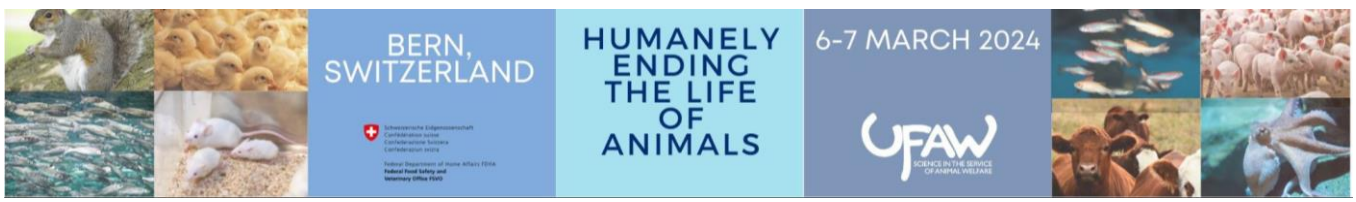
4th FSVO/UFAW Symposium –
Humanely ending the life of animals

Scientific Programme



Welcome to the 4th FSVO/UFAW Symposium

The Swiss Federal Food Safety and Veterinary Office (FSVO) and UFAW (the Universities Federation for Animal Welfare) are pleased to welcome you to the 4th symposium on Humanely Ending the Life of Animals.

The focus of the previous meeting (FSVO/UFAW/HSA 2020) was to identify, refine and implement alternative methods to CO_2 for humanely ending the life of animals to improve welfare in the laboratory, the slaughterhouse and during emergency depopulation.

The 4th symposium seeks to expand on the previous areas of interest and you will hear a range of talks, arranged into five topical sessions, considering: 1) Animals in Research, 2) Humane control of 'Pests' / Schädlinge, 3) Slaughter of livestock, 4) Depopulation and emergency killing, and 5) Humane killing of aquatic species for food.

As well as the usual opportunity for questions following each talk, we have also built into the programme a longer Q&A period at the end of each Session, during which all presenters who gave a talk in the previous session will be available for questions. This is to allow for greater discussion and exploration of each topic.

To ensure that we reach as wide an audience as possible, the meeting will also be live-streamed to nearly 500 people, from over 50 countries, who have registered to attend online. Recordings of select talks will also be made available after the event – please visit the event website for further information in due course.

We would like to thank all those who are contributing to the meeting, as speakers, poster presenters and chairs, as well as delegates. We hope that you enjoy the symposium.

Finally, please do let us know what you think of the meeting. Please fill in the post-symposium online survey and, if you have any specific comments, please email bern2024@ufaw.org.uk.

UFAW and FSVO Organising Committee

Antonio Velarde - IRTA

Claudio Zweifel – FSVO

Hans van de Vis – Wageningen University

Huw Golledge – UFAW/HSA

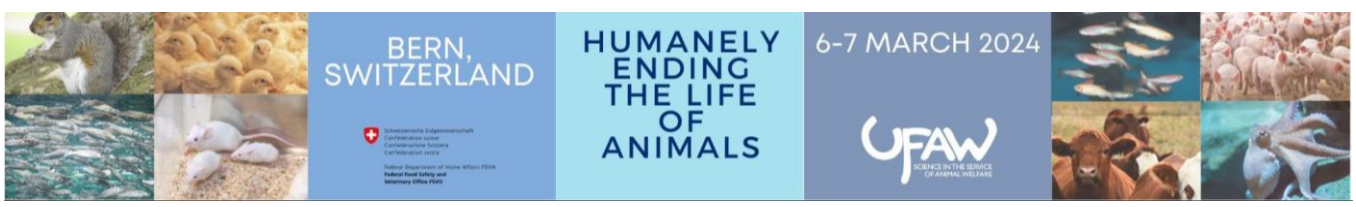
Liv Sigg - FSVO

Ngaio Beausoleil – Massey University

Otto Maissen - FSVO

Patricia Turner – Charles River

Petra Seebeck - University of Zurich



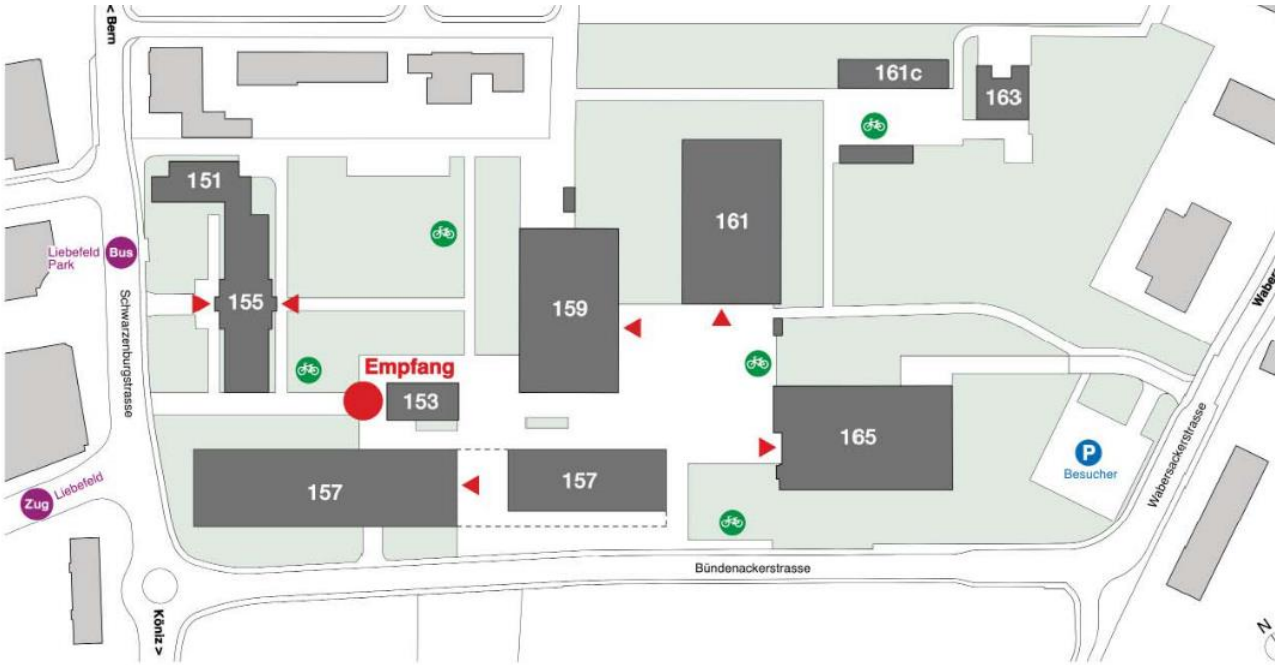
General Information for in-person delegates:

Meeting venue:

FSVO, Liebefeld Campus
Schwarzenburgstrasse 153
3097 Liebefeld
Bern, Switzerland

Link for Contact & Location

FSVO Campus Liebefeld



Registration:

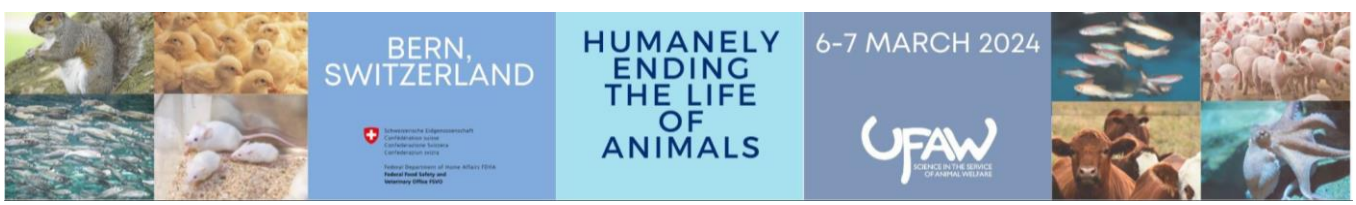
Registration will take place in building number 153 (called EMPFANG) from 8.30am on Wednesday 6 March. The meeting will start promptly at 09:15am.

On registering, delegates will receive a badge and a lunch voucher. Your badge allows access to the meeting and to lunch and refreshments. Please ensure that you always wear your badge whilst at the meeting.

Organisers and/or helpers have been allocated a red coloured badge and Speakers a blue coloured badge.

Please note that only delegates that are registered can attend the scientific programme and that registration is for an individual, not an institution, and is not transferable (unless this has been agreed in advance with UFAW and FSVO).

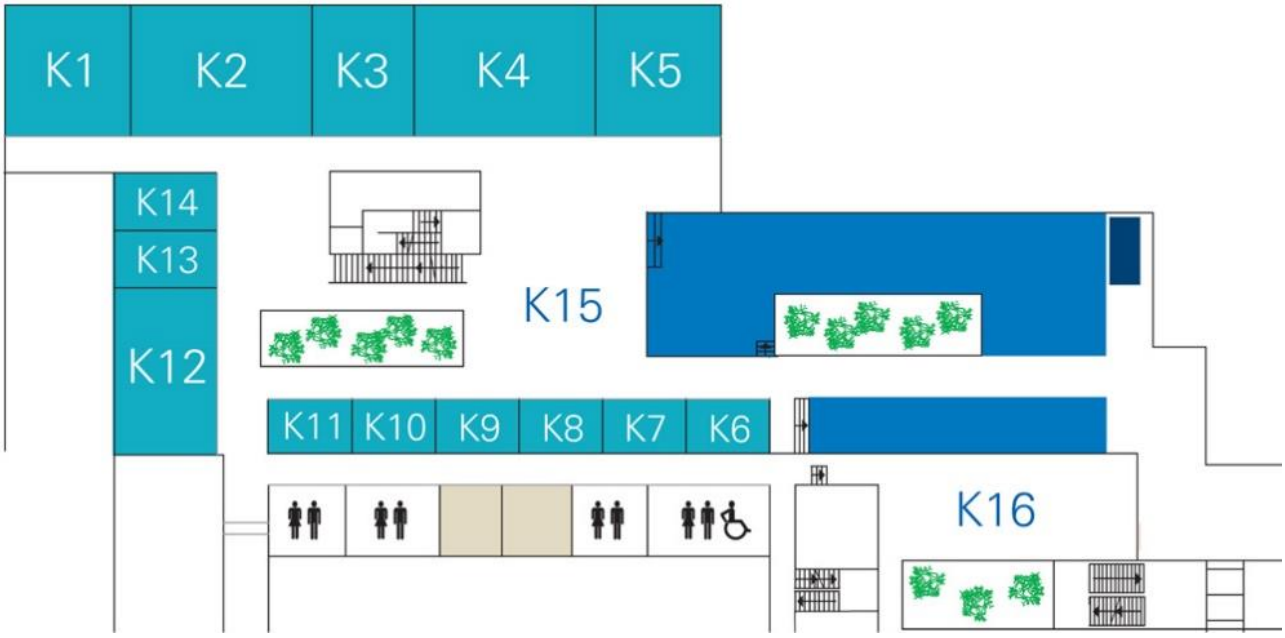
Cloakroom facilities are available; however these facilities are not attended, therefore any items left are at your own risk.



Talks will take place in rooms K4-K5, next to the lobby, with the poster session taking place at the back of room K5. Refreshments will be available in front of room K5. Lunch will be in room K16. Both refreshments and lunch will be held at the time indicated in the timetable.

Delegates with any general questions or queries should address these to the staff at the registration desk in the first instance.

Floor plan building 153



The conference programme is a very busy one and delegates are requested to take their seats in plenty of time before the start of each session. These will start promptly at the time indicated in the programme. The Symposium Programme and Abstract booklet will only be available on-line therefore if you would prefer a hard copy, please print one out in advance.

Posters

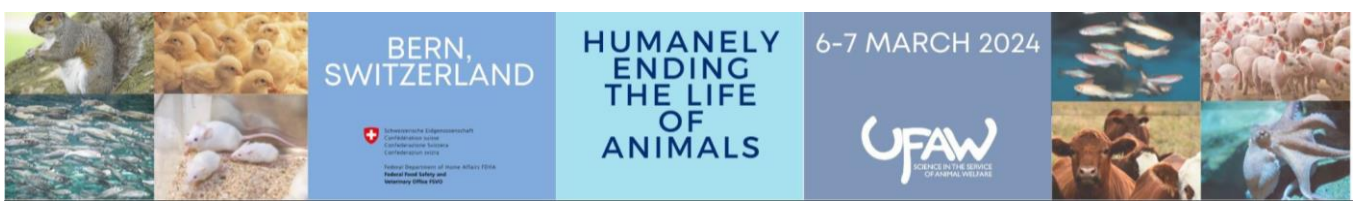
Poster presentations will be held in room K5.

Poster Presenters – please set up your poster before the start of the meeting on one of the boards provided. Attachments will be supplied and there is no specific order as to where your poster needs to be placed. Additionally, please can you be present at your poster, from 13:45 during the lunchtime break on both days, to answer any questions that other delegates may have.

Catering:

Tea and coffee will be served in front of room K5 at the break times indicated in the timetable.

Lunch will be served in the FSVO Campus restaurant and additional seating may be found in room K16, which has been reserved for delegates to use during this time. The lunch voucher that was given to you at registration may be used to obtain a lunchtime meal from the Campus Restaurant.



Internet access:
The FSVO has free pubic Wi-Fi available during the meeting. Delegates wishing to log-on to this network should click on 'gov-public' and follow the instructions. You will then receive an SMS message with a password, giving you access.

Network name: gov-public

Password: received via sms

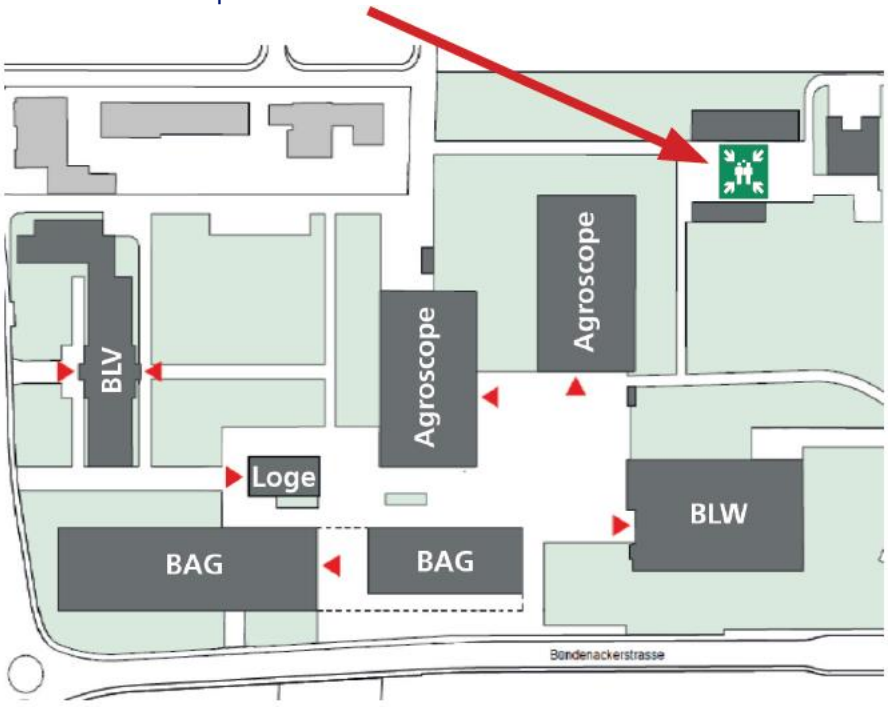
Social media:
Delegates are kindly requested to not take multiple photographs or record talks during the conference, as this is distracting for others.

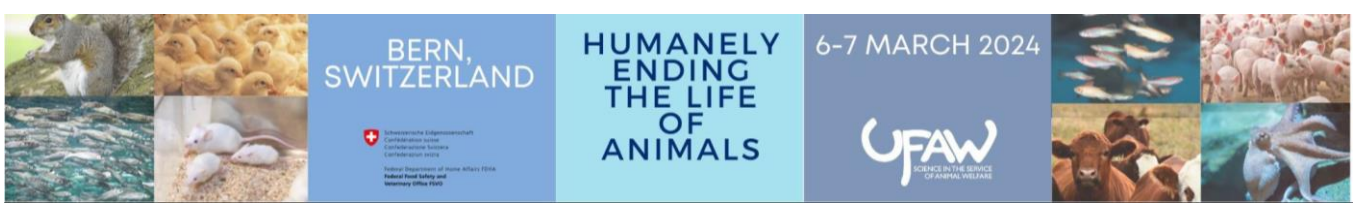
The hashtag for the conference is **#FSVOUFAW24**

Please be advised that video recording and photography will be taking place during the symposium due to online streaming and documentation of both the presentations and the meeting in general. It is possible that delegates may be recorded or photographed during these times and that these images may be used in promotional or educational materials, such as on our website or social media.

Additionally, the event is being live-streamed to an online audience and recorded – select presentations will be available after the event, please check the event website in due course for further information.

Safety:
In the event of a fire or other emergency, please leave via the nearest emergency exit. Delegates should gather in the park on the other side of the road from the Agroscope. A check that everyone attending the conference is present will then be made. Do not return to the building unless authorised to do so.





The Universities Federation for Animal Welfare (UFAW)

The Universities Federation for Animal Welfare (UFAW) is an international and independent scientific and educational animal welfare charity and membership society. Our vision is a world where the welfare of every animal affected by humans is maximised through a scientific understanding of their needs and how to meet them.

We try to bring about this change by:

DISCOVERING what matters to animals

DEVELOPING scientific solutions to animal welfare problems

DISSEMINATING evidence-based animal welfare information

Support Science in the Service of Animal Welfare

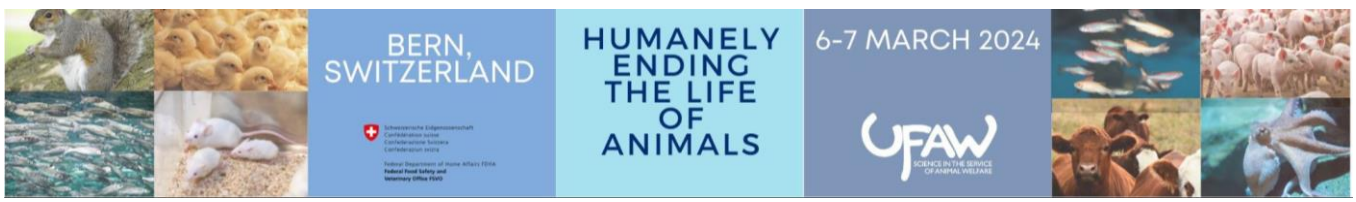
You can help make even bigger strides in animal welfare science by donating, leaving a legacy or becoming a member of UFAW.

Find out more: **ufaw.org.uk/support** or **ufaw.org.uk/membership**

Stay up to date with the latest developments in animal welfare science

There are many ways to stay connected and keep up to date with our latest news and opportunities, including via our website (www.ufaw.org.uk) and social media channels.

To sign up to receive our email newsletter, please visit **ufaw.org.uk/signup**



Federal Office for Food Safety and Veterinary Affairs (FSVO)

The primary task of the FSVO is to actively promote the health and wellbeing of humans and animals. The Swiss Confederation's constitution, legislation, ordinances and treaties provide the basis for this work.

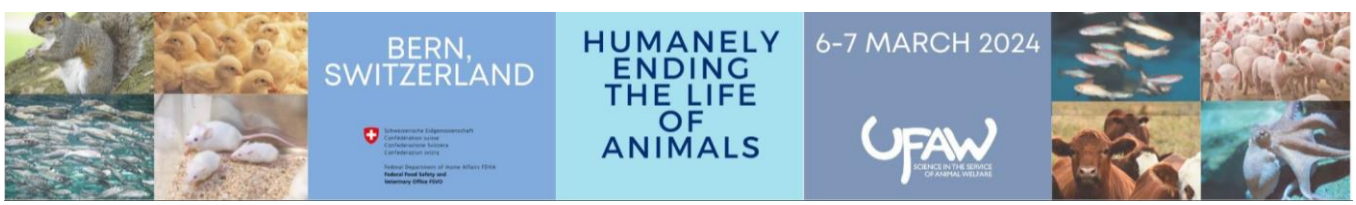
The FSVO creates a framework for guaranteeing a high level of food safety and consumer protection against deception. The Office promotes a healthy diet. It ensures a high level of animal health and welfare in Switzerland and monitors cross-border trade in food and animals. It makes sure that animals are free from diseases, particularly those that could present a risk to humans. The Office supports market liberalisation and makes Switzerland's concerns heard in international bodies. It controls trade in protected species.

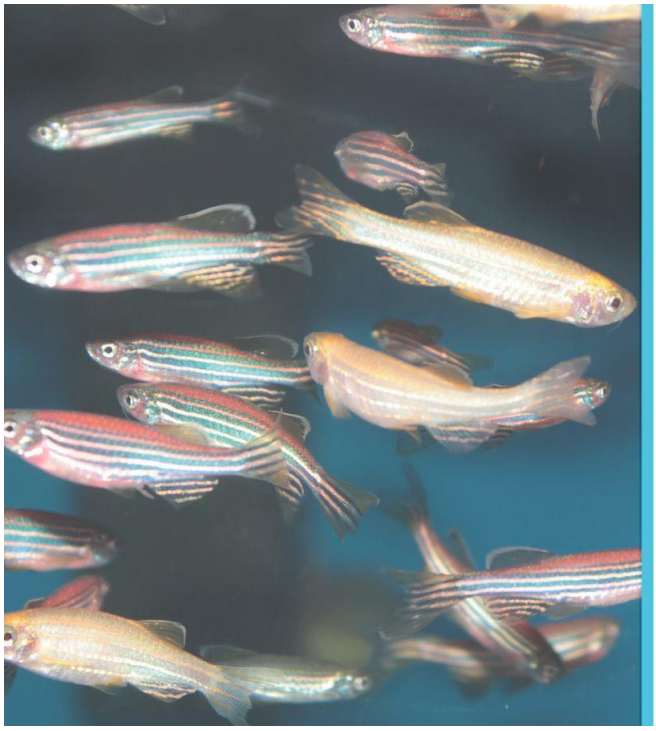
The FSVO Animal welfare is organized into three sectors, with two affiliated external sites dedicated to the proper keeping of animals, the focus remains on ensuring the well-being and dignity of animals.

In this collaborative effort, the Animal Experimentation and Evaluations Sector takes charge of supervising animal experiment licensing and managing the animex-ch specialist unit. Providing valuable advice to cantonal animal protection authorities, animal experimentation commissions, and law enforcement bodies, it offers information on animal experiments and genetic engineering while conducting meticulous evaluations related to animal experiments and primary production.

The commitment extends to providing essential information on animal welfare regarding domestic, wild experiment animals. Here, practical recommendations for the ethical handling and care of animals are developed across diverse areas, including commercial pet care, specialized pet shops, international animal trade, painful procedures, transport of animals, and the killing and slaughter of animals. Actively advising cantonal animal protection authorities, the sector ensures their continuous education in the field of animal welfare through exchange platforms. Moreover, it actively contributes to the basic and further training of official assistants, experts, and veterinarians in accordance with the Ordinance on the training of persons in the public veterinary service.

In a proactive role, the Animal Welfare Division harmonizes legislation enforcement across cantons by drawing up technical directives. Staying updated on the latest scientific findings in the field of animal welfare, the division drives projects that promote the well-being and dignity of animals. This multifaceted approach underscores dedication to the comprehensive betterment of animal welfare across various sectors.





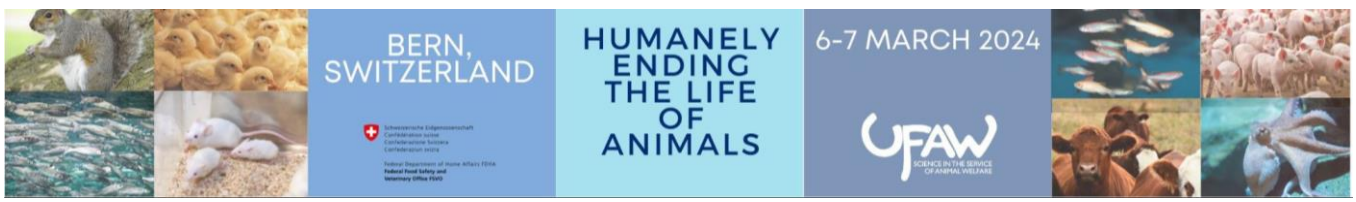
Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Federal Department of Home Affairs FDHA
Federal Food Safety and Veterinary Office FSVO

UFAW
SCIENCE IN THE SERVICE OF ANIMAL WELFARE

Scientific Programme: Talks

Timetable and Speaker Abstracts



Timetable of event

Wednesday 6 March

All timings are GMT+1 / CET

08:30 – 09:15 **Registration and poster set-up**

09:15 – 09:25 **Welcome and Introduction**
Martin Reist *(FSVO, Switzerland)* & Huw Golledge *(UFAW, UK)*

09:25 - 11:20 **Session One: Animals in Research**
Chair: Patricia Turner
(Charles River Laboratories & University of Guelph, Canada)

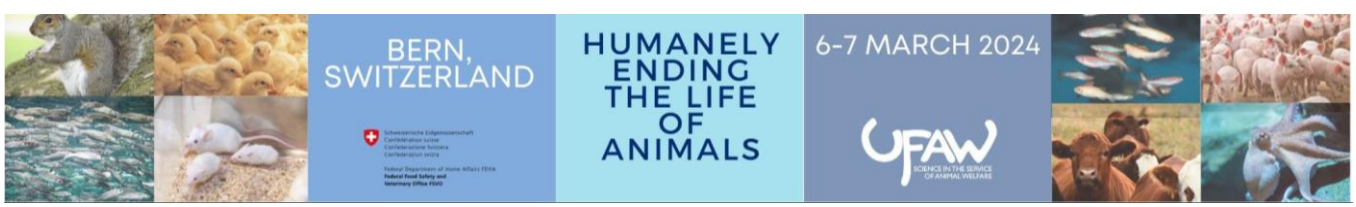
09:25 – 09:45 **Invited Speaker**
Patricia Turner *(Charles River Laboratories & University of Guelph, Canada)*

09:45 – 10:05 **Humanely ending the life of laboratory rats**
CHARLOTTE CALVET[1], Philipp Villiger[1], Tim Buchholz[1], Francesco Prisco[2], Carsten Wagner[3] and Petra Seebeck[1] *([1]Zurich Integrative Rodent Physiology (ZIRP), Institute of Physiology, University of Zurich, Switzerland; [2]Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Switzerland; [3]Institute of Physiology, University of Zurich, Switzerland)*

10:05 – 10:25 **Neurophysiolgical monitoring of the dying brain: the welfare consequences of hypobaric hypoxia and hypercapnic hypoxia in laboratory mice**
JASMINE CLARKSON[1,2], Jessica E Martin[2,3], Ross S Muers[4], Matthew C Leach[5], Julian Sparrey[6] and Dorothy E F McKeegan[1] *([1]School of Biodiversity, One Health and Veterinary Medicine, College of Medical Veterinary and Life Sciences, University of Glasgow; [2]School of Natural and Environmental Sciences, Newcastle University; [3]The Royal (Dick) School of Veterinary Studies and The Roslin Institute, University of Edinburgh; [4]Centre for Neuroscience, University of Glasgow; [5]Comparative Biology Centre, Newcastle University; [6]Livetec systems Ltd, Wrest Park, Silsoe, Bedford)*

10:25 – 10:50 **Short talks:**

Refining the drop method for isoflurane induction in mice
MAYA BODNAR, Joanna Makowska and Daniel Weary *(Animal Welfare Program, Faculty of Land and Food Systems, The University of British Columbia, Canada)*



10:25 – 10:50 **Short talks:**

Etomidate Effectively Euthanizes Zebrafish (*Danio rerio*)
KENZIE SCHWARTZ[1], Kyna Byrd[1], Monika Huss[2], Katechan Jampachaisri, Patrick Sharp[3] and Cholawat Pacharinsak[1] *([1]Department of Comparative Medicine, Stanford University, California, USA, [2]Department of Mathematics, Naresuan University, Phitsanulok, Thailand, [3]University of California, Merced; Murdoch University, Western Australia)*

10:50 – 11:20 **Q&A / Discussion of Session One topics**

11:20 – 11:50 **Break**

11:50 – 13:00 **Session Two: Humane control of 'Pests' / Schädlinge**
Chair: Huw Golledge *(UFAW, UK)*

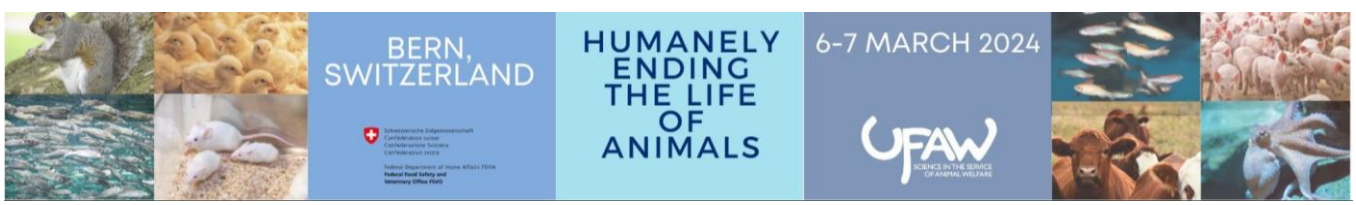
11:50 – 12:00 **Introduction to session**
Huw Golledge *(UFAW, UK)*

12:00 – 12:20 **Incorporating animal welfare into pathways for developing selective toxins for control of introduced animals**
NGAIO BEAUSOLEIL[1], Erica Hendrikse[2] and Clive Marks[3] *([1]Animal Welfare Science and Bioethics Centre, School of Veterinary Science, Massey University, New Zealand; [2]Wildlife Ecology & Management, Manaaki Whenua Landcare Research, New Zealand; [3]Nocturnal Wildlife Research Pty Ltd, Australia*)

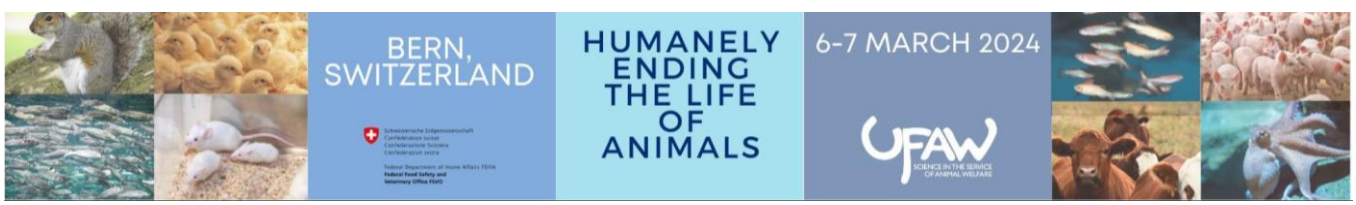
12:20 – 12:40 **Using a naturally occurring sterility gene to humanely control house mouse pests**
ANDRI MANSER, Salomé Friry, Caspar Goedecker, Ellen Gonzales and Anna Lindholm *(Department of Evolutionary Biology and Environmental Studies, University of Zurich, Switzerland)*

12:40 – 13:00 **Q&A / Discussion of Session Two topics**

13:00 – 14:30 **Lunch**



14:30 – 17:00	**Session Three: Slaughter of Livestock** Chair: Claudio Zweifel
14:30 – 14:40	**Introduction to session** Claudio Zweifel
14:40 – 15:00	**The role of the EURCAW in refining the assessment of poultry and rabbit welfare at slaughter** ALEXANDRA CONTRERAS-JODAR[1], V. Michel[2], A. Varvaró-Porter[1] and A. Velarde[1] *([1]Animal Welfare Program, Institute of Agrifood Research and Technology (IRTA), Spain; [2]Direction of Strategy and Programmes, French Agency for Food, Environmental and Occupational Health & Safety (ANSES), France)*
15:00 – 15:15	**A review of indicators and sensor technologies to assess pig welfare at the slaughterhouse** ANGELA RAMON PEREZ[1], Björn Forkman[2], Xavier Manteca[1] and Pol Llonch[1] *([1]Department of Animal and Food Science, Universitat Autònoma de Barcelona, Spain; [2]Section of Animal Welfare and Disease Control, Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark)*
15:15– 15:35	**Comparison of 8 different inert gas (mixtures) to CO_2 for stunning pigs at slaughter** JONAS KNÖLL[1], Julia Gelhausen[2], Thyra Friehs[2], Tony Krebs[2,3], Daniel Mörlein[2], Jens Tetens[2] and Inga Wilk[1] *([1]Institute of Animal Welfare and Animal Husbandry, Friedrich-Loeffler-Institut, Germany; [2]Department of Animal Sciences, Georg-August-University Göttingen, Germany; [3]Institute of Organic Farming, Johann Heinrich von Thünen Institute, Germany)*
15:35 – 16:00	**Break**
16:00 – 16:20	**Improving automatic electric stunning systems for pigs as a viable & more ethical alternative to CO_2** LESLEY MOFFAT, KEES SCHEEPENS, Madelaine Looije, Michel Oosterhuis, Roy Melsert and Jan Voordouw *(Eyes on Animals, The Netherlands)*
16:20 – 17:00	**Q&A / Discussion of Session Three topics**
17:00	**End of day one**



Thursday 7 March

All timings are GMT+1 / CET

09:00 – 09:05 **Welcome and Introduction**
Otto Maissen *(FSVO, Switzerland)*

09:05 – 11:00 **Session Four – Depopulation and emergency killing**
Chair: Antonio Velarde *(Institute for Food & Agricultural Research & Technology, Spain)*

09:05 – 09:20 **Invited Speaker**
Antonio Velarde *(Institute for Food & Agricultural Research & Technology, Spain)*

09:20 – 09:40 **More humane stunning and euthanasia in pigs and poultry based on nitrogen, with high expansion foam technology**
NICOLE KREFTING and Sebastian Strand *(Heft AB, Sweden)*

09:40 – 10:30 **Short Talks:**

Animal Disaster Management Decision-Making: Applying an Animal Welfare Science-Ethics Guided framework within One Health
RAYMOND ANTHONY[1] and Andreia De Paula Vieira[2] *([1]University of Alaska Anchorage, USA, [2]Veterinarian and Independent Researcher, Curitiba, Brazil)*

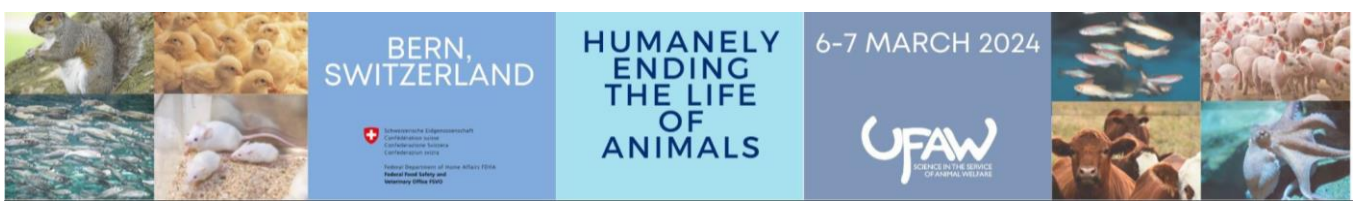
Towards a more humane depopulation of poultry in case of Avian Influenza
Louise Kremer[1,2], Antonio Velarde[1,3], LEONARDO JAMES VINCO[1,4], Tiziano Bernardo[1,4] and Virginie Michel[1,5] *([1]European Reference Centre for Animal Welfare for Poultry and other Small Farmed Animals (EURCAW-Poultry-SFA), ANSES, France; [2]French Reference Centre for Animal Welfare (FRCAW), INRAe CODIR, France; [3]Institute of Agrifood Research and Technology (IRTA), Spain; [4]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Italy; [5]Direction de la Stratégie et des Programmes, ANSES, France)*

Humane methods for killing day-old chicks
BIRTE NIELSEN and Charlie Mason (Humane Slaughter Association, UK)

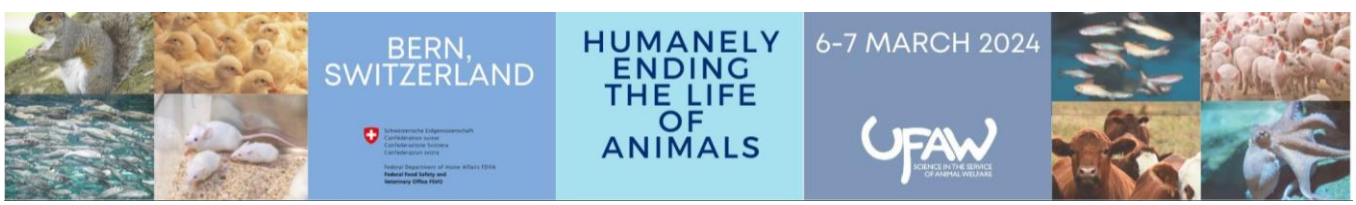
A Sustainable Approach to Eliminate Male Chick Culling in the Egg Industry (online)
ELIRAN KADOSH *(Head of Research, eggXYt, Israel)*

10:30 – 11:00 **Q&A / Discussion of Session Four topics**

11:00 – 11:30 **Break**



11:30 – 13:05	**Session Five – Humane killing of aquatic species for food** Chair: Hans van de Vis *(Wageningen University and Research, Netherlands)*
11:30 – 11:50	**Invited speaker** Hans van de Vis *(Wageningen University and Research, Netherlands)*
11:50 – 12:10	**Challenges of determining sensibility in fish** JENNY BOUWSEMA[1], Maureen Ellis[1], Amaya Albalat[1], Silvere Santos[1], James Turnbull[1] and Jeff Lines[2] *([1]Institute of Aquaculture, University of Stirling, UK; [2]Ace Aquatec, UK)*
12:10 – 12:35	**Short talks:** **Optimisation of electrical stunning in the decapod crustacean Norway lobster (*Nephrops norvegicus*)** AMAYA ALBALAT[1], Endre Putyora[1], Nasser Ayaril[1], Maureen Ellis[1], Sonia Rey Planellas[1] and Douglas Neil[2] *([1]Institute of Aquaculture, University of Stirling, UK; [2]School of Biodiversity, One Health and Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, UK)* **Assessment of the efficiency of electric stunning on the behaviour, physiology, and meat quality of the American lobster *(Homarus americanus)*** Rodrigo A. Lorenzo[1], Pol Llonch[2], Joel González[3], José A. García del Arco[1], Maria Font-i-Furnols[3], Òscar Chic[1] and GUIOMAR ROTLLANT[1], *([1]Institut de Ciències del Mar, Spanish National Research Council (CSIC), Spain; [2]Department of Animal and Food Science, Universitat Autònoma de Barcelona, Spain; [3]Institute of Agrifood Research and Technology (IRTA), Spain)*
12:35 – 13:05	**Q&A / Discussion of Session Five topics**
13:05 – 14:30	**Lunch**
14:30 – 16:00	**Session Five continued – Humane killing of aquatic species for food** Chair: Hans van de vis *(Wageningen University and Research, Netherlands)*
14:30 – 14:50	**Impact of electrical shock and analgesia in Norway lobster *(Nephrops norvegicus)*** ELEFTHERIOS KASIOURAS[1], Guiomar Rotllant[2], Albin Gräns[3], Per Hjelmstedt[3] and Lynne U Sneddon[4] *([1]Department of Biological and Environmental Sciences, University of Gothenburg, Sweden; [2]Institut de Ciències del Mar, Spanish National Research Council (CSIC), Spain; [3]Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Gothenburg, Sweden; [4]Department of Biological and Environmental Sciences, University of Gothenburg, Sweden)*



14:50 – 15:10 **Optimisation of in-water electrical stunning in tropical prawns *(Penaeus vannamei)***

ENDRE PUTYORA, Jenny Bouwsema, Nasser Ayaril, Maureen Ellis, Sonia Rey Planellas and Amaya Albalat *(Institute of Aquaculture, University of Stirling, UK)*

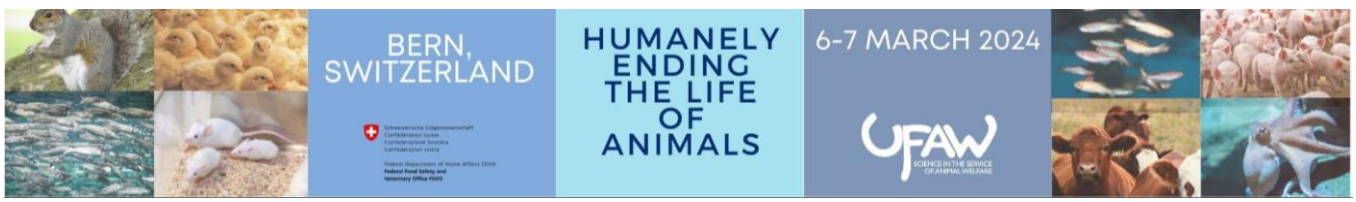
15:10 – 15:30 **Stunning and killing of European lobster *(Homarus gammarus)* and Edible crab *(Cancer pangurus)***

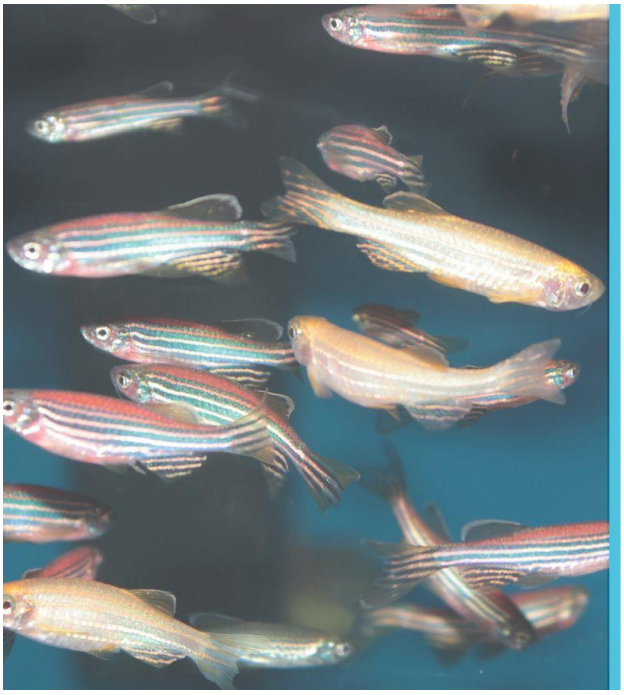
RAGNHILD AVEN SVALHEIM[1], Bjørn Roth[1], Endre Grimsbø[2], Henny Reimert[4], Gro van der Meeren[3], Luca Pettinau[4] and Hans van de Vis[4]

([1]Nofima AS, Norway; [2]UiT The Artic University of Norway, Norway; [3]Institute of Marine research, Austevoll Research Station, Norway; [4]Wageningen University & Research, The Netherlands)

15:30 – 16:00 **Q&A / Discussion of Session Five topics**

16:00 **Meeting Close**





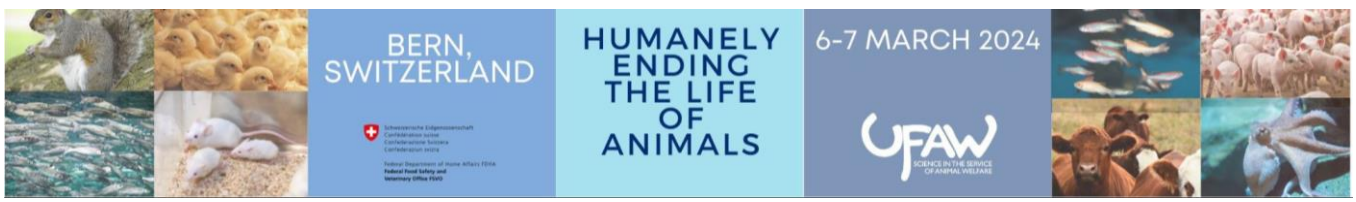
Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Federal Department of Home Affairs FDHA
Federal Food Safety and Veterinary Office FSVO

UFAW
SCIENCE IN THE SERVICE OF ANIMAL WELFARE

Scientific Programme: Talks

Timetable and Speaker Abstracts



HUMANELY ENDING THE LIFE OF LABORATORY RATS

CHARLOTTE CALVET[1], Philipp Villiger[1], Tim Buchholz[1], Francesco Prisco[2], Carsten Wagner[3] and Petra Seebeck[1]

[1] Zurich Integrative Rodent Physiology (ZIRP), University of Zurich, Switzerland
[2] Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Switzerland
[3]Institute of Physiology, University of Zurich, Switzerland

charlotte.calvet@uzh.ch

Millions of rodents are euthanized each year for research purposes. Recent guidelines (AVMA 2020) recommend the use of 30% to 70% CO_2 displacement rates, but there is limited evidence on the impact of CO_2 euthanasia on animal welfare. While CO_2 is commonly used, some studies have suggested that it could be aversive for rodents, but no superior alternatives have been identified that are causing less distress than CO_2 and providing a fast and reliable way to induce unconsciousness.

Given the existing data gaps and the lack of standardization surrounding laboratory rodent euthanasia, our study aimed to assess the impact of different CO_2 concentrations and to explore the potential of alternative gas agents to provide a more humane euthanasia.

We assessed various physiological parameters in rats euthanized with carbon monoxide (CO), nitrogen (N_2), and isoflurane in comparison to two CO_2 concentrations (30% and 70%). We used a multifaceted approach to assess animal wellbeing: telemetry (EEG, EMG, ECG, blood pressure (BP), temperature, activity), whole body plethysmography, and video recordings for behavioral assessment. In addition, we analyzed different stress parameters in the blood and conducted histopathological examinations of the lungs.

Our findings indicate that anesthesia with isoflurane induced no aversive behavior, no gasping or muscular spasms. Prior to the loss of motion (LOM) we observed a decrease in heart rate (HR), increase in BP and respiratory rate but not tidal volume (TV).

70% CO_2 resulted in a rapid loss of consciousness like isoflurane and a faster death compared to 30% CO_2. Both CO_2 concentrations induced a decrease in HR and increase in BP similar to isoflurane, but an increase in respiratory rate and TV. However, CO_2 euthanasia was accompanied by potentially stressful responses before loss of consciousness like gasping and muscular spasms.

CO did not increase BP but did increase HR, respiratory rate and TV. N_2 induced an increase in all physiological parameters measured. Contrary to previous reports in mice, CO and N_2 but not CO_2 induced seizures which most often occurred before the loss of consciousness. Additionally, lung emphysema was more pronounced with CO and N2 compared to CO_2, further impacting animal welfare.

We conclude that CO and N_2 are not suitable alternatives to CO_2 and that the induction of euthanasia with an anesthetic gas might represent an option to alleviate the negative side effects of CO_2.

NEUROPHYSIOLOGICAL MONITORING OF THE DYING BRAIN: THE WELFARE CONSEQUENCES OF HYPOBARIC HYPOXIA AND HYPERCAPNIC HYPOXIA IN LABORATORY MICE

JASMINE CLARKSON[1,2], Jessica E Martin[2,3], Ross S Muers[4], Matthew C Leach[5], Julian Sparrey[6] and Dorothy E F McKeegan[1]

[1] School of Biodiversity, One Health and Veterinary Medicine, College of Medical Veterinary and Life Sciences, University of Glasgow, UK
[2] School of Natural and Environmental Sciences, Newcastle University, UK
[3] The Royal (Dick) School of Veterinary Studies & The Roslin Institute, University of Edinburgh, UK
[4] Centre for Neuroscience, University of Glasgow, UK
[5] Comparative Biology Centre, Newcastle University, UK
[6] Livetec systems Ltd, Wrest Park, Silsoe, Bedford, UK

jasmine.clarkson@newcastle.ac.uk

Millions of mice are used annually for scientific purposes worldwide, with the vast majority killed either during or after the scientific work. Public trust and acceptance of animal use for biomedical research is underpinned by robust application of the 3Rs and minimisation of unnecessary harms. Approved killing methods are assumed to be humane, and as such, are often referred to as 'euthanasia'. Exposure to a rising concentration of carbon dioxide (CO_2) gas remains one of the most widely used killing methods for rodents, despite robustly evidenced welfare concerns. Therefore, there is an urgent need to find a practical and high-throughput alternative to exposure to CO_2 that provides better welfare outcomes during killing.

We investigated whether hypobaric hypoxia (gradual decompression) offered a reliable methodology and improved the welfare of laboratory mice at killing compared to CO_2 exposure (hypercapnic hypoxia). Behaviourally, we demonstrate that although hypobaric hypoxia is associated with elongated latencies to loss of posture and death, mice exhibit species-typical behaviour during induction suggesting a minimally negative animal experience. To support our interpretation of spontaneous behaviour, we also explored brain activity via electroencephalogram (EEG) recordings and spectral analysis, aiming to define the conscious phase of concern during killing with decompression or exposure to CO_2. We explored two fill methods for exposure to CO_2 (top and bottom fill), given the variation in fill application across the UK and globally.

All terminal treatments resulted in the transition from higher frequency, low amplitude activity to slow wave (delta <4Hz) activity. However, we confirmed that brain activity differs during hypobaric hypoxia compared to hypercapnic hypoxia. CO_2 resulted in a reduction in power in higher frequency bands, until peak power occurred in delta frequency bands which occurred after loss of posture. Hypobaric hypoxia resulted in a progressive change in power resulting in increased power in lower frequency bands from ~105s indicating the dominance of slow wave activity occurring substantially before loss of posture (~280s). These findings support the hypothesis of

early cognitive impairment and gradual loss of consciousness during the longer induction phase of decompression.

Our findings are encouraging, providing further evidence that gradual decompression is associated with better welfare outcomes compared to exposure to CO_2. These findings, along with features such as consistent performance and scalability, support the notion that gradual decompression could be the basis of major refinement for the way that we kill millions of laboratory mice worldwide.

REFINING THE DROP METHOD FOR ISOFLURANE INDUCTION IN MICE

MAYA BODNAR, Joanna Makowska and Daniel Weary

Animal Welfare Program, Faculty of Land and Food Systems, The University of British Columbia, Vancouver, Canada

mayajb17@mail.ubc.ca

Laboratory mice are often euthanized with carbon dioxide (CO_2) alone. Exposure to CO_2 is aversive, but this procedure can be refined by first rendering the animal unconscious with isoflurane. Some facilities lack access to a vaporizer used to administer isoflurane, preventing adoption of this refinement. A practical alternative to a vaporizer is the 'drop' method, whereby a fixed volume of isoflurane is introduced into the induction chamber. Previous work suggests that isoflurane administered at a concentration of 5% via the drop method is effective, but aversive to mice; lower concentrations have not been tested.

We assessed insensibility with induction using the drop method for isoflurane concentrations below 5%. Mice (n=27) were randomly allocated to one of three isoflurane concentrations: 1.7%, 2.7%, and 3.7%. During induction, measures of insensibility and stress-related behaviors were recorded. All mice reached a surgical plane of anesthesia, and mice exposed to higher concentrations did so more quickly; as concentrations increased from 1.7 to 2.7 and 3.7%, the time to recumbency (Least squares means ± SE: 120.5 s ± 8.1, 97.9 s ± 8.1, and 82.8 s ± 8.1, respectively), loss of righting reflex, (149.1 s ± 8.5, 127.7 s ± 8.5, and 100.7 s ± 8.3, respectively), and loss of pedal withdrawal reflex (214.5 s ± 8.3, 172.2 s ± 8.3, and 146.4 s ± 8.3, respectively) all declined. Rearing was the most frequently performed behavioral response and was most pronounced immediately after isoflurane administration for all treatments.

In a subsequent study, we assessed mouse aversion to this method using a light-dark conditioned place aversion (CPA) paradigm, based on the innate preference of mice for dark versus light spaces. Mice (n=28) were randomly allocated to the same three isoflurane concentrations: 1.7%, 2.7%, and 3.7%. Before and after conditioning sessions, mice underwent an initial and final preference assessment; the change in the duration spent in the dark (+ isoflurane) chamber between preference tests was used to calculate a CPA score. Aversion increased with increasing isoflurane concentrations; from 1.7% to 3.7% isoflurane, mean ± SE CPA score decreased from 19.6 s ± 20.1 , to -25.6 s ± 23.2, and finally to -116.9 s ± 30.6.

We conclude that the drop method provides a practical alternative to effectively anesthetize mice with isoflurane concentrations as low as 1.7%, and that the use of lower concentrations (between 1.7 and 2.7%) can further refine CO_2-based euthanasia methods.

ETOMIDATE EFFECTIVELY EUTHANIZES ZEBRAFISH *(DANIO RERIO)*

KENZIE SCHWARTZ[1], Kyna Byrd[1], Monika Huss[1], Katechan Jampachaisri[2], Patrick Sharp[3] and Cholawat Pacharinsak[1]

[1] Department of Comparative Medicine, Stanford University, California, USA
[2] Department of Mathematics, Naresuan University, Phitsanulok, Thailand
[3] University of California, Merced; Murdoch University, Western Australia

kenzies@stanford.edu

Drawbacks of common zebrafish *(Danio rerio)* euthanasia methods include human safety, difficulty in preparation, and questionable pain/distress caused to the fish. We assessed the efficacy of the anesthetic, etomidate, to euthanize zebrafish. The aims were to investigate: 1) the effectiveness of 2 different etomidate concentrations; and 2) the effects of 2 different immersion densities. Aim 1- Fish (n=10 fish/group) were randomly assigned to 30-min immersion in 1 of 2 treatment groups: 6 (Eto-6) or 10 (Eto-10) mg/L etomidate. All fish were video recorded throughout the procedure and scored by 2 blinded observers experienced with zebrafish euthanasia. Parameters monitored included: 1) Loss of righting reflex (LORR); 2) undulation cessation; 3) cessation of operculation (COO); and 4) loss of startle reflex. Any aversive behaviors were also noted. Each fish was immersed in its assigned preparation for 30 min and then removed by net and transferred to a recovery tank. In recovery tanks, fish were monitored for signs of recovery (righting, operculation, body movement, and response to tap) once every 5 min for 60 min. Successful euthanasia was defined as absent signs of recovery for at least 60 min in recovery tanks. Aim 2– 5 or 10 fish were immersed in a 6 mg/L concentration of etomidate. Results – Aim 1, there was no significant difference in time to LORR between the two groups. The time to undulation cessation, time to COO, and time to loss of startle reflex was significantly longer in the Eto-6 group compared to Eto-10 group. Fish in both groups were effectively euthanized. Aim 2, the time to LORR and the time to undulation cessation was significantly longer in the 10 fish/L group compared to the 5 fish/L group. There was no significant difference in time to COO or the time to loss of startle reflex in the 5 fish/L group compared to the 10 fish/L group. No aversive behaviors were noted and there were no signs of recovery in any fish. This study indicates that 6 or 10 mg/L etomidate effectively euthanizes zebrafish. Density may play a role and we recommend euthanizing zebrafish at 5 fish per liter.

INCORPORATING ANIMAL WELFARE INTO PATHWAYS FOR DEVELOPING SELECTIVE TOXINS FOR CONTROL OF INTRODUCED ANIMALS

NGAIO BEAUSOLEIL[1], Erica Hendrikse[2] and Clive Marks[3]

[1] Animal Welfare Science and Bioethics Centre, School of Veterinary Science, Massey University, Palmerston North, NZ
[2] Wildlife Ecology & Management, Manaaki Whenua Landcare Research, Auckland, New Zealand
[3] Nocturnal Wildlife Research Pty Ltd, Australia

N.J.Beausoleil@massey.ac.nz

Globally, many millions of sentient mammals are killed every year because they have detrimental impacts on commodities, environments or other species that humans value. Controlling these 'pest' animals relies heavily on the use of broad-spectrum lethal toxins, such as 1080, and anticoagulants, which are becoming progressively less accepted.

Aotearoa New Zealand (NZ) has no indigenous predatory mammals, but a number of introduced mammals have significant environmental and economic impacts. NZ is thus a world leader in lethal pest animal control and has set the objective to become 'predator free' by 2050. A key mechanism proposed to achieve this goal is the development of species- or taxon-selective toxins. NZ's government* has recently funded a large multi-disciplinary programme of research aimed at discovering novel pest-selective toxins and refining existing toxins to make them more selective for mammalian predators and/or less toxic for valued indigenous species.

The key benefit of more selective toxins is reduced mortality and impacts on the welfare of non-target animals. However, it is imperative that any new or refined toxin is also less harmful to the welfare of the target animals, at least compared to the worst toxins currently in use. Thus, scientific assessment of animal welfare must be built into every component of the toxin development pathway, meaning that teams developing new toxins should include personnel with specific animal welfare science expertise and that pathways should be designed to fail early if poor welfare impacts are predicted.

The ways in which animal welfare can be built into toxin development pathways will depend on the knowledge available about the toxic mechanism of action, pathophysiology, and the target animal. For refinement pathways, existing knowledge of the toxin's mechanism and pathophysiological effects may be coupled with emerging knowledge of species-specific factors to systematically predict welfare impacts in the target animals. For entirely novel toxins, emphasis should be put on developing non-animal models to explore the toxin's mechanism of action and molecular targets (e.g. fungal assays for 1080) and on replacing lethal animal assays with less harmful sub-lethal assays (e.g. identifying early predictive indicators of lethality or indicators of lowest observed adverse effects).

In this programme of research we will seek to embed scientific approaches to improving animal welfare and the 3Rs throughout selective toxin development pathways, improving the welfare of both target and non-target animals.

*This research is funded by the NZ Ministry Business Innovation and Employment.

USING A NATURALLY OCCURRING STERILITY GENE TO HUMANELY CONTROL HOUSE MOUSE PESTS

ANDRI MANSER, Salomé Friry, Caspar Goedecker, Ellen Gonzales and Anna Lindholm

Department of Evolutionary Biology and Environmental Studies, University of Zurich, Switzerland

andri.manser@ieu.uzh.ch

House mice are a major ecosystem pest, particularly threatening island ecosystems as non-native invasives. Current methods to control invasive populations have serious limitations. The aerial broadcasting of anticoagulant poisons, currently the method of choice, has raised serious animal welfare concerns, causes off-target effects by killing other species in a target area, and is difficult to apply in areas inhabited by humans. In our research group, we investigate the possibility of using a naturally occurring version of mouse chromosome 17, called t haplotype, as a humane alternative to control mouse pests. The t haplotype has promising characteristics that may allow us to control pest populations by targeting fertility rather than killing animals. First, males that carry two copies of the gene (t/t homozygotes) are completely sterile. Akin to sterile insect techniques, the release of t/t sterile males could be utilised to hamper reproductive rates by directly raising the frequency of unproductive matings. Second, males that carry only one copy of the t (+/t heterozygotes) are not only fully fertile, but pass the gene on to 95% of their offspring rather than to the 50% expected under Mendelian inheritance. This systematic transmission bias, also known as gene drive, may facilitate the genetic propagation of the sterile t in a population via the release of +/t heterozygote males. I will present a theoretical model assessing the general feasibility of sterile t releases to control target populations via a shortage of fertile males. The model will identify key factors that determine the promise of the method, such as population demography, female mating behaviour, and the ability of released males to establish in existing populations. I will then present some early laboratory results on the characteristics of the sterile t, and finally provide a brief outline of the work planned in our research project, ranging from laboratory experiments to field populations. The sterile t approach would offer advantages over current methods. The method is humane as mice are not killed but just no longer able to breed. The method is exclusive to house mice, and would not harm humans or other animals in a target area. Furthermore, t haplotype carrying mice occur naturally, so carrier mice could potentially be bred and released at low cost. Hence, in contrast to currently discussed synthetic gene drive solutions, the method does not require genetic modification, which would greatly reduce ethical and regulatory barriers regarding its use.

THE ROLE OF THE EURCAW IN REFINING THE ASSESSMENT OF POULTRY AND RABBIT WELFARE AT SLAUGHTER

ALEXANDRA CONTRERAS-JODAR[1], V. Michel[2], A. Varvaró-Porter[1] and A. Velarde[1]

[1] Animal Welfare Program, Institute of Agrifood Research and Technology (IRTA), Spain
[2] Direction of Strategy and Programmes, French Agency for Food, Environmental and Occupational Health & Safety (ANSES), France

alexandra.contreras@irta.cat

The European Union Reference Centre for Animal Welfare for Poultry and other small-farmed animals (EURCAW-Poultry-SFA) was created in February 2020, in the context of the regulation 2017/625 about official controls (art. 95). The Centre is aimed at supporting the European Commission and the Member States in the implementation of welfare legislation for poultry and other small-farmed animals (e.g., minks, rabbits) on farms, during transport and at slaughter. In relation to slaughter, the Centre has put special focus on the assessment of the state of consciousness. At first, the Centre broke down the 1099/2009 legislation into legal requirements (i.e., small pieces of legislation) and for each legal requirement, a list of animal-based, resource-based and management-based indicators was made for all those related to the assessment of the state of consciousness. In addition, the Centre has carried out literature reviews aimed at providing practical guidance on the main welfare aspects of different stunning methods. These reviews also included the results of surveys of Competent Authorities (CAs) and Official Veterinarians (OVs) aimed at describing the current stunning methods used in the European Union, the indicators used for the assessment of the state of consciousness and to identify possible knowledge gaps. The results of these surveys showed that there is heterogeneity in the methods and indicators used for the assessment of the state of consciousness, and in the key electrical parameters applied. For this reason, scientific studies have been carried out in commercial slaughterhouses with the aim to refine the method and the list of indicators for the assessment of the state of consciousness to ensure consistency of controls in EU slaughterhouses. The refinement consisted in the assessment of the prevalence and the inter-observer repeatability of the most valid and feasible indicators of the state of consciousness. In addition, the efficiency of stunning was also evaluated and recommendations to spare avoidable pain, distress and suffering at the time of killing based in on-field observations have been reported. Webinars are organised for competent authorities and official veterinarians to disseminate the results found. In addition, factsheets with refined methods and indicators and good practices found in commercial slaughterhouses are produced. On the other hand, the Centre also provide technical assistance to CAs by its service Q2E (Query to EURCAW). Reviews, study results, webinars, factsheets and Q2E answers are freely available on the Centre's website (https://www.eurcaw-poultry-sfa.eu/).

A REVIEW OF INDICATORS AND SENSOR TECHNOLOGIES TO ASSESS PIG WELFARE AT THE SLAUGHTERHOUSE

ANGELA RAMON-PEREZ[1], Björn Forkman[2], Xavier Manteca[1] and Pol Llonch[1]

[1] Department of Animal and Food Science, Universitat Autònoma de Barcelona, Spain
[2] Section of Animal Welfare and Disease Control, Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

angela.ramon@uab.cat

Pigs, as any other meat-producing animals, end their lives at the slaughterhouse. The abattoir represents a crucial place for the collection of numerous data relating to a large number of animals, including their welfare status. A systematic review following PRISMA guidelines was conducted to identify studies using animal-based and resource-based indicators to assess pig welfare (including farm, transport, and slaughterhouse) in three literature search engines. Once the relevant indicators and methodologies for assessing pig welfare at the slaughterhouse were obtained, a commercial search was performed to identify technological solutions capable of evaluating these indicators. A total of 1583 papers were initially identified, selecting 119 for analysis, resulting in 57 specific studies focusing on assessment at the slaughterhouse. Fifty-three different welfare indicators were identified, including 16 related to on-farm welfare issues and 37 related to welfare during transport or at slaughterhouse. Twenty-eight antemortem indicators were found, for assessing consciousness after stunning (n=9), handling stress (n=6), heat stress (n=4), restricted movement (n=3), undefined stress (n=2), prolonged thirst (n=1), group stress (n=1), gastro-enteric disorders (n=1), and locomotor disorder (n=1). The remaining 25 indicators were to be performed postmortem, monitoring undefined stress (n=8), gastro-enteric disorders (n=2), respiratory disorders (n=4), soft tissue lesions and integument damage (n=5), other disorders (n=3), restricted movement (n=1), prolonged hunger (n=1), and muscle exhaustion (n=1). Most of these indicators were assessed by a human observer (n=40), while a small group of biomarkers were required to be analysed at the laboratory (n=9), and a few were evaluated by sensors such as computer vision (n=2) and infrared cameras (n=1). It should be noted that 12 indicators which were assessed by a human observer were also evaluated by using image analysis technologies. From the commercial search, two different computer vision technologies located at the slaughter line were found, one for assessing tail lesions and length, and another for assessing signs of pneumonia and pleurisy in the lungs. Additionally, two prototype technologies were found, one computer vision camera for automatically assessing consciousness after stunning using corneal reflex, and another for monitoring bleeding through thermal image analysis. This review provides a detailed description of the state of the art in assessing pig welfare at the slaughterhouse and elucidates the potential for sensors to enhance the welfare assessment.

COMPARISON OF 8 DIFFERENT INERT GAS (MIXTURES) TO CO_2 FOR STUNNING PIGS AT SLAUGHTER

JONAS KNÖLL[1], Julia Gelhausen[2], Thyra Friehs[2], Tony Krebs[2,3], Daniel Mörlein[2], Jens Tetens[2] and Inga Wilk[1]

[1] Institute of Animal Welfare and Animal Husbandry, Friedrich-Loeffler-Institut, Celle, Germany
[2] Department of Animal Sciences, Georg-August-University Göttingen, Germany
[3] Institute of Organic Farming, Johann Heinrich von Thünen Institute, Westerau, Germany

Jonas.Knoell@fli.de

Despite concerns over its aversiveness, stunning of pigs at the time of slaughter using high concentration of CO_2 is the most common method in Europe. Inert gases and mixtures of inert gases with low concentration of CO_2 have been proposed as an alternative, but have so far not been considered market-ready due to concerns of gas stability, meat quality, costs and stunning effectiveness.

As part of the project for Testing Inert Gases in order to Establish Replacements for high concentration CO_2 stunning for pigs at the time of slaughter (TIGER), experiments were conducted in a commercial Dip-Lift system using a new gassing system allowing residual oxygen concentrations <1%. In a selection phase, pigs were stunned and slaughtered using argon, or nitrogen-argon mixtures with 0-30% CO_2 content or high concentrations of CO_2 (control) to screen for their suitability in terms of animal welfare and meat quality. In an optimisation phase, argon and the nitrogen-argon mixture without CO_2 were compared to high concentrations of CO_2 in more detail.

For each of the eighteen measurement days, video sequences of the stunning procedures were randomized and cut to a new video. Aversive and other events were captured by two observers according to an ethogram. Aversive reactions before loss of balance were statistically significantly reduced ($p<0.05$) for all argon-based gas mixtures and for nitrogen without CO_2 compared to high concentration CO_2.

Stunning effectiveness was closely monitored for at least three minutes after ejection from the stunning system. Exposure time was dynamically adjusted for each gas mixture. Using a binomial model, exposure times to reach a likelihood of inadequate stuns of < 0.5% were estimated. Exposure times were shortest for high concentration CO_2 stunning, followed by the pure inert gases without CO_2 content, and they were longest for mixtures with 20% CO_2.

Meat quality parameters (pH, temperature, and electrical conductivity) were assessed in loin and ham 40min and 24h *post mortem*. Drip loss, cooking loss, shear force and color were examined for the loin (selection phase) and for the ham (optimization phase). In both phases, petechial hemorrhages in the hams were recorded. Small but statistically significant differences ($p < 0.05$) were found for pH_{45min} and pH_{24h} in ham and loin during selection and for pH_{45min} in the loin during optimization, without evidence of PSE meat formation. Additionally, the incidence of hemorrhages in the ham was increased in both phases for the N_2 mixtures.

In conclusion, all tested inert gas mixtures were beneficial with respect to reduced aversiveness in the induction phase compared to high concentration CO_2, with gas mixtures with less CO_2 showing less or shorter aversions. While longer exposure times were needed for inert gas atmospheres compared to CO_2 in high concentrations, safe levels for stunning can be reached. The meat quality of loin and ham is considered on par with conventional CO_2 stunning. Yet, the increased incidence of hemorrhages in the ham under N_2 based mixtures requires further investigation.

IMPROVING AUTOMATIC ELECTRIC STUNNING SYSTEMS FOR PIGS AS A VIABLE AND MORE ETHICAL ALTERNATIVE TO CO_2

LESLEY MOFFAT, KEES SCHEEPENS, Madelaine Looije, Michel Oosterhuis, Roy Melsert and Jan Voordouw

Eyes on Animals, The Netherlands

lesley@eyesonanimals.com

CO_2 gas stunning of pigs causes fear, pain and breathlessness. However, for economic reasons and a so called “lack of a viable alternative”, CO_2 gas stunning continues to be in use more than ever. CO_2 stunning is often promoted as a “welfare-friendlier” system as pigs can be moved in groups and do not go through the stress of single-file restraint, but the price they pay is at the end; every pig stunned with CO_2 inevitably experiences 20 seconds of suffering to death. But have we not forgotten about automatic electric-stunning? When performed correctly, electric stunning is immediate and painless. Could it not be a viable and more humane alternative for large slaughterhouses, if more time and energy was invested in fine-tuning it? Eyes on Animals made crucial changes to the design of automatic electric-stunning systems and to the methods of herding pigs through them. Reducing the tempo per stunner, moving smaller numbers of pigs at a time, changing the ingrained habits of the workers and getting rid of all unnecessary distractions and noise are just some of the important changes made. Their presentation illustrates how to reach the full potential of making electric-stunning the most humane option available today. In the eyes of Eyes on Animals, there is no need to further delay the phasing out of CO_2 stunning.

MORE HUMANE STUNNING AND EUTHANASIA OF PIGS AND POULTRY BASED ON NITROGEN WITH HIGH EXPANSION FOAM TECHNOLOGY

NICOLE KREFTING, Sebastian Strand

HEFT AB, Sweden

nicole.krefting@heftinternational.com

HEFT was founded to further develop and research the nitrogen foam method to find a more humane and commercially sustainable stunning and euthanasia method. An early discovery was that to consequently obtain and maintain an anoxic atmosphere whereof creating a closed system was a necessity. The method is based on the rapid displacement of oxygen by purging the air from a container and replacing it with nitrogen. The high-expansion foam is used to distribute the nitrogen, thus increasing the effectiveness by significantly reducing the filling time compared to only flushing nitrogen. By building the foam from the bottom and up, the foam works as a blanket pushing the air out through air plugs in the ceiling of the container. The animals are instantaneously transited from atmospheric 21% oxygen air to an anoxic environment as their heads are covered by the foam, which leads to loss of posture and loss of consciousness within 10-20 seconds.

Stunning and euthanizing pigs and poultry with nitrogen is well documented and was described by EFSA in 2004 while deemed to have great future potential. However, due to technical difficulties, very few/no commercial products are available. Unlike the commonly used gas carbon dioxide, nitrogen easily mixes with air, thus making it difficult to harness. Attempts to control the gas sparked the idea of encapsulating it in foam, covering the animals and thereby exposing them to the nitrogen gas. At the beginning of the 2000s, different studies involving capturing nitrogen in foam were conducted and proof of principle was later confirmed, both pigs and poultry are euthanized by prolonged anoxia due to the high nitrogen content, not by occlusion of airways from the foam. Characteristics and parameters of the method are established to improve animal welfare and the process. Some of the most profound characteristics and parameters are gas purity, exposure time, filling time, maintaining oxygen level below 2%, bubble size, expansion ratio, and foam agent composition.

The method can be applied to pigs and poultry regardless of size, age, and breed. The technology is scalable and can be used for individually sick and/or injured animals as for large-scale depopulation.

HEFT is in the process of being included in the Council Regulation (EC) No 1099/2009 on the protection of animals at the time of killing for emergency euthanasia and depopulation situations. EFSA's scientific opinion is in preparation.

Concurrent research studies with independent institutions have been conducted with results proving and strengthening the benefits of the method, both published and in preparation.

ANIMAL DISASTER MANAGEMENT DECISION-MAKING: APPLYING AN ANIMAL WELFARE SCIENCE-ETHICS GUIDED FRAMEWORK WITHIN ONE HEALTH

RAYMOND ANTHONY[1] & Andreia De Paula Vieira[2]

[1] University of Alaska Ancorage, USA
[2] Veterinarian and Independent Researcher, Curitiba, Brazil

rxanthony@alaska.edu

For animal disaster management professionals (e.g., veterinarians and responders), operational needs can often supplant ethics or values considerations in importance during a disaster response, potentially impacting negatively on human and animal welfare. An Animal Welfare Science-Ethics Guided framework within One Health enables moral clarity, critical dialogue-reasoning, and creative and inclusive problem-seeing and -solving around competing normative demands that stem from conflicts in moral-professional convictions, identities and duties and situational elements of a disaster. We propose Ethical Guardrails, Animal Welfare Science-Ethics Guided Training and Content Development (AWS-EGTCD) and Communitarian Values be central to animal disaster management decision-making.

Ethical guardrails are vital to responsible practice. They help: i) identify moral issues, ii) inform decision makers about implications or violations of values, norms or duties and evidence-based practices, iii) structure conceptualizations of trade-offs in decision-making, and iv) underscore ideas or principles of respectful and healthy human-animal-environment relationships.

Integrating AWS-EGTCD within the workflow of animal disaster management decision-making provides the basis of good governance, democratic engagement and public trust, and enables outcomes that will be most conducive to the welfare of all interested parties. Content that is well-informed by and inclusive of welfare science, epidemiological data and ethics advances norms of conscientiousness, transparency and professional trustworthiness. Training that incorporates AWS-EGTCD within One Health can resolve tensions around difficult choices, including method of killing animals and when, to mitigate negative welfare and public health consequences.

Animal disaster management is a public health and safety matter grounded in social justice. It requires weighing interests of stakeholders against the contexts of disaster preparedness, mitigation, response and recovery. A pivot to communitarian values ahead of individual interests is necessary but may result in clashes of moral-professional identities and values, moral burden and distress. Emphasizing communitarian values within a One Health framework that centers the abovementioned pairing can serve as an effective means to protect animal disaster professionals from negative impacts of their responsibilities since it clearly communicates the risk mitigation outlook to different publics.

Where possible, we recommend consulting veterinarians, public health ethicists, epidemiologists and animal welfare scientists for an independent and trustworthy voice and to nurture a decision-making structure that empowers animal disaster management professionals to use their ethical-technical voices and confront motivated reasoning and bias. Supporting disaster management plans that are well-structured as above can inform how professional services (e.g., veterinary, civil defense) minimize conflicting courses of action and act with competence and effectiveness.

TOWARDS A MORE HUMANE DEPOPULATION OF POULTRY IN CASE OF AVIAN INFLUENZA

Louise Kremer[1,2], Antonio Velarde[1,3], LEONARDO JAMES VINCO[1,4], Tiziano Bernardo[1,4] and Virginie Michel[1,5]

[1] European Reference Centre for Animal Welfare for Poultry and other Small Farmed Animals (EURCAW-Poultry-SFA), ANSES, Maisons-Alfort, France
[2] French Reference Centre for Animal Welfare (FRCAW), INRAe CODIR, Paris, France
[3] Institute of Agrifood Research and Technology (IRTA), Spain
[4] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Italy
[5] Direction de la Stratégie et des Programmes, ANSES, Maisons-Alfort, France

louise.kremer@inrae.fr

Many EU Member States (MSs) keep recording new outbreaks of Highly Pathogenic Avian Influenza (HPAI), a highly contagious disease which can be lethal and zoonotic, depending on the viral strains at stake. When HPAI is detected, the competent authorities must take the appropriate actions to ensure its eradication. One of the key actions consists in depopulation of the infected flock. To ensure its efficiency, depopulation must be conducted without delay and with strict biosecurity measures to avoid the disease spread. In practice, the sense of emergency in which the depopulation takes place may jeopardize bird welfare, an issue acknowledged by the MSs, who expressed their desire to better integrate the animal welfare dimension into their depopulation operating procedures. To meet this need, the EURCAW-Poultry-SFA has launched a study aiming at making recommendations on the type of on-farm killing methods to adopt in order to ensure the most humane depopulation. Recommendations will be tailored to the characteristics of the farm (e.g., housing system) and birds (e.g., species) at stake. Two sub-objectives have been defined: 1) the inventory of the depopulation methods used across the EU, and 2) the assessment of the effectiveness (in terms of death induction) and welfare consequences of the identified methods. Sub-objective 1 has been attained by sending out a generic survey to the competent authorities of each MS in March 2023. Based on the responses of twenty MSs, fourteen depopulation methods were identified as having been used since 2018. The five most common methods are (from the most to the least used) containerized gassing, cervical dislocation, lethal injection, whole-house gassing without foam, and captive bolts. Sub-objective 2 has been partially achieved. Depopulation experts from eighteen MSs were invited to a workshop to talk about the main bird welfare issues encountered on depopulation site, and discuss practical solutions found to tackle the said issues. Method-specific surveys were also sent in October 2023 to all competent authority contact points, to collect additional information regarding the context of application (e.g., farm characteristics), the efficacy (e.g., failure rate) and the welfare consequences (e.g., cold stress) of each method. Based on the responses obtained, the EURCAW-Poultry-SFA intends to give context-specific recommendations regarding the most humane depopulation method to opt for, and to suggest "best-practices" for each of these methods. The "best-practices" will be refined and validated by depopulation experts – either during workshops or through guided interviews. Deliverables will be available in 2025.

This research was funded by the EURCAW-Poultry-SFA (European Commission Grant number: Project: 101144023 — EURCAW-Poultry SFA 2023-2024 — SMP-FOOD-2023-EURL-EURC-AG-IBA) and the FRCAW (French Reference Centre for Animal Welfare, French Ministry for Agriculture and Food Sovereignty Grant C-2023-013).

HUMANE METHODS FOR KILLING DAY-OLD CHICKS

BIRTE L NIELSEN and Charlie Mason

Humane Slaughter Association, United Kingdom

birte@hsa.org.uk

Millions of day-old layer chicks are killed each year in hatcheries because they are males and thus not needed for egg production. In addition, if chicks are sickly or deformed, they will need to be killed upon hatching to prevent their welfare being further compromised. In the UK and EU legislation, all day-old chicks killed in hatcheries must be killed humanely. Currently, two such methods exist.

One is controlled atmosphere killing by exposure to specific gas mixtures. The principle of gas killing is to deprive the brain of oxygen through inhalation of a gas mixture with very low oxygen content. As the killing of chicks with gas mixtures does not result in an immediate loss of consciousness, it is important to ensure the induction of unconsciousness does not cause distress to the chicks. A humane and efficient gas mixture must therefore be non-aversive, induce loss of consciousness as rapidly as possible, and be capable of killing chicks. Neonates, such as day-old chicks, are very resistant to oxygen deprivation. It is therefore necessary to expose the chicks to gas mixtures with very little (less than 2%) residual oxygen for long enough to ensure that chicks are killed, and not just rendered unconscious (stunned).

The alternative is to use a mechanical apparatus that causes immediate death via crushing or maceration. Although aesthetically unpleasant, instantaneous mechanical destruction (IMD) is a humane and effective killing method for day-old chicks provided the equipment is used, managed, and maintained correctly.

In some EU countries, the routine killing of day-old male chicks in hatcheries has been banned. It is arguably an ethical problem that the production of eggs for human consumption currently involves the breeding of some birds that are killed soon after being hatched. However, it is not an animal welfare issue, provided the day-old chicks are killed humanely. Equipment for humane killing of newly-hatched but moribund chicks always needs to be available in commercial hatcheries.

A SUSTAINABLE APPROACH TO ELIMINATE MALE CHICK CULLING IN THE EGG INDUSTRY

ELIRAN KADOSH

Head of Research, egg XYT, Israel

eliran@eggxyt.com

The global consumption of table eggs reaches approximately 1.5 trillion per year, sourced predominantly from over 7 billion laying hens that have undergone extensive breeding for enhanced productivity. However, their male counterparts, being non-egg-layers and lacking sufficient muscle for the meat industry, face a distressing fate of immediate culling - either through shredding in metal grinders or gassing - resulting in the disposal of around 7 billion 1-day-old male chicks. In response to this ethical and environmental concern, eggXYt is pioneering a revolutionary technology aimed at eliminating male chick culling at the earliest possible stage, right after the egg is laid. This innovative approach requires no prior incubation time and is conducted in a non-invasive manner - without the need to puncture the shell. eggXYt's solution comprises two key components: a sex-detectable chicken line incorporating a fluorescent bio-marker at a safe harbor locus on the male (Z) chromosome using CRISPR/Cas9, and seXYt - an electro-optical scanning device capable of conducting an automatic and comprehensive scan of all eggs entering the hatchery. Through this process, eggs containing fluorescently labeled embryos, indicative of male offspring, are accurately identified and removed prior to incubation. Crucially, eggXYt's approach requires the gene-editing solely in males, while laying females and their eggs remain 100% genetically identical to wild-type hens/eggs and do not harbor the fluorescent bio-marker or any other genomic modifications. In summary, eggXYt's solution is accurate, non-invasive, implemented on day-0, and boasts high throughput. By selectively retaining only eggs containing female embryos in the hatchery, it effectively eradicates the necessity for the subsequent post-hatch culling of male chicks.

CHALLENGES OF DETERMINING SENSIBILITY IN FISH

JENNY BOUWSEMA[1], Maureen Ellis[1], Amaya Albalat[1], Silvere Santos[1], James Turnbull[1] and Jeff Lines[2]

[1] Institute of Aquaculture, University of Stirling, UK
[2] Ace Aquatec, Dundee, UK

jenny@aceaquatec.com

The ability to assess sensibility is essential to ensure a humane slaughter for all animals, including fish. Much of the research in fish has been conducted on salmonids, but even in salmonids challenges remain and translating findings to other species is not straight forward. Here we report studies on two commonly farmed species of fish which are both anatomically and evolutionarily distinct from salmonids. The species are tilapia *(Oreochromis niloticus)* and pangasius *(Pangasianodon hypophthalmus)*. We used behavioural indicators and electroencephalograms (EEG) to assess sensibility, by following fish through a sequence from in-water electrical stunning, recovery, to subsequent terminal anaesthesia. We found that behavioural indicators were lost and regained in a relatively consistent sequence within each species. In terminal anaesthesia, the fish passed through the various stages of anaesthesia rapidly but stages consistent with surgical anaesthesia and respiratory collapse were identifiable. Time to respiratory collapse (mean +- SD) was 204.6 +- 241.5 seconds in tilapia and 151.5 +- 76.3 seconds in pangasius. Subsequently changes in EEG continued for some time with Visual Evoked Responses (VER), persisting for up to 15 minutes in tilapia and 41 minutes in pangasius, after respiratory collapse. In separate fish which were anaesthetised, pithed and exsanguinated, heartbeat persisted for more than 60 minutes beyond respiratory collapse. While changes in EEG were observed over both recovery from stun and in terminal anaesthesia, so far, no clear correlation has been detected between the EEG and the clinical state of the fish. We have not yet found an EEG threshold which can be reliably used to determine sensibility, highlighting the continued challenge of unequivocally determining sensibility in fish

OPTIMISATION OF ELECTRICAL STUNNING IN THE DECAPOD CRUSTACEAN NORWAY LOBSTER *(NEPHROPS NORVEGICUS)*

AMAYA ALBALAT[1], Endre Putyora[1], Nasser Ayaril[1], Maureen Ellis[1], Sonia Rey Planellas[1] and Douglas Neil[2]

[1] Institute of Aquaculture, University of Stirling, UK
[2] School of Biodiversity, One Health and Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, UK

amaya.albalat@stir.ac.uk

In the last decade, public interest in the welfare of decapod crustaceans has increased in many parts of the world. In wild caught animals, welfare threats during capture and slaughter can be substantial, especially depending on the type of fishery. The crustacean decapod Norway lobster (*Nephrops norvegicus*) supports a very valuable fishery, which is substantially dominated by catches in Scotland, UK (>50% catch share). Nephrops can be sold live, fresh and frozen whole, but around 50% of the animals caught in Scotland are destined for the 'scampi' market, with animals having their tails separated from their heads (so-called 'tailing') while still alive on the fishing vessel at sea. In this presentation, we will report efforts made by our research team to optimise and validate an electrical stunning method that can be used to render the animals insensible before the 'tailing' procedure is carried out, thus making it more humane. Our initial recordings of nerve activity using extracellular recordings have shown that electro-stunning can indeed render Nephrops rapidly insensible. Criteria for successful stunning based on behavioural indicators are now also being established, and will be presented here. In terms of product quality and subsequent shelf life, results at day 7 based on a combination of a quality index method, total bacteria counts, muscle pH and bacteria-related metabolites indicate no significant differences between electro-stunning versus placing animals on ice.

ASSESSMENT OF THE EFFICIENCY OF ELECTRIC STUNNING ON THE BEHAVIOUR, PHYSIOLOGY, AND MEAT QUALITY OF THE AMERICAN LOBSTER *(HOMARUS AMERICANUS)*

Rodrigo A. Lorenzo[1], Pol Llonch[2], Joel González[3], José A. García del Arco[1,] Maria Font-i-Furnols[3], Òscar Chic[1] and GUIOMAR ROTLLANT[1]

[1] Institut de Ciències del Mar, Spanish National Research Council (CSIC), Spain
[2] Department of Animal and Food Science, Universitat Autònoma de Barcelona, Spain
[3] IRTA, Qualitat del Producte, Spain

guio@icm.csic.es

Decapod Crustacean sentience has taken the spotlight as one of the most recent debated topics within Animal Welfare due to a lack of formal legislation that protects their well-being, particularly at the moment of killing. Electrical stunning has been proven to avoid or minimise the pain and distress before killing. Studying the effects of this methodology on the quality and recovery of the animals is crucial to provide recommendations for legislation development. This study aims to test the feasibility of applying the Crustastun™ device to stun the highly commercialised American lobster (*Homarus americanus*) in order to: i. Determine the effect of the electrical stunning duration on the recovery and behaviour; ii. Asses the physiological disturbances of a stunning protocol (and its recovery); and iii. Analyse the effects of the electrical stunning on the quality of the 'end' product. For i., animals were electro-stunned with a short cycle (5s, S), long cycle (10s, L) or control cycle (placed inside the device for 10s with no current applied, C). For these groups, the righting response and activity were registered by video recordings and later processed by movement algorithms. To test the physiological disturbances, only the S cycle was used (with a corresponding C group) and glucose and lactate were measured before, and every hour (h) after stunning, for 12h, once the animals returned to aquaria. For objective iii, four different treatments were done: two of them processing the lobsters right after emersion (with (a) and without (b) stunning), and two simulating a 2.5h transport and being cooked afterwards (again, with (c) or without (d) stunning, before transport). Meat quality was assessed by cooking loss parameters, texture and sensory analyses. Regarding behaviour, the righting response was significantly different for each stunning (S & L) cycle and the control, being immediately righted on C, delaying 34±7 and 301±58 min on the S & L cycle, respectively. The S cycle showed metabolic disturbances in both glucose and lactate, with only recovery to basal levels after 12h on lactate (8.2±0.5 vs 3.6±0.9 mM, 3 vs 12h, respectively). The meat quality was improved by the electric stunning showing less chewy and softer meat on an instrumental analysis of it. These results show that the Crustastun™ stunning is reversible and improves the quality of the American lobsters' meat, yet it could be improved by shortening the stun duration to 1s for complying with humane killing methods.

IMPACT OF ELECTRICAL SHOCK AND ANALGESIA IN NORWAY LOBSTER *(NEPHROPS NORVEGICUS)*

ELEFTHERIOS KASIOURAS[1], Guiomar Rotllant[2], Albin Gräns[3], Per Hjelmstedt[3] and Lynne U Sneddon[4]

[1] Department of Biological and Environmental Sciences, University of Gothenburg, Sweden
[2] Institut de Ciències del Mar, Spanish National Research Council (CSIC), Spain
[3] Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Gothenburg, Sweden
[4] Department of Biological and Environmental Sciences, University of Gothenburg, Sweden

eleftherios.kasiouras@bioenv.gu.se

The concept of humane killing stipulates that death ensues without pain, suffering or distress and is highly relevant in fisheries where large numbers of animals are caught for food. The Norway lobster is captured by small to large scale commercial fisheries but methods for humane killing for this, and other crustacean species, are largely unexplored. Scientific evidence for the capacity of decapods such as the Norway lobster to experience pain, stress and suffering would provide powerful arguments that humane killing should be enforced for this animal group. In this study, the impact of electric shock on behavioural and physiological responses was investigated by applying increasing electric fields to Norway lobster. We found that exposure to >2 Vrms cm-1 appeared to cause the animal to go into a tetanus or cramp like state. Electric shock of 1 Vrms cm-1 caused a significant adverse reaction, and the lobsters displayed an escape response during and after the electrical stimulus was applied. To determine if this escape response was nociceptive or potentially painful, we observed behavioural responses prior to the shock and for two hours after the shock with or without analgesia. Animals were randomly assigned to a no shock control group, shock group, shock with either lidocaine (local anaesthetic) or aspirin. Any potential effects from the drugs were determined by observing animals exposed to lidocaine or aspirin without an electric shock. Haemolymph and nervous tissues were sampled to investigate stress indicators and gene expression. Shocked animals without analgesia groomed their body significantly more after the shock over the two hour period. Both drugs prevented this response, but lidocaine appeared to have a sedating, rather than analgesic, effect on the animals. Electric shock resulted in an immediate reduction in activity not seen in the other groups and activity continued to decline over time. All other groups increased their activity immediately after the treatment. These findings suggest that the Norway lobster was affected by exposure to an electric field of 1 Vrms cm-1 and that higher field strengths will be necessary to stun this species. Behaviour was affected for a prolonged period after the shock and was modulated by drugs that provide analgesia. Future studies should explore the effects of electric field strengths above 2 Vrms cm-1 and measure central nervous system responses to determine if the animals become unconscious before considering the use of a confirmatory killing method.

OPTIMISATION OF IN-WATER ELECTRICAL STUNNING IN TROPICAL PRAWNS *(PENAEUS VANNAMEI)*

ENDRE PUTYORA, Jenny Bouwsema, Nasser Ayaril, Maureen Ellis, Sonia Rey Planellas and Amaya Albalat

Institute of Aquaculture, University of Stirling, UK

endre.putyora@stir.ac.uk

Following recognition by the UK government of decapod crustaceans as 'sentient beings' under the Animal Welfare (Sentience) Act, studies looking at welfare (especially during capture and slaughter) are of great importance. Our team has recently begun looking at the optimisation of electrical stunning parameters by focusing on both behavioural and electrophysiological (EEG) measures in an important farmed species: *Penaeus vannamei*. Electrical stunning has the capacity to render animals insensible as well as to kill (irreversible stunning) depending on the voltage and duration of time the current is applied. In this project, insensibility was evaluated via EEG measures while simultaneously validating behavioural observations for non-invasive, on-farm assessment. Behavioural work on stunning parameters applied a voltage on the animals (n=32) of between 2.0-2.5 V/cm with a duration of 1, 15 and 60 s. Video recordings were made with cameras placed on the side of the tank. A total of 40 videos were analysed and behaviours scored using BORIS software. The primary indicator of successful stunning was the loss of the righting reflex however, overall observed behaviours were categorized into 4 stages: Stage 1 is uncoordinated movement, Stage 2 is initial coordinated movement, Stage 3 is righting, and Stage 4 is fully coordinated movement. Animals stunned for either 15 or 60 s did not recover. Additionally, EEG recordings were taken on animals using a voltage between 2-3 V/cm with a duration of between 1-5 s. The level of insensitivity was assessed by performing 4 different tests (mechanical, visual, olfactory and optomotor). The findings of this work are key in ensuring that electrical stunning is capable of rendering tropical prawns insensible and that behavioural observations of a stunned state serve as accurate proxies for confirmation of this.

STUNNING AND KILLING OF EUOPEAN LOBSTER *(HOMARUS GAMMARUS)* AND EDIBLE CRAB *(CANCER PANGURUS)*

RAGNHILD AVEN SVALHEIM[1], Bjørn Roth[1], Endre Grimsbø[2], Henny Reimert[4], Gro van der Meeren[3], Luca Pettinau[4] and Hans van de Vis[4]

[1] Nofima AS, Norway
[2] UiT The Artic University of Norway, Norway
[3] Institute of Marine research, Austevoll Research Station, Norway
[4] Wageningen University & Research, The Netherlands

ragnhild.svalheim@nofima.no

The welfare of decapod crustaceans has become a prominent concern, with legislation in place for humane slaughter in some countries and ongoing discussions in others. Electrical stunning shows promise as a humane method under specific conditions, but scientific data on its effectiveness in different crustacean species are limited. Current criteria for assessing stunning effectiveness rely heavily on behavioural observations, which may not consistently reflect neural insensibility. This study provides crucial neurophysiological insights into the efficacy of electrical stunning, using a laboratory instrument, in two commercial decapod species: the Edible crab *(Cancer pagurus)* and the European lobster *(Homarus gammarus)*.

We employed EEG to measure neurological signals, with a specific focus on simultaneous monitoring of ganglia and heart signals. Neural activity was recorded alongside motor activity and sensory responses. A 50Hz sinusoidal AC current was used for electrical stunning, following a validated protocol. We also examined wet stunning by immersing animals in saltwater while applying current at field strength of 650 V/m. Deviations from the "resting signal" were indicative of nervous system disruption and potential loss of consciousness. After 5-10 minutes, the animals were euthanized by either freezing at -20°C in saltwater brine or boiling, with nerve signal recording during the process.

In both lobsters and crabs, a notable correlation was observed between disrupted nerve signals and motor function and sensory responses using both dry and wet stunning methods. While not all behaviour responses were completely lost, a significant reduction was evident. No return to the "resting signal" was documented during the observation period before euthanasia.

Immersing into boiling water and –20 °C brine did result in death in both species, as evidenced by cessation of ganglion activity and cardiac arrest. The time until death was longer for edible crabs than lobsters, this is most likely attributed to more protective carapace and ganglion.

In conclusion, exposing crabs and lobsters to electrical current equivalent of 240 V on dry and 650 V/m in water provides positive indications that the animals lose consciousness within 1 second. Prolonging the electrical duration to 10 s combined with boiling or cold shock in brine, results in permanent loss of consciousness. Thus, humane stunning and killing of these two species of crustaceans is feasible.

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Federal Department of Home Affairs FDHA
Federal Food Safety and Veterinary Office FSVO

UFAW
SCIENCE IN THE SERVICE OF ANIMAL WELFARE

Scientific Programme: Posters

List of Posters and Poster Abstracts

List of posters

1. **Lateral Vice Cervical Dislocation - A novel method for secondary physical euthanasia in hamsters**
   ALEXANDRIA HICKS-NELSON, Amelia Bothel, Derek Eriacho, Elena Carlson, Brianna Wrightson and Jim Boonyaratanakornkit *(Department of Shared Resources, Comparative Medicine, Fred Hutchinson Cancer Center, Seattle WA, USA)*

2. **Methods of decapod crustacean slaughter; science and practice led evidence and solutions for a sentient species and for industry.**
   BEN STURGEON *(Crustacean Compassion, UK)*

3. **Grinding as a slaughter method for farmed black soldier fly larvae: Empirically-supported recommendations for more instantaneous and humane practice**
   CRAIG PERL [1], Meghan Barrett[2], Chelsea Miranda[3], I Theresse Veloso[4], Casey Flint[5], Austin Martinez[6], Bob Fischer[7] and Jeffery K Tomberlin[8] *([1] Insect Welfare Research Society, Indianapolis, USA; [2] Department of Biology, Indiana University Indianapolis, USA; [3] Department of Entomology, Texas A & M University, College Station, USA; [4] Department of Biology, California State University Dominguez Hills, Carson, USA; [5] Department of Entomology, Texas A & M University, College Station, USA; [6] Department of Biology, California State University Dominguez Hills, Carson, USA; [7] Department of Philosophy, Texas State University, San Marcos, USA; [8] Department of Entomology, Texas A & M University, College Station, USA)*

4. **Effectiveness of repeated exposure to anaesthesia in the Norway lobster (*Nephrops norvegicus*)**
   ENDRE PUTYORA, Sonia Rey Planellas, Maureen Ellis, Nasser Ayaril and Amaya Albalat *(Institute of Aquaculture, University of Stirling, Stirling, UK)*

5. **The fair-fish database: welfare assessment of aquatic species in aquaculture and fisheries**
   FAUSTA BORSANI[1], Jenny Volstorf[1], Caroline Marques Maia[2], María Cabrera-Álvarez[2] and Sebastian Scholz[1] *([1] fair-fish, Uster, Switzerland; [2] FishEthoGroup association, Olhão, Portugal)*

6. **Towards a humane way to euthanize commercial bumblebees**
   GEMA MARTIN-ORDAS[1] and Matthijs Metselaar[2] *([1] University of Stirling, UK; [2] Aquatic Vets Ltd, UK)*

7. **The value of welfare: What can we expect from consumers if we increase their exposure to more facts about wild-caught fish welfare?**
   THEMISTOKLIS ALTINTZOGLOU[1], Ragnhild Aven Svalheim[1], Michelle Boonstra[2], Mike Breen[3], Bjørn Roth[1], Endre Grimsbø[4] and Hans van de Vis[5] *([1] Nofima AS, Muninbakken 9-13, Breivika, P.O. Box 6122, N-9291, Tromsø, Norway; [2] Michelle Boonstra Consultancy, Tweede Jan van Heijdenstraat 64, 1074 XW Amsterdam, The Netherlands; [3] Institute of Marine Research, P.O. Box 1870 Nordnes, NO-5817 Bergen, Norway; [4] UiT, The Arctic University of Norway, P.O. Box 6050 Langnes, 9037 Tromsø, Norway; [5] Wageningen University & Research, P.O. Box 9101, 6700 HB Wageningen, The Netherlands)*

8. **Humane killing of an economically important decapod crustacean, the signal crayfish.**
   ELEFTHERIOS KASIOURAS[1], Lisa Carlsson[1], Albin Gräns[2] and Lynne U. Sneddon[1] *([1] Department of Biological and Environmental Sciences, University of Gothenburg, Sweden; [2] Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Gothenburg, Sweden)*

LATERAL VICE CERVICAL DISLOCATION - A NOVEL METHOD FOR SECONDARY PHYSICAL EUTHANASIA IN HAMSTERS

ALEXANDRIA HICKS-NELSON, Amelia Bothel, Derek Eriacho, Elena Carlson, Brianna Wrightson and Jim Boonyaratanakornkit

Department of Shared Resources, Comparative Medicine, Fred Hutchinson Cancer Center, Seattle WA USA

ahicksne@fredhutch.org

Hamsters are listed in the header for the laboratory rodent section of the AVMA Euthanasia Guidelines, but without a tail and with thick necks, they are traditionally not considered for cervical dislocation. As there is no guidance on methods for secondary physical euthanasia specific to the species, many institutions provide guidance on guillotine, prolonged CO2 exposure, or bilateral thoracotomy. While providing euthanasia guidance to a lab investigating RSV in a hamster model our clinical veterinarian noted lab members needed more rapid confirmation of euthanasia due to the delicacy of the tissues they intended to harvest. In brief, the lab noted variance in titers and autolysis of tissues following prolonged CO2 exposure. Utilizing a variety of modalities hamster carcasses were assessed for novel methods of cervical dislocation, culminating in a lateral vice cervical dislocation (LVCD) method. In deeply anesthetized hamsters, LVCD resulted in immediate cessation of breathing, loss of corneal reflex, voiding, and irregular and rapidly decreasing heartrate. Dental radiographs showed cervical dislocation and displacement of the resultant vertebral ends without skin penetration. Manual palpation of hamsters that received LVCD by vet care staff resulted in 100% identification of cervically dislocated hamsters when compared to prolonged CO2 and bilateral thoracotomy controls. On gross necropsy of LVCD hamsters the spinal cord was severed typically at the atlantoaxial joint. We submit this method as a useful secondary physical method with relevance for labs utilizing hamsters in respiratory research as it is trachea sparing and lung sparing, or labs working in BSL2+ as a general method to minimize sharps usage, as all instruments utilized were non piercing surgical steel.

METHODS OF DECAPOD CRUSTACEAN SLAUGHTER; SCIENCE AND PRACTICE LED EVIDENCE AND SOLUTIONS FOR A SENTIENT SPECIES AND FOR INDUSTRY

Ben Sturgeon

Crustacean Compassion, UK

ben.sturgeon@crustaceancompassion.org

Following their inclusion in the Animal Welfare (Sentience) Act 2022 in the UK, decapod crustaceans were legally recognised as sentient with the ability to feel fear, pain and pleasure. This represented a huge step forward from an encompassing Animal Welfare Act (2006) which only recognised and continues to recognise vertebrate animals.

Despite this, specific means of humane killing is not within legislation and not considered by large parts of the fishing industry. The lack of effective legal protections and practical solutions means billions of these vulnerable animals endure unnecessary suffering every year to supply the UK market alone. The primary reasons provided are that the 'single' best and welfare appropriate technique is yet to be established, and practical solutions, including on board vessels, is yet to be agreed upon.

The poster highlights the different current methods of dispatch and provides science led humane evidence to make recommendations; and presents the practical solutions currently available.

Evaluation of over 300 peer reviewed and open source papers led to the conclusion that decapods crustaceans including lobsters, crabs, nephrops and prawns – eaten in huge quantities globally are and should be considered sentient.

Following the 'Precautionary Principle' it would be expected that such species are then only slaughtered or killed using methods that result in either instantaneous* death or instantaneous* insensibility to pain and distress until death occurs. However, the current methods of killing including boiling, dismemberment, high pressure processing, spiking and splitting, fresh water drowning, high salt solution immersion (NaCl and KCl), CO_2 hypercapnia, and chilling or freezing cause significant and negative welfare impact on decapod crustaceans evidenced through behavioural indicators including autonomy, physiological measures of stress, nerve function changes in synaptic transmission, and in eventual time to death.

Each method is presented highlighting the method of slaughter, efficacy, evidence of welfare compromise; and hence suitability. Lastly evidence and use of electrical stunning (and killing) methodology is given with current research utilising on board practicality and systems adopted in some industrial platforms. These are presented as 'best practice' with further recommendations for research and development.

*within one second

GRINDING AS A SLAUGHTER METHOD FOR FARMED BLACK SOLDIER FLY LARVAE: EMPIRICALLY-SUPPORTED RECOMMENDATIONS FOR MORE INSTANTANEOUS AND HUMANE PRACTICE

CRAIG PERL[1], Meghan Barrett[2], Chelsea Miranda[3], I Theresse Veloso[4], Casey Flint[5], Austin Martinez[6], Bob Fischer[7] and Jeffery K Tomberlin[8]

[1] Insect Welfare Research Society, Indianapolis, USA
[2] Department of Biology, Indiana University Indianapolis, Indianapolis, USA
[3] Department of Entomology, Texas A & M University, College Station, USA
[4] Department of Biology, California State University Dominguez Hills, Carson, USA
[5] Department of Entomology, Texas A & M University, College Station, USA
[6] Department of Biology, California State University Dominguez Hills, Carson, USA
[7] Department of Philosophy, Texas State University, San Marcos, USA
[8] Department of Entomology, Texas A & M University, College Station, USA

admin@insectwelfare.com

At least 200 billion black soldier fly larvae *(Hermetia illucens; Diptera: Stratiomyidae)* are reared each year as food and feed, and the insect farming industry is projected to continue rapidly growing. Despite interest by consumers, producers, and legislators, no empirical evidence exists to guide producers in practicing humane – or instantaneous – slaughter for these novel mini-livestock. Many methods may be employed to slaughter black soldier fly larvae, such as freezing, boiling, or grinding; however, standard operating procedures (SOPs) and equipment design may affect the likelihood of instantaneous death using these different methods. We tested how larval body size at time of slaughter and particle size plate hole diameter affect the likelihood of instantaneous death for black soldier fly larvae that are slaughtered using a standard meat grinder. Larval body size did not affect the likelihood of instantaneous death for larvae that are 106 - 175 mg in mass. However, particle size plate hole diameter had a significant effect on the likelihood of instantaneous death, with only 54% of larvae experiencing an instant death when using the largest particle size plate (12 mm hole diameter) compared to 84% using the smallest particle size plate (2.55 mm). However, a higher percentage of instantaneous death (up to 99%) could be achieved by reducing the proportion of larvae that become stuck in the machine and are thus incompletely slaughtered. We conclude by outlining specific recommendations to support producers in achieving a 99% instantaneous death rate through specific standard operating procedures to be used with similarly-designed machines. We also develop a protocol for producers that wish to test their own grinding machines and SOPs to determine the percentage of larvae that experience an instantaneous death in their facility.

EFFECTIVENESS OF REPEATED EXPOSURE TO ANAESTHESIA IN THE NORWAY LOBSTER *(NEPHROPS NORVEGICUS)*

ENDRE PUTYORA, Sonia Rey Planellas, Maureen Ellis, Nasser Ayaril and Amaya Albalat

Institute of Aquaculture, University of Stirling, UK

endre.putyora@stir.ac.uk

The welfare of decapod crustaceans has recently become a topic of high importance, especially with their recognition by the UK government as sentient beings within the Animal Welfare (Sentience) Act. Recent work in our team has begun for the optimisation of electrical stunning practices prior to slaughter by incorporating behavioural and electrophysiological measures. These measures require brief periods of anaesthesia to attach recording implements. While initial doses of commonly used anaesthetics such as Eugenol are effective, no studies have reported the effects of subsequent exposure of anaesthetic agents in decapod crustaceans. In this research we hypothesised that a second dose of anaesthetic given shortly after initial exposure might result in delayed or reduced anaesthetic effectiveness, an effect reported in some cases for fish species. The experiment consisted of a 3x3 factorial design whereby animals (n=54) were exposed to 600 µL/L of either mint oil, clove oil or no-anaesthetic (control group) for 10 min to ensure complete anaesthesia. Animals were then allowed to fully recover before being re-exposed to the same initial anaesthetic either 1, 3 or 24 hours later. Video recordings were used to monitor and assess overall behaviour and scaphognathite activity as well as electrocardiograph (ECG) measures to measure cardiac activity. The findings of this study are key to ensuring appropriate welfare practices in research laboratories that perform protocols with decapod crustaceans requiring the use of anaesthesia, as well as the use of anaesthesia for the purposes of killing and unravelling the mechanisms associated with the loss of anaesthetic efficacy in these species.

THE FAIR-FISH DATABASE: WELFARE ASSESSEMENT OF AQUATIC SPECIES IN AQUACULTURE AND FISHERIES

FAUSTA BORSANI[1], Jenny Volstorf[1], Caroline Marques Maia[2], María Cabrera-Álvarez[2] and Sebastian Scholz[1]

[1] fair-fish, Uster, Switzerland
[2] FishEthoGroup Association, Olhão, Portugal

fausta@fair-fish.net

In aquaculture and fisheries, the welfare of hundreds of billions of finfish individuals is affected each year, the final point of which involves stunning and slaughter. In the online fair-fish database (fair-fish-database.net), we collect scientifically available welfare-related knowledge on fishes in aquaculture - and since late also in fisheries. By comparing the fishes' behaviour and needs in the wild with what they are provided with in captivity, we assess the welfare potential that fishes may experience under basic and high-standard farming conditions. For our assessment, we focus on 10 criteria we identified as most pressing regarding the welfare of fishes in aquaculture (home range, depth range, migration, reproduction, aggregation, aggression, substrate, stress, malformations, stunning/slaughter). For stunning and slaughter, there is no wild benchmark to compare to. Instead, we compile what the common slaughter method is and whether there is a standard slaughter protocol available that makes sure stunning happens immediately and slaughter is done effectively and while the individual is still unconscious. In the catch branch of the database, we assess 9 different criteria that go along welfare hazards during steps of the catching process, with the 10th criterion also being stunning/slaughter and following the same idea as for our aquaculture assessment.

Of the 80+ aquaculture species currently covered in the fair-fish database, we identified around 20% with having an established slaughter protocol. For the rest, we either did not find any data or we found laboratory studies that still await verification for the farming context or we found protocols for related species with the potential to be applied also to the focus species. We expect to find even less data for applied slaughter protocols in fisheries.

The contribution of the fair-fish database is to serve as an open-access source of overview of which species may already be slaughtered as efficiently as possible - and which not. This overview may be used in different ways: a) inform the interested public and NGOs to raise awareness, b) give scientists hints to knowledge gaps they can tackle, c) point policy to missing areas of application, d) challenge companies to develop species-specific solutions, etc.

TOWARDS A HUMANE WAY TO EUTHANIZE COMMERCIAL BUMBLEBEES

GEMA MARTIN-ORDAS[1] and Matthijs Metselaar[2]

[1] University of Stirling, UK
[2] Aquatic Vets Ltd, UK

gema.martin-ordas@stir.ac.uk

Over a million of commercially produced bumblebee *(Bombus terrestris)* colonies are imported annually on a global scale for the pollination of greenhouse crops as well as for research purposes. Under current animal welfare law in the UK for both commercial and research use invertebrates, which include bees, are not considered a protected animal. Evidence suggest that they can feel pain. As such, the Association for the Study of Animal Behaviour (ASAB, 2023) states that researchers should endeavour to minimize potential harm to them. ASAB guidelines suggest researchers and veterinarians consult the American Veterinary Medical Association (AVMA, 2020) recommendations for killing invertebrates - including insects. The AVMA states that freezing alone should not be used and a two-step process (i.e., use of anaesthetics and then, freezing) provides a more humane way to kill insects. However, the most frequently used method for killing bees currently in laboratory and farming settings in the UK is freezing a colony at –20C. It is estimated that it takes at least 2 hours for a bee colony to die in these conditions. Bees show nociception—the sensation of direct responses to harmful or potentially harmful stimuli such as sensitivity to elevated temperatures and freezing. Evidence also suggests bees could be sentient. As such, consideration should be taken in how these animals are treated and euthanised, so it is done in the most humane way possible.

Given this, it is vital to revise the current killing method. Here we compared a two-step (anaesthetic and freezing) procedure with a no anaesthetic control (only freezing. In particular, the effect of 3 different anaesthetics (i.e., isoflurane, ether and eugenol) widely used in research with insects were examined. Groups of 10 bees were exposed in sealed chambers to one of the 3 anaesthetics for 2 hours, 3 hours and 4 hours. In order to establish the optimal anaesthetic, different doses were used to anaesthetize the bees. Our goal was to find the dose that ensured that bees would be anaesthetized for the minimum time that it is estimated that it takes bees to die in -20C. Preliminary results show that both ether and isoflurane were effective anaesthetic—with a dose of 0.03 ml bees stop moving within a minute. This research is fundamental for developing a standard way to euthanize bumblebees- a method that could potentially be extended to other insects and invertebrates. Importantly, our findings will also have implications for potential amendment of the laws governing insects, internationally.

THE VALUE OF WELFARE: WHAT CAN WE EXPECT FROM CONSUMERS IF WE INCREASE THEIR EXPOSURE TO MORE FACTS ABOUT WILD-CAUGHT FISH WELFARE?

THEMISTOKLIS ALTINTZOGLOU[1], Ragnhild Aven Svalheim[1], Michelle Boonstra[2], Mike Breen[3], Bjørn Roth[1], Endre Grimsbø[4] and Hans van de Vis[5]

[1] Nofima AS, Tromsø, Norway
[2] Michelle Boonstra Consultancy, Amsterdam, The Netherlands
[3] Institute of Marine Research, Bergen, Norway
[4] UiT, The Arctic University of Norway, Tromsø, Norway
[5] Wageningen University & Research, Wageningen, The Netherlands

themis.altintzoglou@nofima.no

Animal welfare is an issue that affects consumers in varying levels of intensity. Factors that influence the intensity of consumers' reactions to information about animal welfare indicate a complex psychological landscape of interactions between knowledge level, trust in information, emotional responses, societal standards, and personal experiences, among others. The species under welfare consideration, its size, the habitat it lives in, and its appearance can influence consumer responses to welfare issues. The way information is provided, in terms of format and emotional intensity, is also a crucial communication tool. The combination of the above indicates how consumers perceive the value of life and welfare in various situations.

The Catch Welfare Platform (CWP) is a network of multidisciplinary teams that will use scientific evidence and practical facts to identify industrially feasible solutions to improve the welfare of wild-caught species. This step towards improved welfare will also generate societal attention on catch welfare. This information will vary in familiarity for some consumers and will be new for others. The working groups of CWP will work on identifying potential risks and benefits that may follow this exposure to information about the coming change towards improved welfare. This will result in a toolset of good practices for successful communication by fisheries and associated businesses that aim at improved catch welfare.

This presentation will focus on the first findings of the CWP meeting in November 2023, supported by scientific literature. The main take-home message from this presentation will be a list of actions that can be taken to support a successful enhancement of consumer responses and the development of social norms and culture that assume improved welfare for the animals that we catch and consume.

HUMANE KILLING OF AN ECONOMICALLY IMPORTANT DECAPOD CRUSTACEAN, THE SIGNAL CRAYFISH

ELEFTHERIOS KASIOURAS[1], Lisa Carlsson[1], Albin Gräns[2], Lynne U. Sneddon[1]

[1] Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden
[2] Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Skara, Gothenburg, Sweden

eleftherios.kasiouras@bioenv.gu.se

The signal crayfish, *Pacifastacus leniusculus*, is a commercially important species commercially but also an invasive species across Europe with management programmes seeking to catch and kill this species to conserve other native species. Decapods have been recognized as sentient beings, but there is very little empirical evidence on how to improve their welfare during to the time of killing. Given that the signal crayfish is killed for food and in conservation efforts, it is particularly important to investigate the most humane killing method of this freshwater decapod. In this study, three methods of killing were compared to determine which is the most rapid and to explore any effects on flesh quality. The heart rate of the signal crayfish was recorded while being exposed to each different method; ice slurry (~2oC), clove oil (2ml/L) and electroshock for 30 sec (3.62-9.12 Vrmscm-1; 0.29-0.79 A/dm2; 802-901 uS/cm) (n = 10 per group). The animals were randomly caught and transferred from a stock tank to the laboratory where electrodes were attached to the carapace. The animals were then placed into an observation tank where the killing method was applied. Since there is direct control of the heart by the nervous system when the heart rate stopped the animal was considered dead. After the end of the experiment, animals were retrieved, measured, and the claws were removed and placed in liquid nitrogen. Following the experiments, flesh quality indicators were analysed from the muscle tissue of the claws to determine if the killing methods affected key parameters. The most rapid method to kill crayfish was the electroshock(>1min) and then the clove oil (~45min). Clove oil might affect the muscle quality and taste, but this method could be used in the laboratory for euthanasia, however, this method took a very long time to death and as such may not be the most humane. Ice slurry was not effective probably because the signal crayfish lives in cold environments, so during the three hours after application of ice slurry the heart rate was lower but never stopped. Thus, killing with ice or ice slurry should be avoided in this species. When killing for food use, the results suggest that electroshock is the best option and further studies should explore reducing time to death or employing a second method after the electric shock is applied. Development of electrical stun or kill parameters should enable commercialization of these results.