMRC, 2008).
    - Has the researcher been trained and assessed as competent in aseptic technique, in addition to the actual surgical procedure? How has competency been evaluated and by whom?

AEC's role in promotion of aseptic technique

The AEC has a responsibility to ensure projects comply with the Code and NHMRC Guidelines and should define minimum standards that will achieve this requirement. When evaluating a SOP or description of a surgical procedure, a checklist such as that described above, will help with this process and assist researchers to meet the required standard. Asking researchers to embed photographs of the procedure allows more thorough evaluation thereby "Breaking Down the Laboratory Walls". The Category A member and Animal Welfare Officer are best placed to correlate contemporary standards of veterinary practice with the proposed procedure. At times, a certain amount of pragmatism and adaptation may be required to ensure a surgical procedure can take place e.g. a drape can interfere with the operation of a stereotaxic apparatus.

A formalised training program concentrating on aseptic technique should be considered, ideally with theoretical and practical aspects and an assessment of competency. This should be delivered by someone who is familiar with the practice of aseptic surgery in the veterinary setting such as a veterinarian (Animal Welfare Officer) or a registered veterinary nurse. Once the principles of asepsis are understood and basic surgical techniques have been mastered, further training in specific procedures can be provided by someone familiar with the technique such as a researcher. Researchers who provide further training should have been deemed competent in and practice aseptic technique themselves to prevent perpetuation of poor aseptic technique to the next generation of researchers.

Objections may be received from researchers who have been performing a surgical technique for prolonged periods of time without due regard to the Code and NHMRC Guidelines for aseptic surgery. It is often argued that they have rarely, if ever, seen an infection develop in the animals they have operated upon and therefore they must be using aseptic technique. My personal viewpoint is that these claims should be treated with suspicion. The traditional view that rodents are naturally resistant to the development of infection has been shown to be erroneous. Some of the more recent strains of rodent e.g. NSG mice are severely immunodeficient and therefore more susceptible to infection. Given that Surgical Site Infection (SSI) rates in veterinary and medical practice average between 1-3% (and up to 15% in veterinary practice for long, complex, orthopaedic cases) it surprises me that such low infection rates are reported.

Factors that might explain this apparent discrepancy include:

- It generally takes 2-3 days for an infection to become clinically apparent i.e. observable to the trained eye. If the animal is killed for experimental purposes prior to this time, infection will not be detectable.
- Signs of infection may be missed or misinterpreted e.g. a wound that bursts open a week after surgery (from infection) may be blamed on the animal or cage mate interfering with the wound.
- Necropsies of animals that die suddenly post-surgery or lose weight may not be performed as a result of attribution to another cause.
- The duration of surgery can impact the likelihood of infection developing – very short and simple procedures such as minipump implantation in rodents reduce the chance of inoculation of the wound with bacteria.

In most states of Australia, diagnosis of a pathological condition in an animal, such as a wound infection, is considered a restricted act of veterinary science. This means that only veterinarians can diagnose a wound infection. Unless a veterinarian is actively involved in the care of animals within a facility, wound infections may go undiagnosed.

I suspect Surgical Site Infection (SSI) rates in rodents are underreported. If surgery is being performed in your facility, the AEC should consider the following:

- Do you receive reports of any animals that develop SSI?
- Given the incidence of reported SSI in veterinary and medical practice is 1-3%, what is the SSI rate in your facility?
- Is an SSI considered as an Unexpected Adverse Event (UAE)?
- If an SSI is not considered an UAE, is an expected incidence detailed in the project (beyond which the event would need to be reported as an UAE)?

Summary

The likelihood of the development of infection after surgery is increased when aseptic technique is not undertaken in accordance with the Code and NHMRC Guidelines. Post-surgical infection will not only have health and welfare impacts on the animal but may potentially interfere with the results of research. The risk of post-surgical infection may be minimised by the development of policies and procedures that promote aseptic technique.

Personal Openness: how (and why) to communicate openly about what we do

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Abstract

Working in the laboratory animal industry can often be isolating. Although we do amazing work ensuring the health and welfare of animals involved in research designed to help improve the health of humans, animals and the environment, we are reluctant to talk about it. What do you say to people that ask you what you do for a job? Do you tell people that you serve on an AEC or that you work in a research field involving animals? What we do is important and the more we feel able to capably address it in a social setting, the better off we (and the animals in our care) will be. This talk will be interactive and include helpful tips for speaking to people about what we do and why we do it.

Some of the issues

- Anti-vivisectionists/animal rights groups…..
- Much less violence and more lawyers, lobbyists, and partially sensible arguments that without another side of an argument to consider sound plausible!
- Now the significant risks are from us doing nothing
- The argument is that animals should not be used for research, but what is the risk of not using animals for research….? This needs to be addressed!
- If the public does not support animal research (when justified), then the funding dries up, the important work stops and the health and welfare of all of us suffers.

Why we need to speak up

- People have been allowed the luxury of disconnect for too long. They readily accept the miracle, but we need to show them the reality.
- We do a great job and are caring loving people to our animals
- We need to be proud of what we achieve and what we help others to achieve
- We help save lives and improve the health of humans, animals and the environment
- We deserve increased job pride and satisfaction
- We need to control the image of what we do!

Questions I have gotten from colleagues

- Spoken to various colleagues in our industry
- Some questions/concerns:

- What do I tell my children? Parents at their school?
- What do I say when someone asks what I do?
- How much do I say?
- Coping with jokes about "torturing" animals?
- When do I walk away?

Helpful information and getting the public into our world

- They don't know what we do, but we thought they did…..
- Time to let them know that there are laws and AECs and animal care staff and researchers that care deeply for the animals, often at great personal costs!
- Much information on public responding positively to education about what we achieve and how we care
- Great animal care = great science!
- Start by talking with friends and family, especially a child. If you can answer the questions of an 8-12 year-old, you are good to go! :)
  - Keep it brief, at first
  - Use personal experiences: positive stories from your facility
- We don't know everything about all research and facilities, so don't speak for all of research
  - Most people don't know that there is a whole laboratory animal profession (and that some of us are on call 24/7)! ☺
  - Not all research ends in a cure for a disease
  - Most people will support research for medical purposes (remember, both human and non-human animals benefit, including pets)
  - 'Well, this is a difficult subject and we won't all agree, but I am glad you feel as passionately about animal welfare as we do' Bella Williams' Top 10 tips for speaking about animal research

- Remember that we are looking to shift perspectives, not necessarily change minds. If uninformed people, even ones that don't agree, become ones that understand and don't agree, then that is good!

- Animal research is complex, and there are costs to the animals and limitations to animal models as well as benefits. It's ok to admit that.

The "elevator speech" is important to prepare

Excerpts from, *Developing an elevator speech for discussing animal research* (Jim Newman, Director of Strategic Communications, AMP)

- Consider your audience; what parallels can you draw between science and the occupation/knowledge base of the person with whom you are speaking

- Start with the goal (general) and then go more specific

- Avoid jargon and acronyms (e.g. NIH, AEC, SAHMRI, AWO)

- Define basic vs applied science (if needed)

- It's a conversation, not a speech

- It's OK to share our feelings

- Don't be afraid of tough questions

- Learn from your interactions and practice

Don't be afraid of tough questions

- Do animals die as part of your research? Do you euthanise animals? How can you do that? Do you feel guilty?

- Acknowledge what was asked and then respond. If a loaded word or phrase is used, avoid repeating it.

    - "Good question. Some animals do need to be humanely euthanized as part of our work, because…."

- It's completely OK to say things like "I would not do this if I did not recognize the need."

Always remember this:

“People in this field are the reason that 6 year olds with leukemia get to be 7.”

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- https://www.laboratoryequipment.com/news/2019/03/developing-elevator-speech-discussing-animal-research Developing an elevator speech for discussing animal research (Jim Newman, Director of Strategic Communications, AMP)

- https://www.amprogress.org/raising-voices/ AMP’s Raising Voices, Saving Lives

- A roadmap for a public discussion of the ethics of animal research (Speaking of Research, Allyson J. Bennett)

- Dr Cindy Buckmaster “Stand and Deliver” YouTube video….and all the talks that she has ever done! ☺

How research on wombat disease is a diverse landscape of social and ethical challenges

Scott Carver
Department of Biological Sciences, University of Tasmania

Wombats are among Australia's most iconic fauna and many Australians have strong emotional attachments to these animals. Across their range, bare-nosed (or common) wombats are impacted by a disease called sarcoptic mange. This disease is caused by the parasitic mite *Sarcoptes scabiei*, which was introduced to Australia by European settlers and their domestic animals; and affects many mammal species, including humans where it causes scabies disease. My laboratory has been working on the impacts and control of mange in wombats since 2013. In that time, there has been strong public, media and political interest, including the formation of grass-roots community organisations who attempt to treat wild wombats that are sick. In this presentation I will discuss the research performed by my laboratory and the social and ethical hurdles we have faced along this journey and consequent lessons learned. I will talk about where I see the future of wombat welfare and conservation with respect to sarcoptic mange, in particular the translation of research into practical outcomes for the Australian public.

No manuscript was received for this presentation

Leaving the void: Tonic immobility, anaesthesia, analgesia and surgical procedures in sharks

Jayson Semmens
Institute for Marine and Antarctic Studies, University Animal Ethics Committee Chair,
University of Tasmania

The use of anaesthesia and analgesia during surgery in sharks is very limited compared to other vertebrates, including fish. This is particularly the case for field research involving sharks, where the majority of work has not used any form of anaesthesia or analgesia. This is mostly a consequence of researchers arguing that given many species of shark when placed on their back go into tonic immobility, a reflex that causes a temporary state of inactivity, anaesthesia or analgesia is not required and would cause unnecessary stress. However, it has not been assessed if going into tonic immobility causes any stress to the sharks, so relying solely on this mechanism may not be justified. Using my own experience with anaesthesia, analgesia and tonic immobility in sharks, I will suggest some priorities in this area of research moving forward to enable us to leave the anaesthesia/analgesia knowledge void we are currently in with respect to field-based shark surgery.

No manuscript was received for this presentation

Commissioner for Animals: a missing piece of the openness in animal research puzzle?

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Abstract

There has been significant progress in Europe toward greater openness in animal research, with the implementation of the United Kingdom's Concordat on Openness in Animal Research, and similar initiatives promoting greater openness in Spain, Portugal, Belgium, and Germany. Groups such as the Basel Society, ANZCCART and ANZLAA have advocated for similar progress to occur elsewhere. However, thus far there has been little to no concrete progress in Australasia to affirm general principles of openness in animal research in a broad way across organisations involved in this research.

In 2015, New Zealand was awarded an "A" ranking in World Animal Protection's Animal Protection Index, acknowledging that New Zealand's Animal Welfare Act 1999 was (and remains) one of the most progressive and stringent animal welfare regimes in the world. In 2017, we received a research grant from the New Zealand Law Foundation to assess the theoretical side of how the Animal Welfare Act is intended to work, and then, after analysis, to weigh this against the practical reality of how the Act is enforced: in other words, to assess the gap being the current ideal and practicalities, and where further improvements could be made in further legislative developments. One of the recommendations we make in this research is that New Zealand establish an independent Parliamentary Commissioner for Animals in New Zealand.

In this presentation, we will outline the reasons for this recommendation and how they align with the reasons for greater openness in animal research, teaching and testing. We will consider the role that the Commissioner could play in animal research in New Zealand and provide some comparative remarks regarding establishment of a similar office in Australia. We will focus in particular on the dynamics of the debate over openness in animal research and teaching in New Zealand and how these resemble, or differ from, those jurisdictions where more progress has been made toward agreement on this issue. We will argue that an active independent Commissioner would improve the speed at which progress is made toward greater openness in Australasia. We will also argue that a Commissioner could improve the value of any openness, and openness agreements, beyond that achieved elsewhere.

This paper explores the role that an independent Commissioner for Animals could play in achieving, or increasing the value of, an agreement on openness in animal research among those conducting research. Specifically, we argue that, a Commissioner for Animals, which independently represents the interests of animals, could solve what we've called the openness in animal research puzzle. This the puzzle that, despite apparent agreement among diverse stakeholders that there ought to be greater openness, no formal agreement has been achieved.

To do this, the paper examines the social and political conditions surrounding the UK Concordat Agreement and presents arguments that cover why a Commissioner for Animals ought to be present in the politics of New Zealand and Australia, and why there ought to be openness in scientific research. We begin with the latter argument.

Below is our argument for openness in animal research:

1. An activity has social licence to the extent that the public tolerates, accepts, or trusts the activity and its practitioners, *and* it is fitting to do so.
2. Social licence is necessary for animal research to occur and be most ethically valuable.
3. Openness in animal research is necessary for public toleration, acceptance, or trust to be fitting.
4. The current level of openness is not sufficient for social license.
5. Therefore, *if* animal research ought to occur most valuably, there should be greater openness.

In a full presentation of this argument each of these claims would need argumentative support: reasons and/or evidence to support them. For the purposes of this paper, we will briefly describe and explain this argument, rather than defend it in detail. Some readers may agree with the argument without rational persuasion and those that disagree are invited to regard it as an assumption of the paper. If our argument is unsound, then at most this removes one argument for greater openness in animal research, but others remain (as we shall describe in the next section).

Premise 1 is largely self-explanatory, with the exception of our appeal to 'fittingness' as an important relation between social license and the activity that has it. Social license that is 'fitting' in our sense is that which is warranted, or that there is reason to grant it to an activity. It is possible for people to tolerate, accept or even trust an activity and its practitioners, but that it is not warranted: the truth is that the practitioners are not trustworthy, and they have deceived those in a position to grant social license about key facts about their activities and practices. In this case, it may be true that people tolerate, permit or trust the activity and those who do it, but this trust is not fitting: social license is not warranted. We claim that a necessary feature of ethical animal research is that it is open about its activities, and without this, there is less reason for the public to grant social license.

If animal research possesses a fitting social license, then it will be most ethically valuable. In one sense this is a necessary relation: we think there is greater reason to grant social license to more ethically valuable activities (i.e. those that are conducted in an ethical manner, and have valuable consequences). If animal research is an ethically justifiable way to benefit animals, humans and the environment, for example, then there is reason to grant it social license. In another sense, the value of animal research depends on its having social license. Without it, or if it is merely tolerated, rather than being trusted, it will be more difficult to conduct animal research, and the results of research will be less beneficial if they are not embraced by those who would implement or use them.

Stakeholders argue for greater openness for a range of reasons, some of which support claims of this argument:

- It would benefit animals
- It would benefit researchers and technicians
- It would benefit the public
- It is a necessary condition of its political/social legitimacy
- It is owed to the public in exchange for their financial support
- It is owed to animals in exchange for their use

Yet, despite some limited progress at individual research organisations and representative groups, attempts to gain formal agreement for greater openness in Australasia have so far been unsuccessful. This differs from the UK, where agreement has been achieved, with the signing of the Concordat on Openness on Animal Research in 2014. This followed an initial commitment from 40 life sciences organisations to develop an agreement in 2012, which led to formation of a steering group, which oversaw a working group that represented signatory organisations and their interests. Public dialogue and consultation was an important part of the process, and the resulting agreement launched with 72 signatories, and at the time of writing has over 120.

The UK Concordat on Openness on Animal Research has four foundational commitments:

1. *We will be clear about when, how and why we use animals in research*
2. *We will enhance our communications with the media and the public about our research using animals*
3. *We will be proactive in providing opportunities for the public to find out about research using animals*
4. *We will report on progress annually and share our experiences*

What can be learned for Australasia from this positive step in self-governance among UK research organisations? We note the following positive aspects of the agreement process. First the fact the formal agreement for greater openness was secured, is a huge achievement. The fact that the agreement focused entirely on openness, rather than bundling that with other ethical guidance for research is also instructive. It reduces the complexity of negotiation of agreement. The concordat has resulted in more information being publicly available, with the numerous signatories held to account for their performance, including rewarding those that excel. The agreement accompanied a reduction in violent protest in the UK, although it is impossible to attribute this with certainty to the agreement itself.

Negatively, there is reason to raise questions about the neutrality and disinterestedness of the agreement itself, and the way it's implemented in practice. A majority of the steering group and working party were people with an interest (i.e. a stake) in animal-research – including the research organisations themselves who are signatories. It is, of course, appropriate for these interests to be represented, but there is the potential for conflict between research interests and openness about some aspects of animal research. Moreover, Understanding Animal Research, who facilitated the process have been criticised as lacking the requisite neutrality, and favouring research.

Immediately prior to working on the development of the agreement, public polling showed a dip in positive regard for animal-based research, particularly with respect to trust in researchers. Although this dip did not persist after the release of the agreement, there is no clear positive change

in public views about the use of animals in scientific research in UK – e.g. "Secrecy" is still a primary characteristic of research organisations in polling, and there is little clear evidence of the public being more informed, or feeling more informed.

How did the UK get agreement? The conditions preceding it were long-running, sometimes violent, protest against animal use in research, there was pressure on research organisations from funders, and political pressure on research organisations, along with the aforementioned drop in public trust in animal research dropped in a 2012 Ipsos survey.

The UK experience may explain the puzzle of why agreement on openness in animal research has not been reached in New Zealand and Australia. First although there is some agreement for greater openness in animal research, there is evidence that this is not universal and that there is disagreement about what sort of information should be made provided openly. Secondly, Australasian researchers and research organisations have fewer reasons to agree than UK: the nature of protest is less harmful and controversial, there is less evidence of political & funder will for openness, and public views about research that could be influenced by the degree of openness are less well characterised compared to the UK – there is no established, repeated polling of these are there is in the UK.

We argue that an independent political office of a Commissioner for Animals in either or both countries (in whatever jurisdiction) could change the conditions to make them more conducive to agreement for greater openness in animal research. Although, if we are correct, this is a reason to favour there being a Commissioner for Animals in the relevant jurisdiction, this is not a good argument for why there should be a Commissioner. That requires an independent political argument. Our argument is as follows:

Animal interests ought to be represented politically

1. Representatives ought to have minimal interests that conflict with those interests they represent.
2. Representation ought to be proportional.
3. Representation ought to be independent.
4. A Commissioner for Animals can best provide this political representation.
5. Therefore, there ought to be an office of the Commissioner for Animals.

It is outside the scope of this paper to argue for each of these premises here, and the unpersuaded reader can regard it as an assumption of the rest of this paper that either this argument is correct, or that there is some other argument that is. Or regard it as an interesting hypothetical to consider what the effects of independent political representation of animals could be for openness in animal research, if it did exist.

If sufficiently resourced, and appropriately established, a Commissioner for Animals could raise the public and political profile of openness in animal research (among the other focuses of such a role). They would be well-placed to conduct research into public views about animal research, which could reveal whether there are public attitudes, or interests, that provide reason for animal researchers, politicians and research funders or organisations, to take more seriously the need to provide greater openness about animal use in research. A Commissioner for animals could act as an independent facilitator of an agreement, which would not be open to criticism for having a pro-

research bias. A Commissioner could also provide oversight and facilitation of openness in animal research, as Understanding Animal Research does in the UK.

There is much more to say in arguing for the above conjectures and considering counterarguments. The purpose of this paper is to put forward a novel idea that could change the dynamics of the debate over greater openness in animal research in Australasia in a way that could increase the possibility of success in gaining an agreement here, as has been reached in the UK. Ideally this would result in an agreement that increases the ethical value of animal research (aligning the practice of animal research and research organisations with ethical requirements, be they for openness as such, or something broader) and allows the development of (fitting) social license for animal research: a trust in research that is warranted on the basis of evidence.

Presentations given

on

Thursday 25th July

Natural disease models, wild immunology and Tassie devils

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Abstract

Wild immunology has been described as the "missing link between laboratory-based immunology and human, wildlife and domesticated animal health" (Pedersen and Babayan, 2011). Although experiments on inbred strains of laboratory animals have made great contributions to the field of immunology, the highly controlled laboratory setting ignores the effects of genetic and environmental diversity, including natural pathogen exposure. These elements of diversity are central to wild immunology which proposes that studies which embrace rather than exclude the genetic and environmental complexities of the natural world can have improved translational capacity for human and veterinary medicine.

Wild immunology speaks to the principles of ethical animal use in science as it encompasses the use of natural disease models in place of experimental models of disease. Animals naturally infected with disease can be studied in the wild or captivity and the research questions these natural disease models provide insights to include:

1. How particular species resist diseases that others succumb to e.g. bats as viral reservoirs; hyenas and rabies virus.
2. How animal diseases compare to their human equivalents e.g. feline immunodeficiency virus and human immunodeficiency virus.
3. The pathogenesis of disease that occurs in hosts infected by natural transmission e.g. bovine tuberculosis in badgers; avian influenza in wild birds.

Wild immunology and natural disease models are becoming increasingly relevant with the rise of emerging infectious diseases and the disease-induced decline of wild animal populations. The WHO estimates that 75% of pathogens to have emerged in the last 10 years are zoonoses. Understanding the pathogenicity of these diseases in their natural animal hosts is critical to addressing their effects on people. On a similar note, diseases continue to decimate wild populations of amphibians (chytridiomycosis), bats (white nose syndrome) and Tasmanian devils (devil facial tumour disease), to name a few. Studying these natural disease models will ideally benefit wildlife conservation along with advancing medical knowledge.

Devil facial tumour disease (DFTD) has attracted much scientific interest since it was first identified as a transmissible cancer in 2006. The conservation value of Tasmanian devils, which are threatened with extinction by DFTD, has been a driving force behind DFTD research. However, DFTD and other transmissible cancers are fascinating disease models because they are simultaneously a cancer, infectious disease and an allograft. They provide a natural model for studying cancer evolution, immune-evasion mechanisms and host immune responses. One of the inherent limitations in studying wild animals and the diseases that affect them is a lack of specific reagents and annotated genomes. This is continuing to be addressed in the DFTD research field.

Introduction

The limitations of traditional experimental animal models are widely acknowledged (van der Worp et al., 2010, Mak et al., 2014, McGonigle and Ruggeri, 2014). Natural disease models and wild immunology are research fields based in the "real world" and could therefore better serve translational veterinary and human medical research. These fields might also have long term conservation benefits for wildlife. Tasmanian devil (*Sarcophilus harrisii*) immunology research encompasses both fields and can be used as a case study to demonstrate what is achievable within the research constraints associated with these fields, and how the constraints are being overcome.

Natural disease models

The rise of 'omic technologies (i.e. genomics, transcriptomics, proteomics, etc) has prompted researchers to reconsider the use of experimental animal models (FitzGerald et al., 2018). Fitzgerald et al. point out that human medical research could be better served by incorporating the biological data sets produced from the 'omic analyses of DNA and RNA samples with human phenotypic information. This could determine disease risk and response to therapy on both an individual and population level.

Flies et al (Flies, 2018), in response to the essay by Fitzgerald et al, suggested broadening this perspective to include natural disease models. They said that such models whereby disease occurs in animals in their natural environment rather than being experimentally induced, have numerous benefits over more traditional laboratory-based experiments using traditional animal models. These benefits include: 1) a more diverse and representative study population; 2) generation of relevant research questions and insights unlikely to arise in the artificial laboratory setting; 3) the opportunity to positively impact on conservation issues e.g. where wildlife populations are threatened by disease.

Natural disease models have long been considered and can be divided into at least three subsets (Swearengen, 2018):

1) Negative models: species which are resistant to diseases that other species succumb to e.g. bats and > 50 viruses (Calisher et al., 2006, Zhou et al., 2016); Spotted hyenas (*Crocuta crocuta*) and rabies virus (East et al., 2001)

2) Surrogate models; diseases which have equivalents that affect other species e.g. feline immunodeficiency virus (domestic cat) and human immunodeficiency virus (Miller et al., 2018); canine transmissible venereal tumour and devil facial tumour disease (Siddle and Kaufman, 2012)

3) Models of natural disease transmission; e.g. bovine tuberculosis in badgers (Gormley and Corner, 2017); avian influenza in wild birds (Keawcharoen et al., 2011)

Wild Immunology

Wild immunology shares common ground with natural disease models, not least in its use of non-traditional study species. The premise of wild immunology is to embrace the genetic and environmental diversity that traditional immunology studies seek to minimise or eliminate. It has been described as "the missing link between laboratory-based immunology and human, wildlife and domesticated animal health"(Pedersen and Babayan, 2011). The complexity of the immune system is staggering; wild immunologists recognise that the depth of knowledge and understanding of the immune system could not have been achieved without the traditional reductionist experiments of classical immunology using genetically modified rodents in the controlled laboratory environment. However, technological advances now allow understanding of immunology and the study of immune responses in the "real world" amongst environmental and genetic diversities and complexities. There are any number of wild immunology studies being undertaken by dedicated laboratories across Australia, the UK and USA. Some results have implications for how laboratory-based study results are interpreted e.g. that immune defences of wild mice are more appropriately regulated than those of their domesticated laboratory cousins (Babayan et al., 2011).

The major limitations affecting both natural disease models and wild immunology studies are logistics and a lack of species-specific reagents. The ease and speed of developing species-specific reagents has been improved as genome sequencing has become more affordable and available.

Case study – Tasmanian devil immunology research

Tasmanian devil immunology became an exciting research field when it was demonstrated in 2006 that devil facial tumour disease (DFTD), a disease threatening the devils with extinction, was a transmissible cancer (Pearse and Swift, 2006). Transmissible cancers are immunological phenomena whereby the infectious cancer cells are passed directly between individual animals without being recognised as "foreign" by the animal's immune system and are therefore not rejected. Tasmanian devils and domestic dogs (*Canis lupus familiaris*) are the only mammalian species to be affected by such cancers (Metzger et al., 2016). The canine transmissible venereal tumour is an ancient lineage (Ni Leathlobhair et al., 2018), compared to the very recently emerged DFTD. The lack of rejection of the DFT cells by the devil's immune system raised three main hypotheses for their successful transmission: 1) devils lack sufficient genetic diversity to recognise the tumour cells as foreign; 2) devils have an inadequate immune system; 3) the immune escape mechanisms of DFTD drive transmission.

In time it would be shown that DFTD's immune escape mechanisms were primarily responsible for its transmission (Siddle et al., 2013). However, in 2006, very little was known about the devil immune system and so the Tasmanian devil immunology research group was established by immunologist Professor Greg Woods at the Menzies Institute for Medical Research, University of Tasmania. The group set out to describe the devil's immune system and assess its function. The paucity of available immune reagents for devil immunology was very apparent. The group made use of three species cross-reactive reagents: CD3 to identify T lymphocytes; CD79b to identify B lymphocytes; and MHC-II to identify antigen presenting cells. They also prepared their own polyclonal devil IgG. These reagents, in conjunction with some standard immunology techniques such as immunohistochemistry and mixed lymphocyte reactions allowed for the assessment of cellular and humoral immune responses, and the histological analysis of lymphoid tissues (Kreiss

et al., 2008, Kreiss et al., 2009a, Kreiss et al., 2009b). The group showed the devils had a functional immune system comparable to that of other mammalian species.

In 2012 the genome of both the devil and DFTD were sequenced (Murchison et al., 2012). This opened the door to reagent development and genomic, transcriptomic and proteomic studies (Morris and Belov, 2013, Howson et al., 2014, Patchett et al., 2015). A method to synthesise devil-specific recombinant proteins and cytokines has been developed (Flies, paper in prep). These FAST (fluorescent adaptable simple theranostic) proteins are being used to explore receptor ligand interactions in devils that are known to occur in humans and mice. The method is easily adapted to produce proteins for most eukaryotic species, thus enabling studies on other non-traditional species.

In summary

While not all research questions can be answered with natural disease models, their use should be considered because of the potential benefits offered by such models. These benefits include the likelihood of improved translational results for veterinary and human medical research; the unique insights raised; and the opportunity to positively impact wildlife conservation.

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A stimulating story – exploring how magnets can influence brain circuitry

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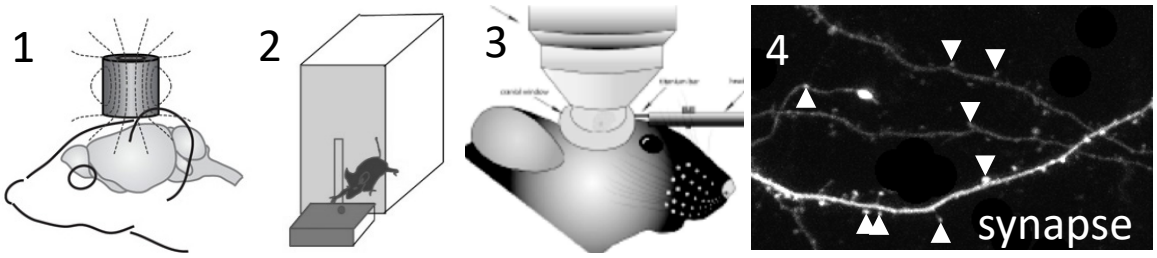
Modulation of brain circuitry with repetitive transcranial magnetic stimulation (rTMS) is gaining popularity in both clinical and non-clinical settings. Typically used as a treatment for depression, or by 'gamers' to enhance their performance, repeated exposure of the brain to complex stimulation patterns have been shown to induce long lasting effects. Somewhat surprisingly, the biological mechanisms underpinning any functional changes to the brain remain poorly understood.

To understand how magnetic stimulation influences the brain, we position a custom-built coil directly over the mouse head to generate low intensity magnetic fields in the underlying brain (Fig 1). The magnetic field interacts with electrically active nerve cells thereby changing their activity. We have combined the use of the magnetic coils with behavioural tests and live brain imaging to try to understand how the magnetic fields change the connectivity of the brain.

To determine whether low intensity (LI) rTMS alters behavioural performance, we delivered daily stimulation or sham stimulation as a priming stimulus to mice completing 10 consecutive days of skilled reaching training. Mice were placed in a clear plexiglass chamber and trained to reach through a narrow slit to retrieve a chocolate pellet (Fig 2). Relative to sham, by applying LI-rTMS before each training session, mice showed increased skill accuracy (~9%) but did not alter the rate of learning over time. In contrast, by applying the LI-rTMS after each training session, there was a small increase in the rate of learning over the 10 day trial. These results suggest that LI-rTMS can alter specific aspects of skilled motor learning in a manner dependent on the timing of intervention.

We have also combined magnetic stimulation with live imaging of the mouse brain. To see the brain circuitry in the mouse brain, we surgically implant a window overlying the brain of a transgenic mouse with fluorescent neurons. By placing an anaesthetised mouse under a multiphoton microscope we can see the neurons in the upper most parts of the brain, the cerebral cortex (Fig 3,4). High magnification imaging allows us to see individual synapses, communication points that link neurons together. These connection points can change over time, and in response to experience and learning. Using this imaging technique, we can directly observe how brain connectivity changes in response to magnetic stimulation.



No manuscript was submitted for this presentation

Managing disease in the Orange- bellied Parrot metapopulation

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The Orange-bellied Parrot (*Neophema chrysogaster*) is a critically endangered psittacine species that migrates annually between south-west Tasmania and south-east mainland Australia. Its decline over three decades or more has been attrubuted to a number of threatening processes including predation, competition from other avian species, breeding and foraging habitat loss and modification, dimished genetic diversity and significantly disease.

Orange-bellied Parrots sourced from multiple captive breeding institutions are translocated to supplement the wild population as one of a number of managment interventions in an attempt to mitigate species decline. Hence disease management and consideration of transmission risk needs to consider in both captive and wild birds as a metapopulation. Orange-bellied parrots from captivity and the wild are monitored and screened for signifcant disease threats and the program is advised by the expert Veterinary Technical Reference Group. A number of diseases and health issues have been identified which has on occasion resulted in morbidity and mortality events. Disease risk assessment is used to determine optimal viable health screening protocols and can also be used to guide disease management. Disease should be considered as one of a number of stochastic events with potential to result in further decline in Orange-bellied parrots, therefore disease management is integral within a wholistic species management framework.

No manuscript was submitted for this presentation

Bruny Island cat management plan

Jennifer Pelham

Ten Lives Cat Centre

Abstract

Bruny Island is located off the south-eastern coast of Tasmania and is of high environmental value. The island is home to a diverse range of threatened native mammals, bird species and to a population of approximately 600 people. Of the native animals that make Bruny island of conservation importance, at least 13 native mammals and 50 native bird species are at risk from cat predation.

So how do we protect the islands unique native fauna from predation by cats and paradoxically protect the welfare of domestic cats owned by the community?

The Bruny island cat management plan is an exciting project that approaches these problems by the implementation of the Bruny Island Cat By-**l**aw, monitoring, research and community engagement. Animal ethics approval for the plan has been provided by The Department of Primary Industries, Parks, Water and Environment (DPIPWE) Animal Ethics Committee (AEC) to ensure the highest animal welfare standards are met through every stage of the plan.

The plan is a collaborative approach, supported by a range of partners including, but not limited to, the local conservation groups, University of Tasmania and Kingborough council, as well as State and Federal Government. Each partner is vital to the plan and to achieving the common goal. The Ten Lives Cat Centre is proud to be one of the partners of this multidisciplinary team and involved in a management plan that promotes the welfare of both cats and native wildlife.

The Bruny Island cat management plan aims to become a learning tool and framework for other islands, regions or areas- whose ecosystems are threatened by cat predation.

Bruny Island is located off the south-eastern coast of Tasmania and is of high conservation value. The island is home to a diverse range of threatened native birds and mammals, of which at least 13 native mammals and 50 native bird species[i] are at risk from cat predation. Some of these native bird species reside at the Neck, an isthmus of land that connects the north and south of Bruny Island. The Neck is covered by vegetated sand dunes and is key habitat for migratory birds such as the short-tailed shearwater, resident species the little penguins and the endangered hooded plovers.

Bruny Island is of high environmental importance as islands play a critical role in the conservation of Australia's native fauna. The 'Threat abatement plan for predation by feral cats'[1], published by the Australian Government, Department of Environment, outlines that feral cats are among the greatest threats to the conservation of island species. The plan is tied in with the Australian

[1] Commonwealth of Australia (2015). Threat abatement plan for predation by feral cats. Department of the Environment, Canberra. https://www.environment.gov.au/biodiversity/threatened/publications/tap/threat-abatement-plan-feral-cats

Government's 'Threatened Species Strategy'[2]. The Threatened Species Strategy is an action-based approach to protecting and recovering the nation's threatened plants and animals. Under this strategy the Federal government elected Bruny island as 1 of 5 Australian islands to begin feral cat eradication. The island was selected due to its high conservation values, support from community, environmental and research partners. Cohabitating with these native species on this island, is approximately 600 residents, some of which own beloved companion cats, the very species posing a significant threat to the biodiversity of the island.

So how do we protect the islands unique native fauna from predation by cats and paradoxically protect the welfare of domestic cats owned by the community?

The Bruny island cat management plan is an exciting project that is approaching both issues in a holistic manner. The plan is a collaborative approach, with support and funding by a wide range of partners including local conservation groups, University of Tasmania, the local Kingborough council, State and Federal Government. These partners are working together to achieve the goals set by the management plan– which in summary is to minimize the impacts of cats (domestic, stray and feral) on the island's biodiversity and promote responsible cat ownership.

All cats, feral, stray and domestic are the same species, felis catus. However, these titles can be defined according to how they live and their relationships with humans. The definitions and categories used in the 'Threat abatement plan for predation by feral cats' provides a good understanding of the differences between feral, stray and domestic cats.

Feral cats. Feral Cats are those that live and reproduce in the wild and survive by hunting or scavenging; none of their needs are satisfied intentionally by humans.

Stray cats. Stray cats are those found in and around cities, towns and rural properties; they may depend on some resources provided by humans but are not owned.

Domestic cats: Domestic cats are identified as owned, most or all of their needs are supplied by their owners, including shelter, food and social interaction.

Community support and responsible cat ownership

Eradication of feral cats alone does not address the impact that domestic cats also have on the environment. Iimprovements in the management of domestic and stray cats is necessary to prevent them becoming part of the feral cat population. Thus community education and promoting responsible cat ownership is vital to the success of the Bruny Island cat management plan.

Kingborough Council, with funding and/or support from Ten Lives Cat Centre (TLCC), Bruny Island Environment Network (BIEN) and Bruny Island Community Association (BICA) held workshops and consultation with community members to identify how best to both minimize the environmental impacts of cats and address feral cat eradication.

[2]Australian Government's 'Threatened Species Strategy
http://www.environment.gov.au/biodiversity/threatened/publications/strategy-home

The Kingborough Council conducted a survey**,** The Bruny Island Cat Management Survey'[3] of 157 island residents and non-resident rate-payers (24 cat owners, 133 non-cat owners) to measure community attitudes towards the management of cats on Bruny Island. The survey found that the majority of the residents and rate payers are in support of feral cat eradication and domestic cat management on the island.

Bruny Island Cat By-law

To reinforce the goals of the Bruny island cat management plan the Kingborough council had the initiative to create the Bruny Island Cat By-Law 2018[4] . The by-law will begin to phase-in in July 2019 and will be enforced by the Community Ranger. The Bruny island Cat By-law includes the following legislation;

- compulsory desexing and microchipping of domestic cats
- 24- hour containment of domestic cats to owners property
- a limit of 2 cats per household (unless permit is granted for additional cats)
- prohibition on the feeding of stray or feral cats.

The Community Ranger position was created to undertake community liaison and education with the Bruny Island community. The physical presence of the Ranger on the island will motivate cat containment through enforcement and will also promote the legal rights of community members in relation to the humane treatment of all cats.

The collaborative approach from Kingborough Council, Ten Lives Cat Centre (TLCC), Bruny Island Environment Network (BIEN) and Bruny Island Community Association (BICA) has resulted in some exciting projects that support the community needs, through education, employment and ownership; including the following projects;

Inside with Cats[5]

Inside with Cats is a partnership between Kingborough Council, Ten Lives Cat Centre, Tasmanian Conservation Trust and the Bruny Island Environment Network. It is a series of stories that demonstrates the benefits of cat containment from a cat owner's perspective. The goal of the videos is to demonstrate how cat containment not only protects the environment but also benefits the health and welfare of the domestic cat. The videos showcase various approaches (incl outdoor enclosures, fenced yards, etc) to cat containment that meet the physical and emotional needs of the domestic cat.

[3] https://www.kingborough.tas.gov.au/wp-content/uploads/2017/05/Cats-on-Bruny-Survey-Results.pdf
[4] https://oursay-files.s3.ap-southeast-2.amazonaws.com/uploads_production/forum-file/bruny-islandcat-by-lawfinaldec-2018_2077.pdf
[5] https://www.kingborough.tas.gov.au/2018/03/inside-with-cats/

The Bruny Island District School

The Bruny Island District School has created promotional stickers and fridge magnets on responsible pet cat ownership (RPO). The stickers and postcards are targeted to Bruny residents and visitors from a child's perspective. They aim to support long term attitudinal change among current and future cat owners.

Assistance and advice on cat containment

Support is being offered to cat owners to help them and their cat adjust to 24 hour containment. Assistance and advice is being provided on a variety of designs and construction methods of cat enclosures. Advice from animal behaviorists is also available in regards to recognizing any stress induced behaviors that arise during the transition period and ways to address/ alleviate them.

Education on feeding strays/ ferals

The Kingborough council is working with individuals who feed stray and feral cats on the island. This act of kindness results in an increase in the feral cat population, which continue to impact on native wildlife (through predation and disease). Most stray cats are not desexed and may become part of the feral population directly or through following generations.

Ten Lives Cat Centre

The Ten Lives Cat Centre (TLCC)[6] is excited to be a partner of the Bruny island Cat management Plan, as the plan reflects our own philosophies, 'helping cats to be a positive part of the community'. Ten Lives is a not for profit organization dedicated to caring for and re-homing unwanted cats and kittens, improving cat welfare and encouraging responsible cat ownership.

In 2017 we changed our name from the Hobart Cat Centre to the Ten Lives Cat Centre to better reflect what we do, which is represent cats, the environment and the community. Our goals are:

- Promote and improve the welfare of cats and kittens
- Promote responsible cat ownership and humane animal welfare principles
- Shift public attitude towards the welfare of their cats so that there are no stray, abandoned, lost, unwanted or feral cats
- Facilitate successful adoptions of cats and kittens into appropriate homes
- Promote containment of cats- to improve the welfare of cats, community relationships and protects birds and wildlife
- Reduce unnecessary euthanasia of cats and kittens

The cat management plan also reflects our goal to provide education through Tasmanian schools, on responsible cat ownership, cat welfare and protecting the environment. Our Edu.cat[7] program

[6] http://www.tenlives.com.au/about.html
[7] http://www.tenlives.com.au/educat.html

is an Australian- first Feline Education program designed to create social change in how people care for cats and protect wildlife.

To ensure the welfare of all cats from Bruny island are met, a cat assessment and holding facility has been built on the island. The facility is being funded by TLCC and Kingborough Council and will provide a place to assess all cats captured on the island. It will be used as a site to perform de-sexing and microchipping for residents' cats and to hold and care for cats (for re-claiming, adoption or euthanasia). The facility will help to reassure cat owners that any domestic cats trapped under the By-laws or the feral cat trapping program will be safely returned to them.

Since our involvement in the Bruny Island cat management plan we have de-sexed, microchipped and rehomed 41% of stray cats from Bruny Island. Our de-sexing and rehoming program will continue to provide a reduction of the numbers of unwanted and stray cats on the island.

Animal welfare and ethics

The welfare of cats involved in the Bruny Island cat management plan is vitally important. As stated in the Cat Management Act[8], all cats must be treated humanely whenever they are the subject of cat management actions including trapping, holding and euthanasia, regardless of whether they are classed as feral, stray or domesticated.

Holding

The creation of the Bruny island cat holding and assessment facility will greatly reduce stress and improve the welfare of cats on the island, by eliminating the need for long distance travel to Ten Lives Cat Centre. Cats trapped and held for assessment will treated humanely under the Cat Management Act.

Trapping

Any method of trapping cats must be humane. The traps currently used in the plan are rectangular wire cages that are operated by the cat touching a metal plate (treadle) on the floor of the trap with either a drop down or hinged swing-style door. Cats trapped must be transferred to a cat assessment and holding facility within 12 hours, or euthanized (if in applicable under the Cat Management Act 2009).

Euthanasia

The Cat Management Act 2009 outlines legislation regarding the humane euthanasia of cats.

Research and monitoring

An important step in the management of feral cats on the island was to gain better understanding of their density and distribution over the island. Research conducted by Mathew Pauza (wildlife biologist with DIPIPWEs Invasive species branch) *Feral cat density and distribution on North*

[8] https://www.legislation.tas.gov.au/view/whole/html/inforce/current/act-2009-089

Bruny has involved attaching GPS collars[9] to feral cats that have been captured in the Neck area. Six feral cats have been fitted with GPS collars and their locations logged. This data provides new and important information about feral cat's movements, how far they roam and where they spend most of their time. This data helps to monitor patterns and seasonal movements over time. These vulnerabilities can be used to target the best locations and time of the year to place traps, in the most efficient and economical way. in addition to the GPS tracking, remote cameras were placed across sites on North Bruny to monitor for the presence of feral cats. Camera footage of individual feral cats has helped to more accurately estimate their density across North Bruny. The camera data has shown that feral cats numbers are higher in the North than was previously assumed, based on prior research and community cat sightings.

A University of Tasmania honours project by Caitlan Geale[10], in 2017 also deployed remote cameras at and adjacent to the Neck and Whalebone Point seabird colonies. High cat densities were recorded at the Neck, both during and after the shearwater breeding season, which indicates that feral cats use the Neck rookery as a major food source both when shearwaters are present and absent. This information suggests that rodent and penguin populations may be high enough to maintain cat presence in the rookery throughout the year. A secondary honours project by the university of Queensland is underway to estimate the numbers of invasive rodents at the neck and any changes in density and adverse impacts as a result of feral cat control.

In addition to monitoring feral cats, species monitoring programs are underway to gain baseline estimates that can be compared with population numbers after cat eradication occurs. Birdlife Tasmania[11] is undertaking annual field surveys of Short-tailed Shearwaters, Little Penguins and Hooded Plovers at the Neck and adjacent areas. The aim is to assess the impact of feral cat control on these populations over time.

Monitoring and research in conducted as part of the Bruny Island cat management plan to improve our understanding of the distribution and ecology of feral cats across the entire island and assess the impact of cat control on the rest of the ecosystem.

Conclussion

The Bruny island cat management Plan recognizes that the management of cats (feral, stray and domestic) is a shared responsibility between conservation groups, local, state and federal government and the community. This collaborative approach between many partners, has facilitated further research, education and employment. The Plan aims to become a learning tool and framework for other islands and parts of Tasmania, whose ecosystems are threatened by cat predation. The plan also promotes the welfare of domestic cats and the benefits of responsible cat ownership.

[9] The GPS collars are designed with brass and magnesium bolts which corrode over time and break, approximately 18 months.

[10] (University of Tasmania Honours project supervised by Associate Professor Menna Jones and Professor Chris Johnson)

[11] http://birdlife.org.au/locations/birdlife-tasmania

The Bruny Island cat management plan aims to;

- Assess and manage impacts of cats on the island
- develop a long term cat management strategy.
- Promote responsible cat ownership (inc phase-in of the Bruny Island Cat By-laws).
- Conduct monitoring and research of;
  – Short-tailed Shearwaters, Little Penguins and Hooded Plover populations
  – Feral cats and other predator populations and activity at the Neck
  – Tracking feral cats to understand feral cat density and distribution on North Bruny
- Control of stray and feral cats (inc. rehoming of stray and unwanted cats)
- Develop long term community involvement and ownership of the program

Feasibility

A feasibility study[12] has been produced by John Parkes of Kurahaupo Consulting to assess whether feral cat eradication of Bruny Island is viable using current methods without causing legal, social or environmental risks. The study outlines the methods, risks and costs of a range of options, from island wide eradication to the management of feral cats in priority areas across the Island, such as the Neck.

Partners of the Bruny Island Cat Management Plan

- the Kingborough Council
- Department of Environment (DoE)
- DPIPWE (Invasive Species Branch (ISB) and PWS)
- Ten Lives Cat Centre (TLCC)
- UTAS (Biological Sciences)
- Bruny Island Environment Network (BIEN)
- Bruny Island Community Association (BICA)
- Bruny Island District School
- Tasmanian Land Conservancy (TLC)
- weetapoona Aboriginal Corporation (wAC)
- Threatened Species Recovery Hub (TSRH UQ)
- private landowners (incl “Fairyland”)
- Tasmanian Conservation Trust (TCT)
- Birdlife Tasmania (BLT)
- Inala Nature Tours
- Pennicott Wilderness Journeys and Bruny Island Coastal Retreats.

The author would like to thank Kaylene Allan, Cat Management Officer, Kingborough Council, for sharing her extensive information on the Bruny Island Cat Management Plan.

[12] https://www.kingborough.tas.gov.au/wp-content/uploads/2019/03/Bruny-Feral-Cat-Feasibility-Report_FINAL_2019.pdf

Resources

- *Bruny island cat management project*, (august 20, 2018) Article by Kaylene Allan - Cat Management Officer Kingborough Council August 20, 2018 http://www.tasconservation.org.au/tas-conservationist/2018/8/23/bruny-island-cat-management-project
- Kingborough Council Website https://www.kingborough.tas.gov.au
- *The Cat Management Act 2009*, Tasmanian Government https://www.legislation.tas.gov.au/view/whole/html/inforce/current/act-2009-089
- *Tasmanian Cat Management Plan*, Department of Primary Industries, Parks, Water and Environment (2017), Tasmanian Government. https://dpipwe.tas.gov.au/Documents/TASMANIAN%20CAT%20MANAGEMENT%20PLAN%20FINAL.pdf
- *Threat abatement plan for predation by feral cats.* Commonwealth of Australia (2015). Department of the Environment, Canberra. https://www.environment.gov.au/biodiversity/threatened/publications/tap/threat-abatement-plan-feral-cats
- *Threatened Species Strategy*, Australian Government (2015) http://www.environment.gov.au/biodiversity/threatened/publications/strategy-home
- *Bruny Island Cat Management Survey Results* (March 2016) https://www.kingborough.tas.gov.au/wp-content/uploads/2017/05/Cats-on-Bruny-Survey-Results.pdf
- https://www.kingborough.tas.gov.au/wp-content/uploads/2018/12/Bruny-Community-Engagement_Cat-Management_Aug-2018.pdf
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- https://www.kingborough.tas.gov.au/wp-content/uploads/2017/05/The-Law-and-Cat-Trapping.pdf
- https://www.kingborough.tas.gov.au/2018/03/inside-with-cats/
- http://www.tasconservation.org.au/tas-conservationist/2018/8/23/bruny-island-cat-management-project
- *COMMUNITY ENGAGEMENT Bruny Island Cat Management Project*, Dr Lynette McLeod (August 2018)
- https://www.kingborough.tas.gov.au/wp-content/uploads/2018/12/Bruny-Community-Engagement_Cat-Management_Aug-2018.pdf
- https://www.kingborough.tas.gov.au/services/animal-management/cats/cats-bruny-island/
- https://oursay-files.s3.ap-southeast-2.amazonaws.com/uploads_production/forum-file/bruny-islandcat-by-lawfinaldec-2018_2077.pdf
- http://www.tenlives.com.au/about.html
- http://www.tenlives.com.au/educat.html
- https://www.legislation.tas.gov.au/view/whole/html/inforce/current/act-2009-089
- http://birdlife.org.au/locations/birdlife-tasmania
- https://www.kingborough.tas.gov.au/wp-content/uploads/2019/03/Bruny-Feral-Cat-Feasibility-Report_FINAL_2019.pdf
- https://www.bien.org.au/category/project/cat-management/
- http://www.tasconservation.org.au/tas-conservationist/2018/8/23/bruny-island-cat-management-project

Meeting the challenges of monitoring remote sites

Johanna Toia

Category A member on the DAF Qld. Community Access AEC

Abstract

The Department of Agriculture and Fisheries (DAF) maintains two AECs, the Staff Access (SA) AEC and the Community Access (CA) AEC. The Staff Access Committee deals predominantly with applications from departmental staff as well as applications from external applicants wishing to conduct an activity involving the use of animals on a departmental site.

On the other hand, the Community Access Committee predominantly deals with applications from non-departmental investigators wishing to conduct scientific activities involving the use of animals in Queensland. Applications are also received from Departmental Staff and external applicants wishing to conduct fish and pest animal related activities.

Although the Community Access AEC receives a variety of project applications, the majority deal with wildlife and a significant number are wildlife surveys. These can come from virtually anywhere in Queensland providing all kinds of challenges to the AEC in meeting their monitoring responsibilities under the code.

Monitoring of remote sites is a challenge faced by many AECs.

This paper will focus on the monitoring processes utilised by the Community Access Committee particularly when dealing with remote sites.

These processes include:

- Site visits - the importance and benefits of site visits as well as the challenges of coordinating these visits in a range of areas across the state
- The use of photos and videos for reporting processes
- Annual reports
- Final reports
- Dealing with unexpected adverse events

The Department of Agriculture and Fisheries (DAF) maintains two AECs, the Staff Access (SA) AEC and the Community Access (CA) AEC. The Staff Access Committee deals predominantly with applications from departmental staff as well as applications from external applicants wishing to conduct an activity involving the use of animals on a departmental site.

On the other hand, the Community Access Committee deals predominantly with non-departmental investigators wishing to conduct scientific activities involving the use of animals in Queensland. Applications are also received from departmental staff and external applicants wishing to conduct fish and pest animal related activities.

Although the Community Access AEC receives a variety of project applications the majority deal with wildlife. A significant number are wildlife surveys. These, as with all the applications, can come from virtually anywhere in Queensland providing all kinds of challenges to the AEC in meeting their monitoring responsibilities under the code.

Monitoring of remote sites is a challenge faced by many AECs.

This paper will focus on the monitoring processes utilised by the Community Access AEC particularly when dealing with remote sites.

In 2017/18 there were some 124 applications approved by the Community Access AEC, 118 external and 8 from DAF staff.

The majority of applications approved included teaching projects, wildlife surveys, efficiency trials, and demonstration of techniques through workshops. More than 50% came under environmental studies while a further 11% had educational outcomes.

Monitoring the Care of Animals (2.3.17 – 2.3.23)

The Code is very clear regarding monitoring the care of animals used in Institutions such as Universities where there are breeding and holding facilities. However, the requirements for monitoring work done at remote sites is far less clear.

The code states:

> The AEC monitors the care and use of animals by inspecting animals, animal housing and the conduct of procedures, and/or reviewing records and reports.

This first item is relatively easy to implement when dealing with animal houses and research being conducted in Institutions but as indicated, becomes much more difficult when dealing with remote sites. In the majority of projects approved by the Community Access AEC, animals are not held for any extended length of time or for any time at all. These projects include a significant number of wildlife surveys utilising techniques such as observation, photographs, call playbacks, videos and various traps where animals are caught, identified and released at the site of capture.

If we take the item 2.3.17 in isolation, it could be argued that reviewing records and reports could be the options used, but is this in keeping with the intent of the code?

In the case of the observation only type surveys, this may be true and in some extreme situations, this may be the only option.

A better option is to use a number of approaches to monitoring, including records and reports, which are more in keeping with the context of the whole code and in particular, the section on Monitoring the Care and Use of Animals (2.3.17 – 2.3.23). Time for this presentation does not allow an in depth covering of all these points, however they will be referred to with each monitoring approach used by the Community Access AEC.

Records and Reports covered by the Community Access AEC include:

- Annual Reports
- Final Reports
- Unexpected Adverse Event reports

This is no different to other AECs.

Annual Reports

Annual reports do help to create a picture of what is happening in remote sites and a number of investigators do supply photos.

This is a practice to be encouraged. On occasion, investigators have provided video footage of their activities. There have also been times where the AEC has requested a video to provide a much clearer picture of the actual procedures being undertaken.

Final Reports

Final reports provide more extensive information on the total number of animals used and the outcomes from the project including any published articles as a result of the project. Again, photos or videos may be provided.

In both types of reports the AEC will acknowledge those investigators who supply clear and informative reports.

Unexpected Adverse Events

Much can be learnt from unexpected adverse event reports. Obviously, we do not want to see adverse events, but on the positive side when the event is properly investigated and processes are put into place to avoid reoccurrence of the specific event or the risk of adverse events is reduced, this must be a considered a positive outcome.

The AEC has requested updates on the success of implementation of action plans and have at times been able to visit the site or investigators during remote location visits in that region.

Site Visits

The importance and benefit of site visits cannot be emphasised enough. It is not just about seeing the work being done in the field, but it also provides the opportunity to talk directly with investigators and find out what concerns or questions they may have. It also provides the opportunity to clarify any areas of confusion.

In 2017/18 there were some 37 site visits conducted by DAF AECs. When travelling to a location such as Cairns the number of site visits are maximised by looking at programs not only in Cairns but also in the surrounding areas such as Atherton Tableland and Kuranda. This means that several site visits can be completed over a number of days.

Although in this paper the focus is on wildlife surveys and environmental studies, there are a range of projects which include, but are not limited to koala research, control of pest animals including pigs and a number of fish research projects.

Koala projects include the attachment of collars and tracking the movement of koalas in specific areas and studying the effects of Chlamydia infections.

Given that both DAF AECs have projects covering the whole state sometimes the AECs will cover site visits for projects approved by the other AEC. This adds to the capability to cover a wider range of projects and areas.

Applications have been approved in the following regions:

- Brisbane and near Brisbane region
- Bundaberg
- Longreach
- Gladstone
- Rockhampton
- Mackay
- Townsville
- Cairns
- Atherton Tableland
- Weipa
- Remote North Queensland Islands

Many applications submitted by various companies to conduct wildlife surveys are general in their location as they are dependent on what work contracts these companies acquire, meaning they could occur anywhere in the state or region stated on the application.

As well as location, the timing of surveys is also not known at the time of application, making the setting up of site visits an even bigger challenge. The AEC is always notified of surveys but often there is insufficient time to arrange a site visit particularly in more remote locations.

Site visits in 2017 were conducted in a number of areas throughout the state including:

- Brisbane area
- Cairns
- Atherton Tablelands
- Weipa
- Longreach
- Caboolture and Bribie Island
- Gatton
- Emerald
- Townsville
- Oakey

Wherever possible a C or D involved in the site inspections as per the requirements of the code (2.3.20).

The following is a brief overview of the site visit report document. The site visit process was developed in accordance with 2.3.17 to 2.3.22 of the code.

Areas covered by the site visit include but are not limited to:

- Identification of animals;' general health; behaviour and social contact; and monitoring of animals in accordance with the AEC approval;
- Environmental factors such as the state and repair of buildings; cages; pens/ponds/tanks; and fences; yards and handling and restraint facilities;
- Water supply and equipment; food supply and equipment as well as power supply; lighting and ventilation in facilities where animals are held for extended periods of time;
- General hygiene and cleaning.

As well as the above areas, records and documentation are also checked.

This includes:

- Staffing records including competency records to ensure animals are handled by appropriately experienced and skilled staff.
- SOPs
- AEC Approvals
- Emergency Contact Details
- Maintenance Records.

Questions are raised during the visit to ensure compliance with the AEC approval.

The site visit document and process were originally developed to cover sites where animals may be held for periods of time and for locations which can be relatively easily accessed. However, the question remains, what happens when animals are not kept and/or sites cannot be readily accessed? What happens with wildlife surveys where there is an extensive range of locations and notification of survey dates, which does not allow sufficient time to organise a site visit?

A significant number of surveys are observational only with minimal invasiveness. Techniques used include direct observation; spotlighting; photographs and the use of video cameras. In these cases, a direct site visit may not be needed.

Another option used by the AEC, is to visit the offices of the company which may be in one of the centres, or to visit an investigator who may have a home office. This approach has been used for organisations undertaking wildlife surveys through trapping where the AEC cannot attend a survey, or those projects where observational studies are used.

The site visit document is still used but adapted to each situation and can include the following:

- Inspection of equipment being used for surveys
- Investigators may give examples of the use of equipment
- Hygiene protocols are discussed
- Procedures for setting up traps and ensuring records of locations of traps are sighted
- Photographic records of activities undertaken
- Records and Documentation are examined to ensure competency of investigators and the following of protocols as per the AEC approval.

Videos and photographs

AEC can request videos to look at activities and clarification of any procedures at any time. A number of videos have been received which have been extremely informative. The decision to request a video is made on a case by case basis and is not, at this stage, a standard requirement.

The University of Southern Queensland AEC has taken a further step and have included a number of special conditions of approval for off-site projects in Australia. They have also done the same for overseas projects.

One of these special conditions is:

(d)

> Video (and where practical photographic) demonstration of an activity being undertaken within the field by an investigator is to be undertaken. At least one demonstration of each activity undertaken should be made available to the USQ AEC as soon as is practicable after conducting an approved activity or field trip. Video and photographic records are to be uploaded to the QRIS Cloud site for this project.

It then goes on to give the details on how this is done.

Making this a condition of the approval places a direct responsibility on the investigator. Given the available technology such as go-pro this should not be too much of an impost.

An example of a video demonstrating the use of an Elliot trap on site had been provided by a B member of the DAF AEC for the conference presentation. This video is an example of the type of video which can be used for monitoring and covers a number of key points including:

- Description of the trap and its uses
- Factors to be taken into account when using the trap such as location and behaviour of the animal
- Weather, temperature and other environmental factors
- Preparing the bait making sure gloves are worn
- Placing the trap
- GPS recording of location of traps
- Use of tape on trees to indicate location of the traps
- Distance between traps and number of traps set
- Checking traps twice a day and timing
- Identification of any animals caught in the trap and quick release
- Cleaning of traps.

In this paper we have covered a number of options used by the DAF Community Access AEC for monitoring remote sites.

This journey will continue as AECs continue to find more effective ways to meet the requirements of the code and promote best practice in the care of animals used in research and teaching in remote sites.

Biases in animal modelling of stroke

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Abstract

Researchers using animal models as surrogates for human diseases often focus their attention on just one organ. This might be the brain, the liver, kidneys, muscles or pancreas. These individual tissues are avidly collected at the end of an experiment but the remainder are usually wasted. All of the tissues from at least half of the animals from these experiments, the non-diseased control cohorts, are of value to researchers from other disciplines. If a more holistic approach is taken to the research questions being asked, for example how does stroke influence the function the heart or muscles? then the tissues specific to one discipline are also relevant to others. Moreover, tissues from animals used for breeding programs, for example novel transgenic strains of mice, could be used (but are generally not) to better describe the natural history of the diseases they are designed to emulate. Tissue banking and sharing of tissues amongst researchers and institutions can reduce some of this waste. However, the logistics of collecting, storing and making available tissues for later use can be daunting. Tissue microarrays which collect small proportions of each tissue and place them in fixed paraffin blocks which can be organised to suit the needs of secondary tissue users can provide can provide a cost effective solution to these later problems. The presentation will describe the technologies available and make the case for a scalable biobanking and issue microarray program.

The human brain is the most complex product of ~3.7 billion years of evolution[1]. For the last ~1.5 billion years, adaptation to a changing oxygen environment has been a critical driver of our evolution[2]. While advantageous in terms of energy efficiency, reliance on oxidative phosphorylation comes at a cost placing the human brain at enormous risk[2].

Ischemia is a risk to both children and adults. At any age, if blood supply is stopped completely (cardiac arrest, strangulation), or if oxygen is removed from the environment (asphyxiation), death can occur within minutes. Despite improvements in resuscitation, as few as 12% survive out-of-

hospital cardiac arrest[3]. Perinatal ischemia is the biggest cause of non-infectious infant mortality and potential life-long disability[4]. In later adulthood, ischemic stroke is one of the main causes of death and disability[5] and low-level but persistent vascular compromise contributes to the dementias[6]. Finding solutions to these problems is perhaps medicine's greatest challenge.

However, because access to the living human brain is limited, most neuroscience knowledge comes from surrogate studies in other mammals. Unfortunately, translation of this knowledge of other mammal's biology into therapies for human brain diseases has proven particularly difficult over many decades. This is true for stroke, Alzheimer's disease, Parkinson's disease, motor neuron disease, many epilepsies, spinal cord injury and traumatic brain injury.

Two plausible explanations for this are that: 1) Animal experimentation has been compromised by poor construct validity and poor execution of the experiments[7-12] or 2) Experimental animal brains are sufficiently different from human brains in terms of neuron density[13], arbor size[14], dendritic spine density[15], cell types present[16, 17], gene expression[18], alternative splicing[19] and injury responses[20] to compromise human drug discovery.

In this very personal narrative review, I will summarise the evidence for different types of biases in stroke research and how they are likely to have influenced drug discovery for this important disease. While the focus is stroke, many elements of this review are equally pertinent for other fields on neuroscience research.

Stroke is one of the leading causes of death and disability. Worldwide ~15 million people have a stroke each year, 6 million die and ~30% of the estimated 62 million stroke survivors have significant neurological impairment[21]. The cost of stroke is also high, with estimates for the developed world reaching A$1475 billion a year[22]. Despite decades of research we still have only one licensed drug for acute ischemic stroke therapy, thrombolysis with recombinant tissue plasminogen activator (tPA). Unfortunately, because of uncertainty about diagnosis, time of onset and the risk of cerebral bleeding, even in specialized centres, only ~10% of ischaemic stroke patients are treated with tPA. Moreover, half of those treated remain dependent or die[21]. While surgical clot removal has shown promise, there remains substantial unmet need, thus even modest gains from new drugs would provide great benefit[22].

Large numbers of novel drugs appear to show benefit in preclinical models of stroke, but the track record of translation to the clinic is poor[12]. In the past we could posit that this might be explained

in part because clinical trials were being conducted too late, when the therapeutic window identified in animals had closed. This is no longer the case and recent clinical trials conducted early after stroke onset have also failed[23-25].

Since most failed trials have been testing the concept of cyto/neuro protection, it has been argued that this concept is flawed. However, evidence from both stroke and other human diseases contradicts this nihilism. Firstly, the natural history of stroke and the success of thrombolysis and thrombectomy all indicate that in many patients, relatively large volumes of brain tissue survive wide-spread ischaemia for long enough for hospitalisation and therapy to be practical[26-30]. The marked time-dependence of good outcome after thrombolysis[29] contradicts the idea that cell death is particularly rapid but instead suggests a relatively prolonged process providing a window of opportunity for therapy. Viable slice cultures of human motor cortex in which neurons survive can be obtained for up to 8 hours after death[31].

Few forms of transplant surgery would be possible without the protective effects of hypothermia during organ harvest and transfer[32]. Therapeutic hypothermia is routinely used to protect the brain during aortic arch surgery, affording 20-30 minutes of safe surgical time during complete circulatory arrest[33]. It is also used to protect the brain in adults with global ischemia after cardiac arrest[34] and in neonates with hypoxic-ischemic encephalopathy[35]. While it might be argued that the neonatal brain is a special case, neuroprotection of ischaemic adult brains of patients with similar age and risk factor profiles to stroke patients shows that stroke neuroprotection should be possible. If neuroprotection is a reality, we must find another explanation for poor translation in stroke research.

If the concept of neuroprotection is not flawed, what else might explain poor translation in stroke research?

Systematic reviews and meta-analyses suggests that over optimistic reporting of therapeutic potential offers a partial explanation because a high proportion of researchers fail to prevent bias in experimental design[21, 36] or use cohort sizes too small to provide adequate statistical power for their animal experiments[21, 36]. Others perform only those experiments most likely to provide a positive outcome and ignore more difficult experiments in animals with comorbidities prevalent in the clinic (e.g. old age, hypertension, diabetes) and known to yield smaller treatment effects[21]. Both systematic review and meta-analysis and direct experimentation[8, 10] indicate that the presence of such biases probably account for failure of clinical trials of the free radical scavenger NXY-

059[37] and of magnesium as an inhibitor of the NMDA receptor[24]. However, failure of clinical trials of G-CSF[23] and EPO[25] do not seem to be related to overwhelming bias in the animal data[38, 39]. If bias is not the sole problem, what other factors associated with animal modelling could contribute to stroke translational failure?

One largely unexplored possibility is that the animal models of stroke which we use are confounded by surgical and anaesthetic comorbidity which is not present in human stroke and it is this that interferes with effective translation.

The author has used animal modelling of stroke extensively to study stroke cell biology[40], evolution of the ischemic penumbra[41], for testing candidate therapies[11, 42], and has contributed significant improvements to the models themselves[43, 44]. During the course of this work he has come to realise that animal models of stroke are confounded by surgical (and consequentially, anaesthetic) comorbidity.

Opening the skull to allow direct access to the cerebral arteries, as is used in the simplest stroke models[45], is not normally a feature of human stroke. When it does occur (usually within 48 hrs of stroke) it is used explicitly as a therapy, hemicraniectomy, with profound effects on survival after large middle cerebral artery (MCA) strokes in humans[46]. Opening the skull and any pressure relief may therefore provide a falsely positive overlay to assessment of new therapies.

A similar problem seems likely to contaminate the intraluminal thread occlusion models used by >40% of stroke researchers[47] and the methodologically related thrombo-embolic models which both require major surgery (Figure 1) to the musculature and blood vessels of the neck[45]. This again is not a feature of human stroke.

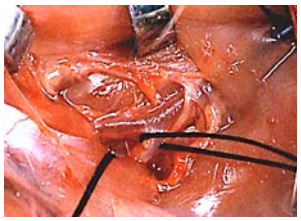

Figure 1. *Surgical access to the internal carotid artery of a rat.*

In these models two unintended co-pathologies that are not features of human stroke probably also contribute to failure of effective drug selection. The first, accidental induction of subarachnoid haemorrhage, further confounds the physiological basis of the model and leading to high mortality and excessive outcome variability[45]. This problem is well recognised and considerable effort has been expended on reducing its incidence[45,44]. In all of these models, the surgical complexity, and in particular the uncertainty as to when the thread has been inserted sufficiently, undoubtedly contributes to the ease with which bias can be introduced and over estimation of effect sizes and in turn under powering of most studies of candidate therapies[21, 36].

Introduction of emboli into the vasculature to mimic the embolic basis of human stroke (usually using the surgical approach used for Thread-MCAO and incorporating many of the same comorbidities) to block flow to cerebral arteries is plagued by higher mortality and variability of infarct size[45]. These embolic methods have mortality from 30-50% within 24 hours and up to 85% if animals are kept for 72 hours, contributing to their poor uptake (<10%) by the stroke research community[45].

The second, surgical induction of inflammation, is largely ignored even though it is well recognised outside the field of preclinical stroke that surgical trauma produces profound immunological dysfunction and morbidity for patients[48]. After traumatic brain injury, the presence of additional peripheral injury exacerbates neural inflammation[49]. Importantly, the processes induced by surgery (for example, hypoxia, activation of resident immune cells, inflammatory cytokine production, endothelial dysfunction, coagulation and systemic coagulopathy, complement activation and delayed immunosuppression)[48] are all directly relevant to the biology of stroke[50].

Inflammation is a highly complex and well-coordinated biological response to harmful stimuli aimed at eliminating harmful agents and helping wound healing. As such, it is induced swiftly. However, there are major differences in the time frame of critical cellular responses in human and induced rodent stroke. Brain neutrophil recruitment peaks at 24 hours in rats but not until day 2-3 in human stroke. Macrophage accumulation peaks at 48 hours in rats and it does not start until day 3 in humans[51]. During Affymetrix gene array experiments to find stroke biomarkers after Thread-MCAO, we found that stroke induction surgery has a profound effect on the systemic inflammatory biology of the rats. Principal component multivariate regression analysis (PCA) revealed a reproducible and time-dependent clustering of expression response (Figure 2A). Plotting the data for individual genes revealed how quickly and consistently expression changed after stroke surgery (Figure 2B). However, expression of 26% of the temporally regulated genes changed in the same way after sham surgery which did not induce infarction. Figure 2C illustrates confirmation of the Affymetrix data by RT-PCR for IL1r2 in an independent experiment using two different sham surgical procedures. Rapid priming of immune responses by stroke induction surgery seems likely to adversely influence drug development. Therefore, this surgical response may provide another veil that obscures stroke specific biology.

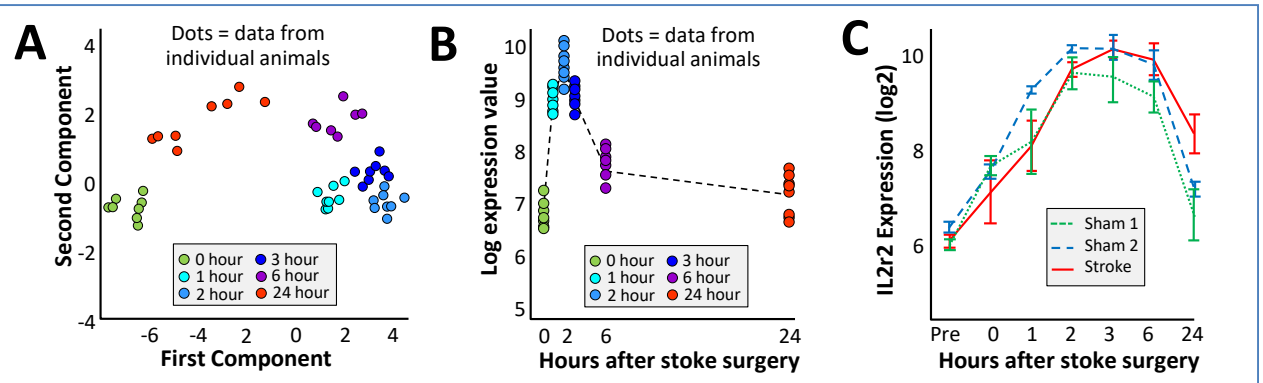

Figure 2. *A) PCA showing time dependent clustering of mRNA expression change after MCAO, B) MRGA10 expression showing hyperacute and highly reproducible change, C) RT-PCR confirmation of overlap of IL1r2 expression in MCAO and two different SHAM experiments.*

The surgery required for almost all stroke models brings with it an additional important confound, the ethically mandated use of anaesthetics.

The potential influence of anaesthetics is complex with both neuroprotective and (in the longer term) neurotoxic properties reported[52, 53]. In the context of ischaemic injury, both pre- and post-treatment with isoflurane (a commonly used anaesthetic), is reported to provide neuroprotection to rats subject to experimental hypoxic/ischemic brain injury or focal brain ischemia[54]. We confirmed this protective effect by exposing two groups of WKY rats to different isoflurane (2% in 50:50 air/oxygen) regimes over the week preceding and 28 days following 90 min MCAo. The MIN group received isoflurane at day -7 (pre-scan, 60min), during surgery (day 0, 90 min) and day 28 (final scan, 60 min). This was the experimental end-point. The MAX group received isoflurane at day -7 (pre-scan, 60min), during surgery (day 0, 90 min), during occlusion and reperfusion (4 hours), and at days 1,3, 7, 14 and 28 (60 mins each). Measuring infarct volume by T2 weighted MRI (4.7T Bruker) confirmed that longer isoflurane exposure in the MAX group resulted in smaller infarct volumes (Figure 3).

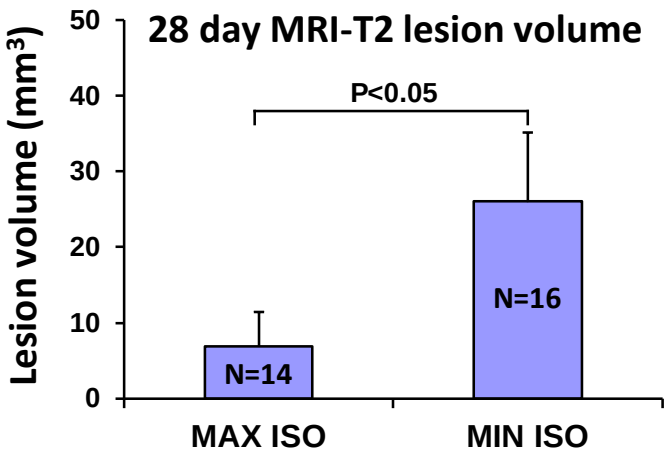

Figure 3. *Infarct volume 28 days after short and long-term isoflurane post MCAo.*

This is supported by early clinical observations that patients under general anaesthesia were more tolerant of ischemia than were un-anesthetized patients[55]. However there are many anaesthetic agents and reports of neuroprotection are found for agents as diverse as nitrous oxide[56] and ketamine[57]. Inhibition of spontaneous depolarization[58], activity as antioxidants[59], antagonism of NMDA receptors[60], GABA potentiation[61, 62], and alteration of cerebral blood flow redistribution[63] have all been posited as explanations for these effects.

Our systematic review and meta-analyses in stroke modelling supports this broad view with the choice of anaesthetic appearing to influence the efficacy of candidate drugs. For example, in studies of nicotinamide[64], melatonin[65], FK506[66] as candidate neuroprotectant therapies, the use of ketamine anaesthesia is associated with better outcomes. Conversely, for hypothermia, higher efficacy was reported in studies using chloral hydrate or pentobarbital but not ketamine[67]. In studies of tissue plasminogen activator, the profile of anaesthetic interaction is different again and suggests that the interaction might differentiate between structural and behavioural outcomes. For infarct volume, efficacy was highest in studies using acepromazine and ketamine combination anaesthesia ($\chi 2=29.4$, df=7, $p<0.003$; Figure 4A). For neuro-behavioural score it was highest in those studies using pentobarbital ($\chi 2=25.5$, df=5, $p<0.002$; Figure 4B)[68]. Similar differences in anaesthetic interactions between structural and behavioural outcomes were seen in studies of stem cell transplantation[69].

The importance of these interactions becomes most obvious when the impact of hypertension, the most common modifiable risk factor for stroke, is considered. In normotensive animals, pentobarbital, halothane and isoflurane are profoundly neuroprotective, but in hypertensive animals they worsen stroke (Figure 4C)[70]. The clinical relevance of this observation becomes clear when it is recognised that 77% of the patients recruited to the unsuccessful SAINT-II trial of NXY-059 had a history of hypertension and that in the supporting animal data, hypertensive animals received substantially less benefit (17.6% versus 47.8%; $\chi^2=29.1$, df=1, $P\leq 0.001$) than their normotensive counterparts[10]. Thus, the influence of anaesthesia, which again is not a feature of human stroke, is also likely to hinder detection of effective drugs for human use.

Conclusions

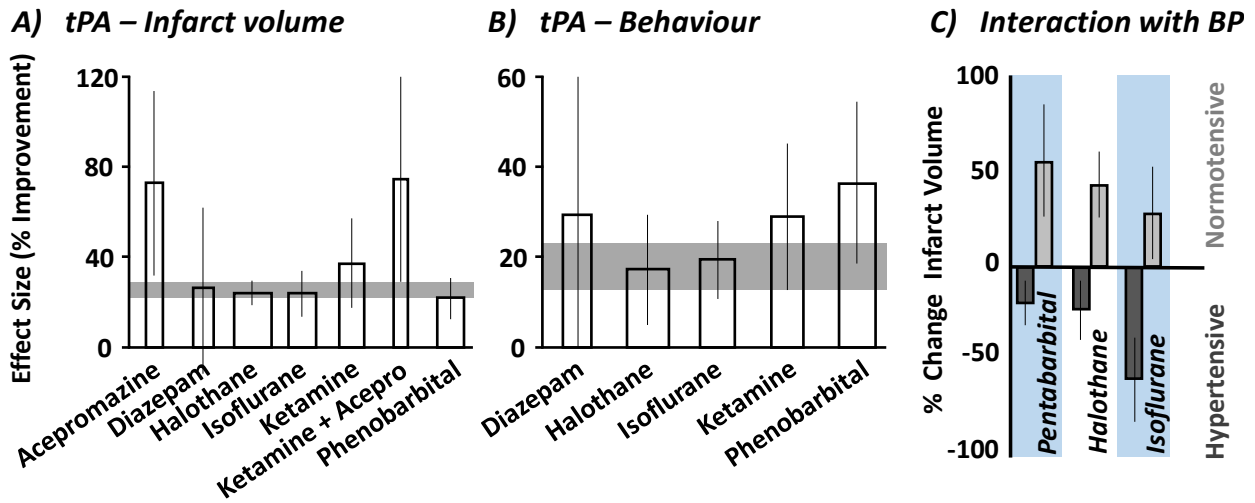

Figure 4. *Differential interactions between anaesthetics and outcome as A) infarct volume and B) Neuro-behavioural score in studies of tissue plasminogen activator therapy after MCAo and C) Differential effects of anaesthetics in normotensive and hypertensive animals after MCAo. Mean ±95% CI, Horizontal grey bar = overall effect size.*

In this review I have highlighted significant problems with the way stroke research has been conducted and deficits in our understanding of how animal models of stroke can be effectively employed. Never-the-less, animal models of stroke have provided a wealth of knowledge about the underlying pathophysiology of stroke and have an important part to play as we move forwards.

To provide a high chance of translational success requires preclinical studies which build on this existing knowledge with scientific rigor, a realistic grasp of what must be overcome in the clinic and most importantly an innovative and coordinated approach to solving the problems of human rather than animal stroke.

The first step in this process needs to be utilisation of the concept that evolution conserves important molecular processes. Finding molecular targets which are highly conserved across evolution and most importantly, specifically present in humans, will increase the probability of identifying effective therapeutic targets.

Existing *in vivo* and *in vitro* human and animal data indicates that stroke recruits' multiple metabolic processes in multiple neural and peripheral cell types. Many of these may change substantially after stroke. Nevertheless, they will represent end-stage effectors of change or will be single steps in multiply redundant pathways and their manipulation is unlikely to yield effective therapies. Others (probably a small proportion) will either occupy key loci that regulate large segments of downstream pathways or, given the energy dependency of the brain, will be so energetically demanding that their impact is disproportionately large. These should be targets for therapeutic intervention.

Stroke also recruits different pathophysiological processes over time but the success of thrombolysis shows clearly that recruitment of later processes is not always inevitable. Identification of the "trigger points" in this recruitment process will also allow development of interventions which break the destructive chain of stroke. The earlier the target is in the chain of post-stroke changes the greater will be its therapeutic potential. However, this does not preclude the possibility that late interventions will benefit patients. Moreover, even partially successful early interventions may maximise the benefit possible from later interventions.

The motherhood statements above are logical and defensible. However, they are insufficient on their own. They need to be used to craft the framework of a strategic plan to better understand stroke pathophysiology, identify potential therapeutic targets, identify ways of promoting beneficial processes and inhibiting harmful ones and then in vivo evaluation that is both methodologically robust and sufficiently broad that candidate drugs will have a high probability of success when subject to the rigors of clinical trial and real world therapeutic use.

It is tempting to promote a plan that starts with a clean slate. However, this would waste past investment in the field and ignore the political reality that those who felt their past work was devalued would not support the overall plan. Moreover, useful evidence comes in many forms. This means that the schema devised must accommodate multiple points of entry but whatever the source of the data we must understand the face validity of the hypothesis it purports to support and understand the rigor with which the data was collected and thus its likelihood of being true.

This is critical to permit rational decisions about which lines of evidence have the potential to deliver therapies soonest.

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Towards an Openness Agreement for Australia and New Zealand

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Abstract

Animal advocacy organisations have argued for over a century that the scientific community should be more open about its use of animals in research. Until recently, however, most research institutions have been reluctant to draw attention to their use of animals – a response undoubtedly driven by past misrepresentation or fears of targeting by activists.

A voluntary program launched in the UK in 2014 has seen a striking reversal of this defensive approach. Known as the UK Concordat on Openness in Animal Research, the program invites research organisations to make a public pledge to be more proactive in informing the broader community about their animal research programs. Signatories to the Concordat now include over 120 of the UK's most prominent research bodies, and reported outcomes are consistently positive. Similar openness agreements are now established or well advanced in several European countries and discussions are underway in the US.

Presentations at recent ANZCCART and ANZLAA conferences have suggested that there is considerable interest in openness agreements such as the UK Concordat in the Australian and New Zealand animal ethics, laboratory animal care and animal research communities.

This presentation will discuss options for the development of an openness agreement in Australia and New Zealand and will provide an update on progress, including results from canvassing key stakeholders.

Introduction

Several countries have either implemented or are actively developing initiatives to support greater openness in animal research. Central to these initiatives is a document known as an 'openness agreement'.

What is an Openness Agreement? Typically, openness agreements take the form of a public pledge signed by organisations involved in animal research. These organisations include universities, research institutes, public and private funders, industry and scientific societies. Another key feature of openness agreements is that they are national (as distinct say from initiatives developed by individual institutions) and, importantly, participation is voluntary.

The longest established openness agreement is the UK Concordat on Openness on Animal Research. Signatories to the Concordat agree to the following commitments:

- Commitment 1: We will be clear about when, how and why we use animals in research.
- Commitment 2: We will enhance our communications with the media and the public about our research using animals.
- Commitment 3: We will be proactive in providing opportunities for the public to find out about research using animals.
- Commitment 4: We will report on progress annually and share our experiences.

The UK Concordat has operated since 2014 and now has over 120 signatories. Examples of other openness agreements can be found at the website of the European Animal Research Association.

What is openness?

In relation to animal research, the concept of openness has at least 3 possible applications:

1) Reporting of research. Advocacy for greater openness – or transparency – in the reporting of research has come about because of the so-called 'research reproducibility crisis': a concern that is being recognised increasingly across many scientific disciplines (not just animal research) when scientists are unable to reproduce experiments published in scientific journals. An important factor underlying the reproducibility crisis is a lack of methodological transparency when research is published in scientific journals. This has led to calls to ensure that full methodological details are presented when publishing scientific work – the ARRIVE Guidelines are an example of the response to this. Although the term 'transparency' is sometimes used in reference to openness agreements, it is better reserved when referring to the reproducibility crisis to avoid confusion.
2) Advocacy. Some openness initiatives deliberately highlight the effort made to support high animal welfare standards and positive outcomes in animal research. This is an understandable approach and has merit in bringing some balance to a public discourse that has often been dominated by selectively negative images of animal research. Yet in my opinion, it can risk introducing bias into public perceptions of animal research.
3) "Openness means openness". This best reflects my personal view of openness in animal research and was a phrase used by Bella Williams from Understanding Animal Research UK at the 2018 ANZLAA conference. As an ethically contentious issue, animal research requires social licence. I believe the intensely polarised debate that has predominated for much of the last 150 years has done little to advance the quality of public discourse on animal research ethics. With true openness, the public should receive a less selective – and therefore less polarised and more nuanced – view of animal research on which to reach their own informed opinion.

ANZCCART and openness

Anti-vivisection protests were a feature of ANZCCART conferences on both sides of the Tasman for much of the 2000s. Although precautionary security measures were implemented, most protests did not cause major disruption and were essentially peaceful and sometimes colourful expressions of a democratic right.


Copied from St George and Sutherland Shire Leader Sept. 28 2004

Activists protest

Grim fears for animals

GRIM REAPER.... Animal rights was the theme for a protest in Brighton-le-Sands on the weekend. Photo: WESLEY LONERGAN

ANZCCART conference protests: Sydney 2004 (left) and Melbourne 2007 (right)

However, there were exceptions. The venue for one conference contacted ANZCCART less than a week beforehand to say it was cancelling the booking because of threats from protestors. Remarkably, a replacement venue and accommodation were found nearby. However, this was not a time for openness: delegates arriving to register at the new venue had been instructed to tell staff they were there for "the McFarlane family reunion"!

Most memorable were the 2003 ANZCCART and ANZLAA conferences in Christchurch. Although not particularly numerous, the protestors intensified their presence with drums and megaphones for many hours, often starting early in the morning to wake up hotel guests. Some attracted attention by chaining themselves to the building. Private security and police maintained a conspicuous presence throughout both conferences and a dog squad was deployed for daily security checks. Protests peaked on the evening of the conference dinner. Knowing that delegates would be leaving the hotel to board buses for the dinner venue, the protestors massed at the hotel entrance. Police formed a double cordon to guide delegates safely to the buses and separate them from the protestors, some of whom were at screaming pitch. I recall looking down on the protestors once inside the bus and concluding that I didn't need to be a lip-reader to work out the names we were being called. Perhaps indicative of the perceived impact of the conference and its protests, a commentary on these events (more from the activists' point of view) emerged some years later as a chapter in a human-animal studies textbook[13].

Faced with such belligerent opposition, one might have expected ANZCCART to withdraw from public attention. Instead, it advocated for openness. Aligning with the conference theme of 'Lifting the veil: Finding common ground', ANZCCART issued a media release arguing:

- "…increased transparency of animal research…would be of value to the public, and that more information should be provided…"
- "…the preferred means for providing this information is by…a plain language summary of all research projects approved by animal ethics committees." And,
- "… this information would allow the public to judge the validity of claims by [activists] and draw their own opinions."

[13] Knowing Animals (Human Animal Studies) Laurence Simmons (Editor), Philip Armstrong (Editor). Chapter 5 "Farming Images: Animal Rights and Agribusiness in the Field of Vision" by Philip Armstrong ISBN-10: 9789004157736; ISBN-13: 978-9004157736

Scenes from the 2003 ANZCCART conference in Christchurch

Openness was also advocated at the 2005 ANZCCART conference in Wellington, which also required a last minute change of venue and substantial police and security officer presence. The report on that conference noted Dr Simon Festing of the UK's Research Defence Society (now Understanding Animal Research) had argued that "It's far better to be open and transparent so that the public can get information about what goes on in research centres, and so that researchers do not give the impression of having something to hide." This was despite UK being viewed as having at that time "the most vitriolic anti-vivisection movement in the world".

Another aspect of openness was considered at the 2013 ANZCCART conference in a paper by Simon Bain and Kelly Debono from Australian National University. In a review of the reporting of animal use statistics, they noted the uniform reporting requirements throughout 27 EU member states and 10 Canadian provinces, and the EU Directive to publish lay summaries of all applications. This was in contrast to the often frustrating inconsistency of reporting between jurisdictions in Australia – something that can encourage misleading speculation on the actual numbers. Aside from enhancing public understanding, Bain and Debono suggested that greater openness in reporting could have practical applications by informing policy, resource allocation and design of training programs.

Interest in openness in the laboratory animal care sector

The Australian and New Zealand Laboratory Animal Association (ANZLAA) is an organisation of approximately 700 members comprising animal technicians, Animal Welfare Officers and laboratory animal veterinarians.

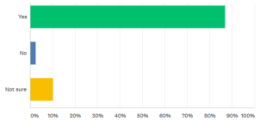
A review of ANZCCART and ANZLAA conference presentations on openness, outreach and related topics shows signs of growing interest in this area with the number of presentations more than triple in the last five years compared to the previous five years:

	2009-12	2013-18
ANZLAA	2	9
ANZCCART	2	5
Total	**4**	**14**

Presentations on openness, outreach and related topics at ANZCCART and ANZLAA conferences.

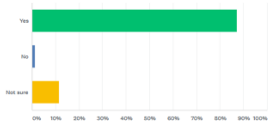
Some ANZLAA members have implemented individual initiatives to promote greater openness. However, in order to gauge the level of interest in openness across the membership, ANZLAA emailed a simple questionnaire to its members. This found a substantial majority (87%) of respondents believed that institutions should be more open about their use of animals, and that Australia and New Zealand should look at establishing an openness agreement along the lines of the UK Concordat:

1. Do you believe that research institutions in Australia and New Zealand should be more open about their research involving animals?



Yes	87%
No	3%
Not sure	10%

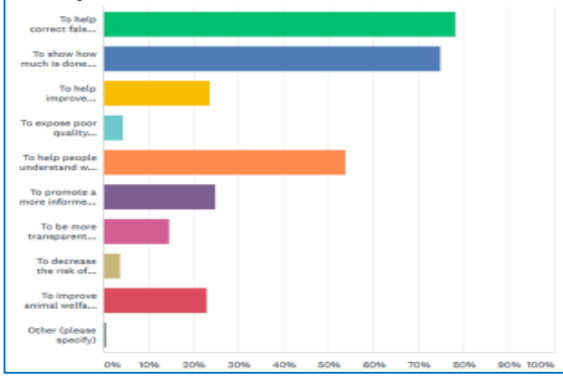
2. Do you think Australia and New Zealand should look at establishing an 'openness agreement' (perhaps similar to the UK' Concordat) that institutions could sign up to?



Yes	87%
No	1%
Not sure	12%

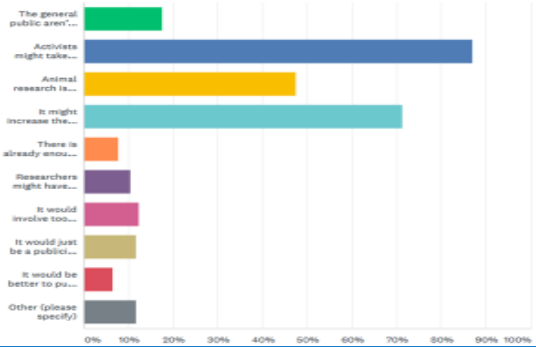
When asked about the reasons for and against supporting greater openness, the prevailing themes related to a desire to improve public understanding of animal research, and concerns about misrepresentation of animal research:

3. Which would you regard at the THREE most important reasons for supporting greater openness in animal research?



1. To help correct false information about animal research: 78%
2. To show how much is done to support the welfare of animals used in research: 75%
3. To help people understand why we still can't replace animal research with alternatives: 54%

4. Which would you regard as the THREE most important reasons for NOT supporting greater openness in animal research?



1. Activists might take information out of context to misrepresent animal research: 87%
2. It might increase the risk of attacks from those opposed to animal research: 71%
3. Animal research is often too complex to communicate easily to a public audience: 47%

Despite an overall response rate of only 22%, the questionnaire suggests there is strong support for greater openness (and an openness agreement in particular) among those most closely involved in the daily care and welfare of animals used in research.

Although not explored in the questionnaire, it is also important to recognise the negative impact on workplace morale that comes from public misunderstanding of animal research due to a lack of openness. Too often I hear animal care staff say they are reluctant to talk about their work in social situations because of the damage done to the image of animal research by years of unchallenged misrepresentation. I think animal care staff deserve better – after all, they enter their vocation because they want to provide practical care for animals.

Attitudes to openness elsewhere in the animal research sector

Although I haven't undertaken any sort of methodical investigation into attitudes to openness elsewhere in the sector, my anecdotal impression is that it is mixed.

I've heard concerns, for example, that greater openness could have an adverse effect on philanthropic support for medical research because of negative public perceptions about animal use. However, such concerns do not necessarily take into account the strong motivation of benefactors and their possible overlap with patient advocacy groups. Indeed, one recent study into the use of animals in personalised medicine found high rates of acceptability both among disease survivors and the general community[14].

My own conversations with members of the research community have also indicated mixed views. I've often found there is in-principle support for openness but a reluctance to take the lead. Yet I have also found that the need for greater openness has been recognised for some years: an email from a senior researcher after a 2004 TV debate between scientists and opponents of animal research said "I agree that the perception of closed doors is out there and we must address it."

Where to next?

I believe the time for an Australian and New Zealand openness agreement has arrived, and I see ANZCCART and ANZLAA being well-placed to take the lead in this. After all:

- ANZCCART's objectives are to promote…"informed discussion and debate within the community regarding these matters"
- ANZLAA aims to promote "The exchange of information with the research and broader community relating to animals used in research"

The next step will be to recruit universities, research institutes, funders, pharmaceutical companies and scientific societies, willing to commit to openness and engage with the community they serve.

I am hopeful that by this time next year, Australia and New Zealand will have taken their place among those countries that already have an openness agreement, and that by working on the

[14] 'Survivor, parent and community willingness to use patient-derived xenografts' Wakefield CE et al (2018) EBio Medicine 37:205-213

principle that “openness means openness”, we will start to see a better informed, less polarised and more constructive discourse on the ethics of animal research.

Achieving the three R's in Atlantic salmon disease research

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Declining natural fish populations, combined with a growing global demand for high quality protein, has seen aquaculture production grow rapidly both in Australia and abroad. Indeed, salmonid aquaculture (including Atlantic salmon) is now Tasmania's largest Agri-business, with a gross value of $700 million in 2015-16. Similar to terrestrial species, aquatic animals are susceptible to a variety of viral, bacterial and parasitic diseases. The mitigation and control of such diseases is essential for maintaining optimal fish welfare, industry sustainability and financial viability of aquaculture. Research and development play a vital in the mitigation and control of disease in aquaculture and remains an ongoing priority for many industry sectors. Here we review and discuss some of the unique attributes of aquatic animals (namely Atlantic salmon) and their relevance to achieving the three R's in aquatic animal health research. We specific focus on amoebic gill disease, highlighting diverse approaches to optimise animal welfare under research operations.

No manuscript was submitted for this presentation

Animal ethics in the wildlife space: Addressing the 3Rs.

Russell Bradford

CSIRO Oceans & Atmosphere

The 3Rs – the cornerstones to Animal Ethics – are often the most poorly addressed components of applications for an Animal Ethics permit. For example, it is not uncommon under Replacement to see something to the effect of "*There is no alternative to using live animals*". At present, this is the case for many studies on wild animals outside of the laboratory walls, but the rather dismissive remarks hint that the applicant may not have taken the time to fully investigate. The same is true for "Reduction" and "Refinement". In this talk I am going to demonstrate how it is possible to fully address the 3Rs in an application by using a body of work I have been involved in.

The CSIRO has a long history of working with southern Bluefin tuna in the Great Australia Bight. Management of the commercial fishery relies on an understanding of recruitment into the catch. This has traditionally come through the use of conventional tagging experiments where we would aim to tag up to 20,000 wild fish each with two tags per year. Through refinements to the management models, developments in new tools for fish tagging and handling, and the implementation of novel genetic techniques, we now aim to tag up to 10,000 per year fish each with a single tag.

No manuscript was submitted for this presentation