

Providing animal  
technicians with the latest  
news from the NC3Rs

# Tech3Rs

Welcome to the latest edition of Tech3Rs. In each issue, we share updates on recent advances in the 3Rs and highlight new resources, research and events.

This newsletter is for animal technicians working in research establishments to help with identifying 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](mailto:tech3rs@nc3rs.org.uk).

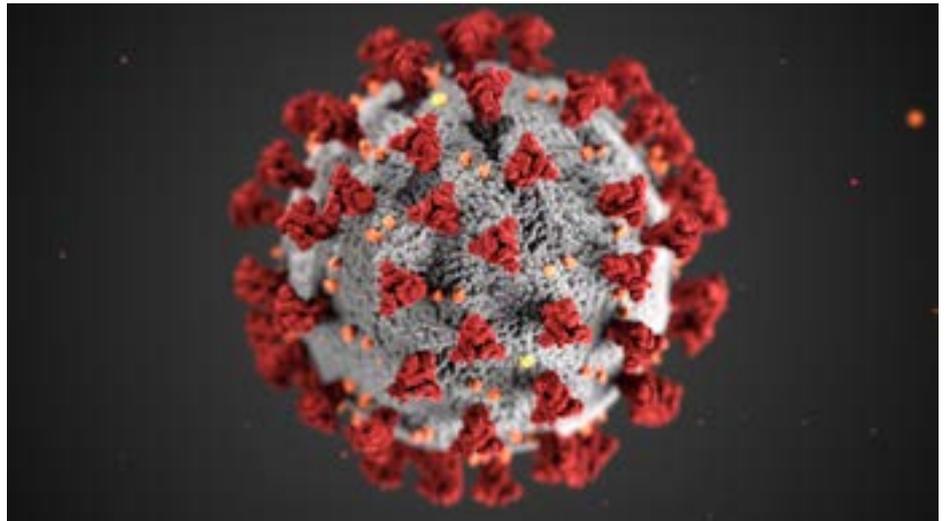
In this issue we take a look at environmental enrichment and new online resources. This issue also includes our first Tech Journeys feature, in which we speak to John Waters, Chief Animal Technician and NACWO at the University of Liverpool, about his career so far.



Don't miss the  
next issue!

We're currently only publishing Tech3Rs online, but UK institutes can request free hard copies of future issues by visiting [www.nc3rs.org.uk/tech3rs](http://www.nc3rs.org.uk/tech3rs).

You can stay up-to-date on the latest issue of Tech3Rs and more via the NC3Rs e-newsletter. Visit [www.nc3rs.org.uk/register](http://www.nc3rs.org.uk/register) to subscribe to our monthly updates.



## The NC3Rs and the 3Rs during the COVID-19 pandemic

How we are continuing to support the research community.

The COVID-19 pandemic has presented the scientific community with a multitude of challenges to navigate, especially for anyone involved in animal research.

We would like to thank animal care staff around the world who are working tirelessly to care for research animals, and who continue to go the extra mile under very difficult circumstances to ensure good animal welfare.

Through these unprecedented times, the NC3Rs continues to operate to support the community with 3Rs advice and information, including through our dedicated hub for animal technicians at [www.nc3rs.org.uk/animaltech](http://www.nc3rs.org.uk/animaltech).

We have practical information on a wide range of topics to help you and your colleagues to continue to do good science by keeping the 3Rs a priority. Topics include housing and husbandry, dosing, sampling, euthanasia and more.

Our staff are working remotely and are on hand throughout the working week to answer any questions you may have about your animals, projects or 3Rs initiatives.

Please email [enquiries@nc3rs.org.uk](mailto:enquiries@nc3rs.org.uk) to be put in touch with the right NC3Rs staff member or team.

Our dedicated COVID-19 page will be updated to show the most up-to-date information, guidance and contact details: [www.nc3rs.org.uk/covid19](http://www.nc3rs.org.uk/covid19).

We understand that the current circumstances could have a significant impact on your mental health. The IAT have published resources to help support your wellbeing, which we encourage you to read and share with your colleagues. Their 'It's OK... Not to be Okay' leaflets, 'Let's Talk COVID-19' and 'Let's Talk Euthanasia', can be found at [www.iat.org.uk/news](http://www.iat.org.uk/news).

# 3Rs champions

We would like to help you share your ideas for putting the 3Rs into practice. In every issue of Tech3Rs we feature animal technicians who are championing the 3Rs at their establishments.

**Julia Bartlett is a Specialist Technician at the University of Bristol. Here she describes how her lab has refined oral dosing for mice and rats.**

## What 3Rs idea have you developed?

Our work involves measuring affective states in rodents with the aim to better understand psychiatric disorders such as depression. The behavioural assays we use are very sensitive and stress caused to the animals during handling and dosing can confound our results. Substance administration involves physical restraint, which is known to be aversive to rodents. We use refined dosing methods that include oral dosing using palatable solutions, eliminating stress and risk to the animal whilst still delivering a precise volume and concentration of drug.

## How have you refined oral dosing?

We use solutions like strawberry milkshake or diluted condensed milk as our vehicle to encourage our animals to drink the drug-solution directly from a syringe. The palatable substance is introduced to the animals a few days before we start the study, so they can overcome any apprehension about the novel food and drinking from the syringe.

Each animal receives a small amount of the vehicle straight after dosing to "rinse out" any remaining drug-solution. This ensures that the entire dose of drug-solution is ingested and prevents any drug aftertaste that may discourage future drinking. They also receive a



second syringe of vehicle solution later in the day to prevent them associating any adverse effects of the drug with the vehicle. The method has been used successfully for both rats and mice.

## What are your future plans?

We are always looking for ways to further refine our dosing and handling methods and encourage and assist others in adopting these methods. We have developed other techniques, including a method for restraining mice that eliminates any tail-handling and a low stress method for intraperitoneal dosing of rats (see reference below). Our hope is that these less aversive methods of substance administration will be adopted by other researchers and technicians, as they are far less stressful for the animals and handlers and don't significantly increase the time required for dosing.

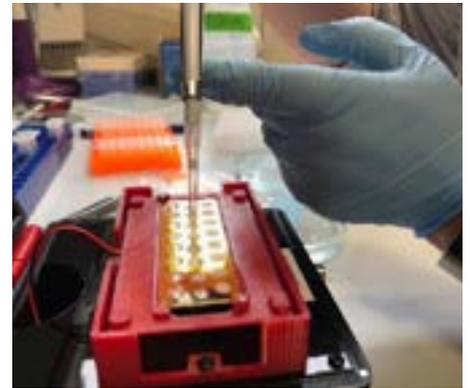
**Stuart SA, Robinson ESJ (2015). Reducing the stress of drug administration: implications for the 3Rs. *Scientific Reports* 5: e14288. doi: [10.1038/srep14288](https://doi.org/10.1038/srep14288)**

**Lisa Van Hateren is the Aquarium Stock Resource Manager and NACWO at the University of Sheffield's Bateson Centre. Here she shares her experience of using the ZEG, a non-invasive technology for obtaining genetic material from zebrafish.**

## What 3Rs idea have you developed?

Recently I have introduced the Zebrafish Embryonic Genotyper (ZEG), from the company wFluidx, into our zebrafish genotyping service. The ZEG unit allows extraction of genetic material from live zebrafish embryos within a few days of their birth and without causing harm, leading to 3Rs and research benefits. This technology will allow us to significantly reduce the number of protected zebrafish held in our facility – in some cases, by up to three-quarters.

With the ZEG we're able to identify and remove embryos with unwanted genotypes early on, ensuring that only



the required animals are raised beyond five days post-fertilisation. The device also offers a significant refinement in fish genotyping as there is no longer a need to fin clip adult fish, which requires the use of anaesthesia.

Embryo genotyping using ZEG offers benefits to researchers too, by allowing them to create new crosses more efficiently and speed up their experiments. It can also reduce costs since fewer fish need to be bred past the early embryonic stages.

## How did you develop your idea?

I found out about the ZEG from a presentation at the Zebrafish Facility Management Workshop in Lisbon in October 2018. I contacted the company about trialling this equipment and realised what a great opportunity it would provide for us to significantly reduce animal numbers and refine the genotyping process.

## What are your future plans?

In our facility, we currently need to genotype up to three mutations in some fish. However, the DNA sample collected after a single run of ZEG is limited to 10 ul. My future plan is to try and improve the technique to allow for genotyping of multiple genes without having to collect more than one DNA sample per fish.

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**Would you like to be featured in our next issue, or find out more about the refinements featured above? Please email [tech3rs@nc3rs.org.uk](mailto:tech3rs@nc3rs.org.uk).**

# 3Rs papers of interest

Each issue we summarise recent 3Rs publications, providing links to the full articles for you to share with your colleagues. This issue we focus on enrichment.



**Mertens S et al. (2020). Effect of a partial cage dividing enrichment on aggression-associated parameters in group-housed male C57BL/6NCrI mice. *Applied Animal Behaviour Science* 224: e104939. doi: [10.1016/j.applanim.2020.104939](https://doi.org/10.1016/j.applanim.2020.104939)**

- Episodes of aggression can occur between group-housed male mice and may lead to mice being housed singly.
- Plastic X-shaped partial cage dividers were assessed for their ability to reduce aggression and stress by providing shelter and minimising visual contact.
- Two groups of mice, provided with either a transparent or black cage divider, were compared against a control group of mice in a non-divided cage with standard enrichment only (a cotton nesting pad).
- Mouse wellbeing and stress levels were evaluated using nest building performance and other behavioural tests (e.g. open field test of anxiety). Blood glucose and faecal corticosterone metabolites, two physiological markers of stress, were also measured.
- Contrary to their initial hypothesis, the authors found that transparent dividers did not influence aggression.
- However, the presence of a black divider significantly increased aggression and stress, leading to an overall decrease in mouse wellbeing.
- The findings of this study highlight the importance of carefully choosing and evaluating the benefits of commercially available enrichment equipment, as even basic characteristics like opacity can have profound effects on animal behaviour and welfare.

**Khoo SY et al. (2020). Nesting material enrichment reduces severity of overgrooming-related self-injury in individually housed rats. *Laboratory Animals* 2020. doi: [10.1177/0023677219894356](https://doi.org/10.1177/0023677219894356)**

- Rats are highly social animals and should not be single-housed without exceptional scientific or animal welfare justification.
- Single housing can have severe adverse effects on their wellbeing and lead to stereotypies (repetitive aberrant behaviours), such as overgrooming.
- This 18-month study, which monitored single-housed rats across multiple projects, aimed to investigate how providing nesting material affected the prevalence and severity of self-injury from overgrooming.
- Rats were housed with either a shelter and a chew toy (unenriched), or with a shelter, chew toy and shredded paper nesting material (enriched). If an unenriched rat showed symptoms of overgrooming, they were subsequently provided with paper nesting material.
- Although there was no difference in the prevalence of injuries between the two groups, enriched rats suffered less severe self-injuries and recovered from these more quickly than unenriched rats. Unenriched rats that had nesting material added to their cages after the onset of injury did not benefit from the faster recovery times that were observed in the enriched rats.
- This study highlights how the relatively simple addition of paper nesting material can reduce the severity of self-injury in single-housed rats. However, this enrichment needs to be permanent for the animals to fully benefit.

**DePasquale C et al. (2020). A standardized protocol for preference testing to assess fish welfare. *Journal of Visualized Experiments* 156: e60674. doi: [10.3791/60674](https://doi.org/10.3791/60674)**

- When working with laboratory animals, it is important to assess their specific enrichment preferences, including their preferred environment.
- The authors present a protocol to investigate housing preferences in zebrafish by giving fish the choice of four different habitat zones.
- They measured the mean number of fish per zone, which was used to calculate a preference score using Jacob's index, a statistical assessment of preference, which ranked the habitats from -1 (avoidance) to +1 (most preferred).
- The authors assessed whether zebrafish preferred the presence or absence of enrichment (plastic plants), water flow, or a combination of the two.
- Fish were significantly more likely to spend time in the zone with both enrichment and waterflow than all other habitat types.
- The protocol can be adapted easily for assessing a variety of enrichment and habitat types across a range of different species.
- However, these types of assessments are limited by the choices presented, of which the animals may simply choose the least unpleasant.
- For others using this protocol, the researchers highlight the importance of sufficient acclimatisation time, thorough between-trial cleaning to remove social cues, and consistency across experimental runs.

# Tech Journeys

Tech Journeys is a new feature focused on the career paths of inspirational technicians who have demonstrated a long-term commitment to the 3Rs. If you have an inspirational story, or know someone who does, please get in touch.

**This issue we're featuring John Waters, whose thirty-year career has seen him rise from trainee to Chief Animal Technician and NACWO for the Mammalian Evolution and Behaviour Group at the University of Liverpool. John is a member of the IAT Council and received the IAT's Andrew Blake Award in 2017. In 2019 he was the UK recipient of the AAALAC International Fellowship.**

I started my career back in 1990, as a trainee animal technician at the University of Liverpool. Back then I knew I wanted to work with animals, but was unsure what path I wished to follow.

In the early days, most technician jobs focused on animal husbandry. After a while, I started to become involved in scientific programmes and to appreciate the importance of providing excellent animal welfare. I had the viewpoint that if animals justifiably had to be used in research, then I was going to do my best to make sure their welfare needs were met to the best of my ability.

## Working with researchers

One thing I have found has helped me as an animal technician has been to show an



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I have always had a personal ambition to make a difference to the animals in my care.”

interest in the work being carried out by the researchers and to try to understand the scientific questions that are being asked. Researchers are more than happy to explain their work, and I have found this really valuable in helping me determine how to provide the best welfare practices in each situation.

As I gained more experience and acquired the IAT qualifications, I became more involved in the welfare aspects of looking after laboratory animals and meeting their physiological and behavioural needs. In 2010, following the publication of non-aversive mouse handling methods by Professor Jane Hurst and Rebecca West, I became involved in promoting this refinement. The most important factor for me was identifying how I could help others to improve the welfare of their mice, for example, by highlighting the mistakes I had made when trialling the non-aversive techniques.

## Making a difference

Being involved in this project gave me the opportunity to present at NC3Rs and IAT symposia and to interact with many technicians from different areas of biomedical research. Working with



The Mammalian Evolution and Behaviour Group also works with wild rodents.

the NC3Rs, I've had the opportunity to help produce an online video tutorial, instructional poster (now translated into French, German and Chinese), Twitter chat session and quite a few practical workshops. Having a passion to improve lab animal welfare has also enabled me to travel to establishments outside of the UK to offer guidance to those wishing to implement cupping and tunnel handling.

To be recognised by fellow professionals for my contribution to improving laboratory animal welfare has to be the highlight of my career as a technician. I have always had a personal ambition to make a difference to the animals in my care, and to win the 2017 Andrew Blake Tribute Award was such a proud moment. Without getting involved in the mouse handling work this award would not have been possible, but it highlights that with commitment and hard work this fantastic award is within the grasp of every animal technician within the UK.



John receiving the 2019 AAALAC International Fellowship, with US recipient Theresa Cunningham-Faughnan

## International recognition

In 2019 I received the AAALAC International Fellowship Award, which is presented to UK registered animal technicians who have made a significant contribution to animal welfare and demonstrated an aptitude to develop and support fellow technicians. This offered me the opportunity of a lifetime, as it included free attendance at the AALAS National Meeting, probably the largest event of its kind in the world.

In the week leading up to the conference, I was privileged to be given guided tours around numerous animal research facilities within the Washington D.C. area and to witness fantastic work being carried out within the USA. I also learnt about differences and similarities in the regulations and standards for animal

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Each and every technician can make a difference – when the opportunity comes around, grasp it and show everyone your commitment to animal welfare.”

care on both sides of the Atlantic. As a technician, to be recognised and rewarded for what I have contributed over the years was very fulfilling.

I am also passionate about promoting the work animal technicians carry out, which led me to apply to be a member of the IAT Council. I help out with social media, the animal welfare group and the IAT Congress committee. I believe that my commitment to improving animal welfare played a role in me being elected as a Council Member.

## John's advice for technicians

I would encourage all animal technicians to highlight the fantastic work you carry out as much as you can. There are

lots of avenues now to highlight your contribution to animal welfare, such as the Steve Moore Memorial Poster Competition, the Janet Wood Innovation Award, NC3Rs events and publications, the IAT Congress and branch activities. It is always important to highlight how you have contributed to finding an excellent solution to a welfare issue. If you manage to help just one technician and their animals in the process, then it is worthwhile and creates a marvellous sense of satisfaction.

I have been very fortunate to get involved in, and be acknowledged for, a fantastic welfare improvement, the non-aversive mouse handling project. Each and every technician can make a difference – when the opportunity comes around, grasp it and show everyone your commitment to animal welfare.

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**To learn more about John's experience attending AALAS and visiting US animal facilities, read his report in the February 2020 IAT Bulletin. Find out more about the AAALAC International Fellowship Award at [www.aaalac.org/awards/fellowship-award](http://www.aaalac.org/awards/fellowship-award), and more about the Andrew Blake Tribute Award at [www.iat.org.uk/abta](http://www.iat.org.uk/abta).**

**Visit [www.nc3rs.org.uk/mousehandling](http://www.nc3rs.org.uk/mousehandling) to find resources on non-aversive mouse handling, including those John helped develop.**





# A new device for embryo culture

The University of Leeds and MRC Harwell are seeking animal care staff and researchers to help test and improve a new device funded by the NC3Rs. Read more about how you can get involved.

In 2016 staff at MRC Harwell posed a challenge to the scientific community to develop a technology that improved the implantation rates of early stage mouse embryos. The aim was to increase the use of non-surgical embryo transfer in the generation of transgenic mice, reducing reliance on surgical methods and thus improving welfare.

The project, referred to as EASE, was funded by the NC3Rs' CRACK IT Challenges programme. A team led by Dr Virginia Pensabene at the University of Leeds won the Challenge contract.

They have developed a microfluidic device that improves the developmental competence of *in vitro*-derived mouse embryos, making them more viable for non-surgical embryo transfer. This new device replaces the standard Petri dish for embryo culture with a microfluidic closed dish.

The team are looking to increase the validation data set for this device and tailor it to the needs of the embryo culture research community. They have put together a short questionnaire for animal care staff and researchers, which

should take no more than five minutes to complete and can be found at [leeds.onlinesurveys.ac.uk/microfluidic-device-for-ivf](https://leeds.onlinesurveys.ac.uk/microfluidic-device-for-ivf).

Once you have completed the survey, you will be able to request a free sample set of devices and a future visit from the team to help you introduce the technology into your work.

You can find out more about the EASE CRACK IT Challenge at [www.nc3rs.org.uk/crackit/ease](https://www.nc3rs.org.uk/crackit/ease).



## Benefits of the device

**Non-toxic to embryos**  
No need to use oil.

**Cheap**  
Does not require additional equipment and integrates easily into an existing laboratory environment.

**Easy**  
No training needed.

**Mouse welfare**  
Does not involve additional procedures that may cause harm to the mice.

# Spotlight on...

## New anaesthesia e-learning resources

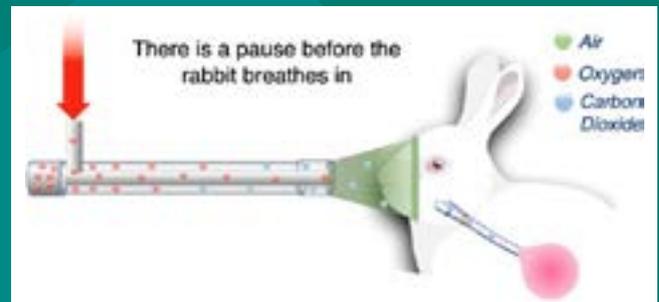
This month we updated our e-learning resources hub to include three new modules on advanced anaesthesia. These modules focus on monitoring anaesthetised animals, anaesthetic breathing systems, and how to understand, and respond to, problems that might occur. They can be found,

along with other modules, at [www.nc3rs.org.uk/elearning](http://www.nc3rs.org.uk/elearning).

The resources were created by Professor Paul Flecknell and Jon Gledhill of FLAIRE Learning. They recently wrote a guest post for the NC3Rs blog, which highlights the value

of e-learning resources and how they can complement traditional training approaches. This can be found at [www.nc3rs.org.uk/elearning-and-3rs](http://www.nc3rs.org.uk/elearning-and-3rs).

You can also watch the recording of a recent webinar by Professor Flecknell: [www.nc3rs.org.uk/elearningwebinar](http://www.nc3rs.org.uk/elearningwebinar).



## Tickling rats for improved welfare

In Issue 4 of Tech3Rs we featured Megan LaFollette and colleagues' work on rat tickling to improve welfare (you can find all our previous issues at [www.nc3rs.org.uk/tech3rs](http://www.nc3rs.org.uk/tech3rs)). Rat tickling mimics rough and tumble play and can habituate rats to their handlers, while reducing behaviours associated with fear. In addition to improving general wellbeing, reducing

the stress of research animals can lead to more robust data.

Like all animal handling techniques, it's important to do rat tickling right. To help you to implement rat tickling effectively in your facility, we have created a website hub which includes:

- An introduction to rat tickling and its welfare benefits

- Articles about the evidence underpinning the technique
- An online course to gain a rat tickling certificate
- A printable flyer available in three languages

You can find the hub at [www.nc3rs.org.uk/rattickling](http://www.nc3rs.org.uk/rattickling).



# Highlights from our news and blog

The NC3Rs blog is a platform to talk about the research we champion and the issues we care about. Recently we highlighted technicians' approaches to evaluating enrichment, as well as new publications on mouse handling and pair-housing of dogs.



## Approaches to evaluating environmental enrichment

From nesting material to chew toys, environmental enrichment is fundamental for good animal welfare. Earlier this year, NC3Rs staff met with animal technicians from different universities around the UK to discuss the ways they introduce new enrichment items into their facilities.

We summarise the discussions in a new blog post, including barriers staff face when it comes to evaluating the impact of enrichment on welfare, and potential solutions to facilitate more formal studies in this area.

Read our blog and share your experiences with evaluating enrichment at [www.nc3rs.org.uk/evaluatingenrichment](http://www.nc3rs.org.uk/evaluatingenrichment).



## International survey on non-aversive handling of laboratory mice

An international survey by researchers at Newcastle University has found that use of non-aversive methods for picking up laboratory mice is greater in the UK than in other countries.

Around 36% of UK respondents used non-aversive methods only (tunnel handling or cupping), 13% used the tail only, and 51% used a combination. The vast majority of participants were convinced that non-aversive handling improves animal welfare and scientific outcomes.

Read more about the survey findings at [www.nc3rs.org.uk/handlingsurvey](http://www.nc3rs.org.uk/handlingsurvey).



## Dogs can be pair housed in ADME studies without scientific compromise

Being able to interact with companions is hugely important for the welfare of laboratory dogs. A Covance-Novo Nordisk collaboration has demonstrated that dogs can be pair-housed during metabolism studies to assess the pharmacokinetic properties of new drugs, without compromising study integrity. Pair housing was achieved by modifying and combining two metabolism cages so the dogs were able to interact with each other whilst maintaining the same amount of floor space per dog.

Read our summary of the paper at [www.nc3rs.org.uk/dogpairhousing](http://www.nc3rs.org.uk/dogpairhousing).