

Pioneering Better Science

Providing animal technicians with the latest news from the NC3Rs

Tech3Rs

Welcome to the latest edition of Tech3Rs.

This newsletter is for animal technicians working in research establishments to help promote opportunities to embed the 3Rs in practice and ensure high standards of animal welfare.

If you have any ideas for future issues or are working on a 3Rs approach you would like us to feature, please get in touch—we would love to hear from you! You can email us at tech3rs@nc3rs.org.uk.

In this issue we share details of our free 3Rs training and we hear from our two Tech3Rs champions on the use of ultrasound imaging to refine studies involving pregnant mice. We also talk to the Named Training and Competency Officer (NTCO) at Newcastle University about how they have made refined mouse handling the norm at their facilities and their plans to offer free in-person workshops on this approach, with the support of the NC3Rs.



Don't miss the next issue!

Tech3Rs is published online – www.nc3rs.org.uk/tech3rs.

If you would like to receive an email when the next issue of Tech3Rs is published, please email us at tech3Rs@nc3rs.org.uk with the subject line "Subscribe".



Did you know the NC3Rs team can deliver free 3Rs training to any research establishment in the UK?

We are excited to offer <u>free</u> <u>3Rs training</u> to any research establishment in the UK, both inperson and online. Sessions range from general introductions to the 3Rs and animal research ethics, good experimental design in research involving the use of animals, animal welfare and workshops based on implementing resources from the NC3Rs.

Whilst some of the material has been developed with a specific audience in mind, many of our training opportunities can be tailored to suit animal technicians, aiming to provide relevant, focused training to help you enhance your knowledge on variety of topics.

One of the current highlighted training opportunities developed with animal technicians in mind is the Evaluating Environmental Enrichment workshop. This workshop aims to complement our resource on **environmental** enrichment which encourages those working in animal facilities to take a more formal approach to assessing the welfare impact that new enrichment items may have. Taking attendees through the resource in an interactive manner, the two-hour session combines talks and activities to ensure that the audience is comfortable with applying the scientific material.

You can find the current training offering on our 3Rs training web page www.nc3rs.org.uk/3rs-training

Tech3Rs Champions

We want to help you share your ideas for putting the 3Rs into practice. In this issue we feature a refined approach to confirm pregnancy in mice that also improves the accuracy of the procedure.

Kirsty Kemp, Named Training and Competency Supervisor (NTCO) and Carolyn Karam, Named Animal Care and Welfare Officer (NACWO), at the MRC Laboratory of Molecular Biology, talk to us about how they have introduced the use of ultrasound imaging to confirm pregnancy in mice.

What 3Rs idea have you implemented?

In our facility there is often the need to identify early pregnancy in mice because some research programmes require the use of mouse embryos. We have established the use of ultrasound imaging to confirm pregnancy in mice as early as embryonic day 5.5 (E5.5) during mouse embryo development. Traditional methods of palpating the abdomen or visually checking the mice do not allow confirmation of pregnancy until later embryonic stages. This has allowed us to reduce the number of mice used for experiments and improve mouse welfare.

How did you develop this idea?

Originally, for experiments requiring pregnant mice, pregnancy would be confirmed through palpation or visual indicators then mice would be taken out of the facility and used by the researchers. However, these traditional methods of confirming pregnancy have limitations as palpation is only possible after E9.5, and even then, it is not always accurate and mice are generally not visibly pregnant until E12.5. As a result, if pups younger than E9.5 were needed, the female mice would be killed regardless of knowing with certainty that they were pregnant. This would lead to potential unnecessary usage of animals and experimental delays. It would also lead to a lot of technician time being lost in setting up new breeding pairs/ trios and plug-checking animals. Being able to confirm pregnancy more accurately and at earlier timepoints using ultrasound means

that non-pregnant mice can be kept at the facility and used in future matings. Apart from this, palpation can be stressful for the pregnant mice and can lead to injury of the pups or cause abortions if not done properly.

We knew we needed to come up with a more accurate and less stressful way of identifying pregnant mice. In March 2017 we were provided with an ultrasound machine and taught ourselves how to use it to determine pregnancy in time-mated mice. Following initial training in using the machine and interpreting the ultrasound images, we could routinely scan as early as E7.5. After retraining at earlier time points in 2023, we are now able to scan and successfully determine pregnancy as early as E5.5.

What was the outcome of this process?

A number of staff at our facility have now been trained to use ultrasound and as a unit we have moved away from using traditional methods to determine pregnancy. When we first started using ultrasound we were only using it to confirm pregnancy in mice for one research group but we have now expanded to multiple groups.

Using this approach, we are able to identify pregnant mice at E7.5 – E9.5 with 91% accuracy (mice correctly identified as pregnant or not pregnant) and at E5.5 – E6.5 with 83% accuracy. We have recently started scanning at E6.5 with an increased accuracy of 95%. The use of ultrasound has led to a 29% decrease in unnecessary animal

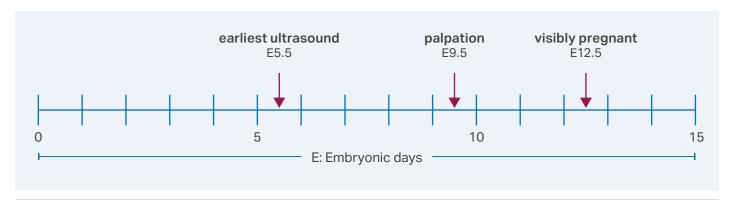






Ultrasound scans of mice at E6.5, E11.5 and E15.5

usage, as we are now able to send out mice with more confidence that they are pregnant and use non-pregnant mice in future matings. Although we cannot predict accurately how many pups a pregnant mouse carries, the use of ultrasound gives us a rough idea of the size of the litter which allows us to decide if it is more appropriate for the female to litter down rather than use a small litter that might not provide enough data. The use of ultrasound also has welfare benefits as the mice have to be restrained for a shorter period of time (less than thirty seconds versus lengthy and possibly multiple palpating sessions) and it puts less pressure on the mouse abdomen, minimising the risk of injury to the pups.



Mouse embryo development timeline

When introducing ultrasound imaging, there is an initial cost associated with buying the equipment, however, it is not uncommon that some facilities have ultrasound machines available that are used for other reasons, for example to scan tumours in mice. If you could get permission to use already existing machines, the initial expense can be minimised, at least until there are funds available to purchase an ultrasound machine to be specifically used to determine pregnancy in mice. There are also less expensive options available such as probes that can be attached to tablets.

"Overall introducing the ultrasound has been beneficial not just for optimising the use and improving the welfare of mice but also to the unit technicians who gain new skills and the researchers who have fewer experimental delays."

Kirsty Kemp



A mouse undergoing ultrasound examination for confirmation of pregnancy



A portable ultrasound device

Making refined mouse handling the new normal

Claire Robinson, Named Training and Competency Officer (NTCO), spoke to us about making refined mouse handling the standard approach for picking up mice, and how animal technicians at Newcastle University want to support other facilities to do this.

Where did you start from and where are you now?

In 2018 Newcastle University began its journey to make picking up mice using refined methods (cupping or tunnel handling) the standard approach. Prior to this, some staff and researchers had used refined handling in specific units or with certain lines of mice, but this was not consistent across all areas. Two years later, the Newcastle University AWERB (the UK institutional ethics committee) approved a policy stating that all staff should use low stress methods instead of tail capture when handling mice and rats.

Since then, we have made refined handling the new normal and Newcastle University can now proudly state that we have technicians within our team who have never picked mice by their tails. Our technicians are advocates for how easy it is to implement refined mouse handling across a large site, even with several satellite units and mouse areas.

We have seen a profound impact on mouse behaviour indicating a substantial positive welfare impact. Mice are less aggressive and reactive or 'pingy', and we have noticed a reduction in overgrooming, stereotypical flipping, fighting and other behaviours associated with high levels of stress. We even have fewer escapes and when we have conducted practice runs to learn how to capture "escaped" mice we found that they followed the staff around and readily entered the tunnel presented to them-this was a big surprise!



A mouse being picked up with a tunnel

Who drove the change?

The technical team, with the support of our Named Animal Care and Welfare Officers and and NTCOs, drove the change in practice. I came from an establishment where refined handling was standard practice and picking mice up by the tail was banned. My experiences led me to encourage my colleagues to engage with the evidence and to challenge the status quo. "Just because we can doesn't mean we should" became the mantra for those who worked alongside the training and competency team.

As the conversation grew among the technical team about the practicalities of moving to 100% refined handling it made sense to bring in an external technician to share their experiences and discuss the barriers to using refined mouse handling. John Waters, a former animal technician involved in the original research on refined mouse handling in Professor Jane Hurst's lab, kindly came to visit us to deliver a workshop that included practical application of the handling techniques and frank discussions with our team members.

Following this workshop a group of very passionate technicians became our 'low stress handling champions' and they delivered a presentation to our AWERB in November 2019 asking them to support a standard for all mice housed at Newcastle University to only ever be handled using refined methods.

How have you embedded this into your culture?

Supporting staff has been paramount. To help staff gain confidence with the practical technique we made a decision early in the process to only teach new staff, researchers and licensees tunnel or cupping for pick-up and capture of mice. Additional practical support, such as John's visit, helped boost staff confidence with their handling skills and allowed them to openly voice concerns and ask questions.

Another important practical step was for us to have a plan. John spoke to us about what we wanted to achieve (refined mouse handling as standard) and helped us to put an action plan in place so we knew our next steps as a team. The support of

our AWERB has empowered both staff and researchers to remind colleagues of the 'new' approach if they accidentally reverted to tail capture. This was more common during initial transition as years of habit are understandably hard to undo, however by having a supportive approach to these incidents we have seen a reduction in their occurrence.

What is next for your refined handling journey?

We understand how important training and support is in this process and so technicians at Newcastle University, supported by the NC3Rs, are providing free in-person workshops to a limited number of UK facilities. We will cover practical aspects relating to the technique itself, barriers to uptake and setting objectives. We would also love to identify refined mouse handling champions within facilities. We believe that all mice used in research should be picked up using low stress, refined methods and we want to share our experiences and support other institutions to make this the standard within their own mouse units.

Would you benefit from practical support in implementing refined mouse handling?

The NC3Rs is providing funding to Newcastle University to deliver in-person workshops on refined mouse handling to a limited number of UK facilities.

This free, half-day workshop is suitable for all animal facility staff, new personal licence holders and early career researchers and will be delivered by animal technicians from Newcastle University at the host institution. A minimum group size of 15 attendees is required.

If you are ready to make refined mouse handling the new normal contact tech3Rs@nc3rs.org.uk for more information.



Mice picked up using refined methods are calmer and easier to handle



Tunnels can also be used as in-cage enrichment

Highlights from the NC3Rs

New resource: How to plan a colony

Following best practice guidance for colony management reduces animal use, but this can be complicated by the number of (sub) strains available and the variation in breeding characteristics. A new web page on our breeding and colony management hub helps animal technicians and researchers to optimise breeding design for maintenance and/or experimental cohorts. The page outlines what strain characteristic information is required to decide on a breeding strategy and features new and existing tools, including an online breeding calculator from the University of Zurich Institute of Laboratory Animal Science.

You can find guidance and practical tools here: www.nc3rs.org.uk/ how-plan-colony

3Rs Champions: Driving 3Rs aspirations towards reality

The role of a '3Rs Champion' is emerging within the animal research community as an individual who proactively elevates the 3Rs within their establishment. The activities of a 3Rs Champion vary between facilities but include leading 3Rs projects such as establishing and promoting specific refinements. For a feature on the NC3Rs blog, **Dr Ellen** Forty spoke to individuals who are pioneering the 3Rs Champion role within three UK research institutions. This included experienced animal technicians Richard Berks (Babraham Institute) and Liz Brown (Fera Science) who gave their perspectives on what having a designated 3Rs Champion brings to their local research environment.

Many animal technicians already promote the 3Rs, particularly through welfare refinements and Liz highlights that "We need someone to drive the projects, because we have got the ideas it's just having somebody who is dedicated to do that". The 3Rs Champion role aims to address this and to give individuals agency to focus on being an advocate for the 3Rs and to seek out and share 3Rs ideas and inspiration. Making 3Rs aspirations a reality requires the right person for the job, and as Richard concludes "Champions should be thinking of the 3Rs everywhere in everything we are doing!"

If you are interested to learn more about how 3Rs Champions could have an impact at your establishment then read our blog post: www.nc3rs.org.uk/news/driving-3rs-aspirations-towards-reality

CRACK IT

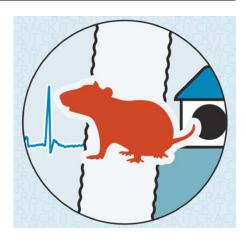
CRACK IT Challenge 49 Rodent Shelter: improving the post-operative care of rodents

The post-operative care of rodents is critical to aid recovery and minimise any pain and distress. Rodents are often singly housed for a period during their recovery and cage enrichment items are removed to avoid injuries. There are opportunities to refine the care of rodents through improved monitoring of parameters such as body temperature and the housing environment after surgery.

The aim of this Challenge is to develop an easy-to-use and affordable shelter that provides warmth and a means to monitor the animals, which can use be used in any cage type to improve post-

operative recovery and monitoring of rodents. Up to £150k is available to solve the Challenge and collaborate with the Sponsors, AstraZeneca. Rodent Shelter will benefit from expertise across disciplines including engineering, wireless technologies, sensors, data handling and animal care.

If you are interested in applying as a lead applicant or as part of a consortium, join us for the launch webinar on Wednesday 18 September, 10.00 – 11.30 (BST) to hear from the Challenge Sponsors and learn more about the application process. Register here: www.nc3rs.org.uk/events/nc3rs-webinar-



rodent-shelter-crack-it-challenge-funding-competition-launch. Visit the Innovation Platform www.nc3rs.org.uk/crackit/rodent-shelter to find out more about the Rodent Shelter Challenge including key aims and deliverables.

*The competition is open to any UK, European Union, European Economic Area and European Free Trade Association body public or private. The competition is run using the Innovate UK Contracts for Innovation process.

Other news





The 3Hs Initiative for laboratory rodents

NC3Rs grant holder Professor Emma Robinson and her team at the University of Bristol have recently launched a website and online training resources based on their 3Hs (Housing, Handling and Habituation) Initiative. The framework is based on the quantification of animals' emotional experiences to build the evidence base for refinements to housing, handling and habituation.

The 3Hs Initiative resource provides details of approaches that Emma and her team have shown to have a positive impact on animal welfare. Each module contains information relevant to rats and mice, tutorial videos and images and validation data where available.

As part of the launch event, we supported Emma and her team to hold a webinar covering the background and principles of the 3Hs Initiative. The webinar featured practical examples of refinements to the housing, handling, and habituation of rats and mice. A recording of the launch webinar is available to watch on YouTube: www.youtube.com/watch?v= nKCWIrBnBU

To find out more about the 3Hs Initiative and Emma's mission on improving the lives of laboratory rodents one lab at a time, please visit the 3H Initiative site: www.3hs-initiative.co.uk

Chat with school students about your work as an animal technician

'I'm a Scientist, Get me out of here' connects people working in science, technology, engineering and mathematics (so-called STEM) roles with teachers and school students from around the UK through instant messaging-style chats. They have launched a new Animal Research theme and are keen for students to find out more about the wide range of career paths that make up the research community. Students are interested in how animals used in research are cared for and the role of animal technicians.

If you would like to share your experiences of your career and help students to understand how animals are used in research, find out more and sign up to take part in I'm a Scientist, Get me out of here: imascientist.org.uk/signup

Interested in taking part but not sure how to discuss your work? You can find public engagement resources and guidance on the NC3Rs website: www.nc3rs.org.uk/communicating-3rs-research. Do not hesitate to contact our communications science manager for further advice and support: genevieve.barr@nc3rs.org.uk.

Here is what I'm a Scientist have to say about taking part:

Unlike visiting lectures or science festivals, the I'm a Scientist activity is student-led. The text-based platform gives every student an equal voice, from the most confident to the most shy. Students are empowered to be curious – you will discover the areas of your research that interest them most, and offer them personal answers and a 1-to-1 experience in return. Based completely online, you can take part in I'm a Scientist from anywhere to engage with classes around the UK. Students have lots of questions about your field of work. Whatever your role, they are eager to connect with you to discuss your experiences.

Using ethograms to improve animal welfare

When making changes to housing and husbandry, recording animal behaviour can give insight into the welfare impact. Ethograms (lists of clearly defined species-typical behaviours) allow behaviour to be recorded in a consistent and objective way. The behaviours can then be compared between conditions to make informed decisions for improving animal welfare.

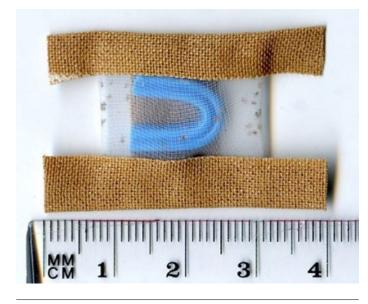
Dr Francesca Nunn (Moredun Research Institute) and colleagues used ethograms to further refine the use of their on-hen poultry red mite device. The on-hen device refines vaccine screening studies by allowing mite infestation to be controlled and reducing the time the animals are exposed to the mites. This method also allows a more accurate assessment of vaccines on a small number of hens prior to large-scale field studies, leading to a reduction in the total number of hens used in mite vaccine development. The device consists of a mesh pouch containing mites and is fitted to the thigh of the hen. Fitting the device requires some feathers to be plucked from the hens' leg, which can be painful.

"The ethogram allowed for a more objective measurement of the hens' behaviours during the procedure. It clearly demonstrated to me that measuring behaviours is a more reliable way of evaluating welfare than relying on experience alone.

I recommend that all researchers would benefit from establishing and using an ethogram to reevaluate the work that they carry out."

Dr Francesca Nunn

Read the publication by Francesca and colleagues here www.f1000research.com/articles/12-715



An 'on-hen' mite feeding device containing 100 mites which is attached to a birds thigh.

To investigate whether the procedure could be further refined using an analgesic cream, hens were randomly assigned to a group with either a placebo or local anaesthetic cream applied to the plucking site. Francesca predefined behaviours associated with the feather plucking procedure, such as the hens looking at their leg, vocalising or trying to flee. She then used an ethogram to record behaviours as either present or absent during the procedure. This allowed behavioural scores to be tallied and compared between the group of hens with and without the topical analgesia. The team concluded that the analgesic cream at 3mg/kg did not cause any adverse effects and that importantly it significantly reduced stress-related behaviours during experimental trials.

Validate your refinements and investigate their welfare impacts by adapting the information in the <u>Evaluating</u> environmental enrichment online resource.



Ultrasonic vocalisations in rats and mice

Rodent vocalisations can give us insight into their behaviour and welfare state, but ultrasonic vocalisations (USVs) cannot be heard by the human ear. Recording USVs is a cheap and non-invasive way to capture these sounds and investigate welfare and social interactions in laboratory rodents. An NC3Rs-supported workshop, organised and hosted by Dr Vincent Bombail (Scotland's Rural College), covered a range of topics related to USVs in rodents, including practical approaches to recording and analysing USVs.

Visit the Positive Animal Welfare (PAW) site to view recordings of the talks from the workshop: www.positiveanimalwelfare.net/usv-workshop

Events







Galleria mellonella workshop

23 August, London, UK

The third annual *Galleria mellonella* workshop will include networking and discussion of methods, models and husbandry techniques.

This year's workshop is organised by NC3Rs-funded PhD student Evgenia Maslova in the McCarthy lab at Brunel University London, in collaboration with the *Galleria Mellonella* Research Centre.

Travel awards are available (including an international award) on the first-come-first-served basis with priority given to participants presenting a talk.

To register for this workshop, please visit www.nc3rs.org. uk/events/galleria-mellonella-workshop-2024

61st LASA Annual Meeting

18-20 November, West Midlands, UK

Registration is now open for this year's LASA Annual Meeting. Topics include animal care and husbandry, animal welfare, facility management as well as innovations in animal science and the 3Rs.

LASA are inviting abstract submissions for poster presentation under the theme: "Husbandry, technical, welfare and care refinements- little steps can make a big difference in animal and staff wellbeing". Young animal technologists and early career researchers are strongly encouraged to participate.

For more information, please visit https://my.lasa.co.uk

2nd European Zebrafish Husbandry Association Meeting

16-18 October, Gödöllő, Hungary

EZHAM 2024 will focus on zebrafish facility management and husbandry with special emphasis on health and welfare. Early bird registration will end on the 31 August but standard registration will stay open until the event with the option to join remotely for those who cannot attend in person.

Hands-on workshop sessions will cover the practical techniques of behavioural imaging, tagging for identification and skin swabbing for DNA sampling. Dr Ceinwen Tilley will run the session on zebrafish skin swabbing for genotyping. Ceinwen is part of an NC3Rs-funded team investigating swabbing as a refinement to fin clipping. She has published on the NC3Rs F1000 Gateway on this topic and contributed the NC3Rs guidance on Skin swabbing for DNA sampling of zebrafish.

To find out more about EZHAM 2024, including how to register, visit www.ezham2024.hu