

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 identify 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 share the outcomes of our reader survey, summarise three papers focused on laboratory birds and speak to two 3Rs champions about the refinements they have introduced in their facilities. We also highlight upcoming in-person and hybrid 3Rs events.



Don't miss the next issue!

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## Reader survey 2022

We would like to say a huge thank you to everyone who responded to our recent survey on the content and design of Tech3Rs. We received responses from readers in the UK (73%) and overseas (27%), working with a range of animal species (see Figure 1 below). These responses will help us to make Tech3Rs as useful and informative as possible. Below we summarise the feedback and outline our plans to implement the suggested changes.

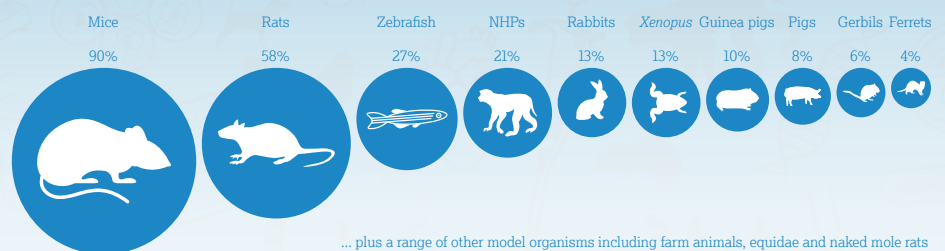


Figure 1. Respondents told us that they work with a wide range of species, the most common are shown here by percentage.

### Supporting you to advance the 3Rs

The primary aim of Tech3Rs is to help animal technicians stay up to date with the 3Rs, and 85% of those who responded agreed this aim is being met. The majority also felt Tech3Rs could be accurately described as "a useful resource for animal technicians" (78%) that "makes a positive impact" (63%). Many of these readers (57%) confirmed they have spoken positively about Tech3Rs to a colleague.

We are pleased to learn that of the 16 3Rs opportunities we requested feedback on, 15 have been implemented by at least one reader after learning about them in Tech3Rs (Figure 2). 63% of those who responded have incorporated the use of grimace scales into their welfare checks to assess pain.

Many respondents were inspired to implement more than one refinement, with over half making the switch to single use of needles (55%) and non-aversive methods of picking up mice (51%). 49% of respondents have applied the NC3Rs recommendations to minimise aggression in group-housed male mice.

### Learning from your feedback

It is great to know that our content makes a positive real-world impact, but we also understand that making changes within your facility is not always within your control. Of the respondents who shared reasons why they have not implemented 3Rs opportunities featured in Tech3Rs, 57% cited a lack of resources and/or wider support from management and researchers.

# Reader survey 2022 (continued)

37% of respondents reported having already employed the 3Rs opportunities featured before reading about them in Tech3Rs. Early adopters of 3Rs initiatives are important in paving the way for other institutions to successfully implement changes within their facilities, and for helping to build the evidence base for refinements. We are always keen to hear from animal technicians who are at the forefront of putting the 3Rs into action, especially when you have overcome challenges to do so. If you have experiences to share on this topic, please get in touch at [tech3rs@nc3rs.org.uk](mailto:tech3rs@nc3rs.org.uk).

Other respondents (33%) explained that the opportunities described in Tech3Rs were not relevant to them; for example, there were no 3Rs opportunities for their species of interest. Readers highlighted this elsewhere in the survey, stating that they would like to see a wider range of species represented. Going forward, we will broaden the diversity of animal species featured in Tech3Rs, while ensuring that we continue to share 3Rs opportunities that have the widest potential impact.

In this issue we have an all-avian line up for our 3Rs papers and our 3Rs champions share their refinements to rabbit handling and zebrafish housing. We will also be making small changes to the design of Tech3Rs, including giving more space for the content you find most useful.

## Sharing 3Rs news with a wider audience

In addition to widening the range of species we feature, we would love to expand and diversify our readership. In past issues we have featured two 3Rs champions from outside the UK (Carina from Denmark in [Issue 9](#) and Chago from the US in [Issue 14](#)) – if you think you could be our first 3Rs champion from your country we would love to hear from you.

Technicians are instrumental in making sure the 3Rs are put into practice, and we are glad to see Tech3Rs is helping with this. Thank you again for your continued readership and support for the 3Rs!

## Figure 2. Top ten Tech3Rs-featured refinements implemented by our readers.

1. Used the grimace scales to assess pain in laboratory animals.
2. Switched to single use of needles.
3. Switched to non-aversive mouse handling.
4. Applied the NC3Rs recommendations to minimise aggression in mice.
5. Introduced rat tickling.
6. Switched to environmental monitoring for rodent health surveillance.
7. Switched to refined oral administration for rodents.
8. Re considered the type of enrichment provided to zebrafish.
9. Used recycled materials to create environmental enrichment for rhesus macaques.
10. Switched to skin swabbing for zebrafish genotyping.



Figure 3. Your words: the larger the word the more frequently it occurred in the survey responses.

# 3Rs papers of interest

Each issue we feature recent 3Rs papers, providing summaries and links to the full articles for further reading. This issue we focus on refinements for laboratory birds.



## Refined method for studying foraging behaviour and body mass in group-housed European starlings

- Starlings are used in studies of foraging behaviour and body mass regulation, which often require frequent capture, handling and single housing of birds.
- The authors aimed to validate their novel technology – the social foraging system (SFS) – for collecting individual-level foraging effort and body mass data from group-housed starlings, without the need for frequent capture and restraint when weighing birds.
- European starlings (*Sturnus vulgaris*) were fitted with microchips glued onto plastic leg rings, which provided individual identification of each bird by a Radio Frequency Identification (RFID) aerial located on the SFS.
- Following training using food rewards, two groups of six birds were housed in indoor aviaries with an SFS for 11 consecutive days, with food accessed by pecking an illuminated key.
- The starlings quickly learned to use the SFS and data was collected remotely from all birds. By reducing the stress associated with human interaction and social isolation, the SFS improves animal welfare and data quality.

Bateson M and Nolan RA (2022). *Animals* 12(9): 1159.  
[doi: 10.3390/ani12091159](https://doi.org/10.3390/ani12091159)

## The impact of acute loud noise on the behavior of laboratory birds

- Low frequency fire alarms are used in some animal facilities to minimise disturbance, primarily to rodents. This study examined the effect of low-frequency alarms on the behaviour of zebra finch (*Taeniopygia guttata*).
- After acclimatisation to experimental cages for three to four days, 24 randomly-selected mixed-sex pairs were exposed to 10 to 20s of fire alarm noise (430 to 470 Hz, 97 dB) or no noise on separate test days. Each pair received both treatments.
- The birds were filmed for one hour before and after the treatments and their behaviour was scored using an ethogram.
- For at least 15 minutes after exposure to the alarm finches decreased their activity and vocalisations and increased stationary and social grooming behaviours.
- Noise stress from routine alarms may confound behavioural studies by affecting behaviour and may impact experimental outcomes by increasing data variability, even when birds are exposed to routine alarms from hatching.

Corbani TL *et al.* (2021). *Frontiers in Veterinary Science* 7:607632.  
[doi: 10.3389/fvets.2020.607632](https://doi.org/10.3389/fvets.2020.607632)

## From the point of view of the chickens: what difference does a window make?

- This study investigated the impact of lighting conditions on the behaviour of chickens (male Cobb 500 broilers).
- 85 chicks were separated into ten pens. Each pen was divided by a blackout curtain into two sides, one illuminated by only artificial light (OAL: 32.4 lux, 6500K LED bulbs) and the other by a combination of natural and artificial light (NAL: 545.5 lux, a glass window and LEDs). Chickens could move freely between the two sides. Their behaviour and location (OAL or NAL) were recorded twice daily.
- At 18+ days old, a greater number of chickens were observed in the area with the NAL than OAL. Birds of all ages were more active and exploratory on the side with NAL than OAL, but inactivity was the most observed behaviour regardless of lighting condition.
- Chickens should have access to daylight and a choice of light levels within their pens, but a glass window alone is not sufficient to address inactivity. Additional environmental enrichment should be provided to further improve chicken welfare.

Sans ECO *et al.* (2021). *Animals* 11(12): 3397.  
[doi:10.3390/ani11123397](https://doi.org/10.3390/ani11123397)

# 3Rs champions

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

**Alicia Kinally is an animal technician at the University of Leicester. She spoke to us about refining rabbit handling.**

## What 3Rs idea have you developed?

Our male New Zealand white rabbits are regularly handled for health checks, procedures and general husbandry. In the past, collecting the rabbits would entail leaning into the cage to catch them before picking them up and securely carrying them to their destination. I was conscious of the fact that this process may not be ideal from the perspective of the rabbit – a sensitive prey animal.

I wanted to explore whether the rabbits would willingly cooperate with capture, and to give them some control over their interactions with us. Now we don't have to manually catch the rabbits; we can hold a pet carrier up to the cage door and they will hop into the box voluntarily. We think of this refinement as being similar to non-aversive mouse handling – our mice have become accustomed to going into the handling tunnel voluntarily, and now our rabbits have a similar option.

We are confident that the rabbits prefer this method. We have noticed that they are calmer and generally more cooperative. They wait by the front of their cage ready to come out, whereas with the previous method some rabbits would actively move away from you.

We now use this approach for transporting all our rabbits. It is particularly useful for their weekly weigh-ins, as they will hop straight out of the carrier onto the scales.

## How did you develop this project?

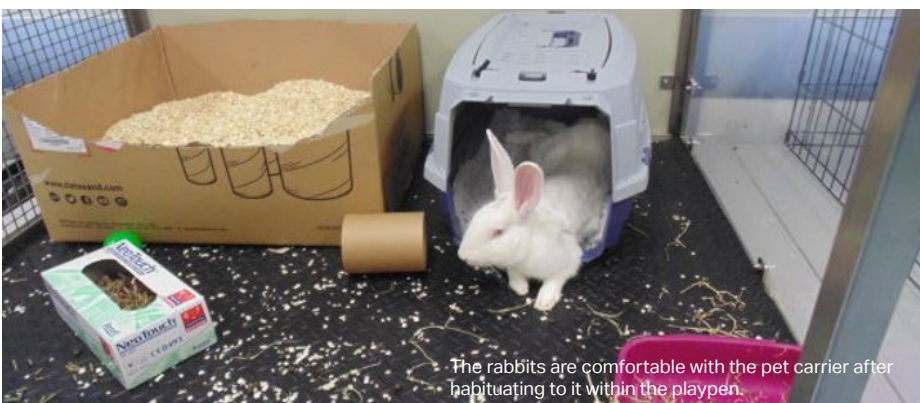
Before we present the carrier to the rabbits for capture and transport, we allow them to become used to it as an environmental enrichment item within their playpen. After around seven hours of playpen time, we begin presenting the carrier to the rabbits at their cage door. This early stage requires patience; it is important not to force them into the carrier as you don't want them to associate it with a negative experience.

I knew it was important to create a positive association with the carrier, so I tried to entice the rabbits with treats, but they weren't interested. I set out to find something that would encourage them into the carrier and was inspired when I noticed that the rabbits would happily sit in a shared bed lined with fleecy bedding. I conducted a small trial using Vetbed® that had either been scented by the individual rabbit, or all the rabbits during access to the shared playpen. I found that placing the group-scented bedding in the carrier seems to encourage the rabbits to get into it. They appear calm but curious and hop right in.

## How has this project impacted you?

It feels good to know that I've done something to improve the welfare of our rabbits, especially because this is a refinement to their daily routine. It has also been very rewarding to engage with the rabbits in this way. They are intelligent animals, and I think the process of investigating the carrier and learning to hop into it from their cage is stimulating for them. I had previously observed the rabbits learning to use the puzzle feeder enrichment devices that we provide for them, so I knew they were smart enough to do this. I am really pleased that they proved me right and we've been able to make this positive change.

**For video guidance on handling and lifting rabbits, including footage of Alicia transporting a rabbit using the method described here, visit our rabbit housing and husbandry page: [www.nc3rs.org.uk/rabbit-handling](http://www.nc3rs.org.uk/rabbit-handling)**



The rabbits are comfortable with the pet carrier after habituating to it within the playpen.



Daily handling is refined by using box handling instead of capture and restraint.

**Peter Gardiner is the research aquarium technical manager at the University of Bristol. He spoke to us about providing environmental enrichment to zebrafish.**

### **What 3Rs idea have you developed?**

We house a number of aquatic animals, including wild type *Danio rerio* (zebrafish) and other *Danio* species on recirculating systems. I felt that we could improve fish welfare by adding ecologically-relevant environmental enrichment into our holding tanks. My goal was to enhance structural complexity, create areas with different light levels and provide more variety for fish seeking refuge.

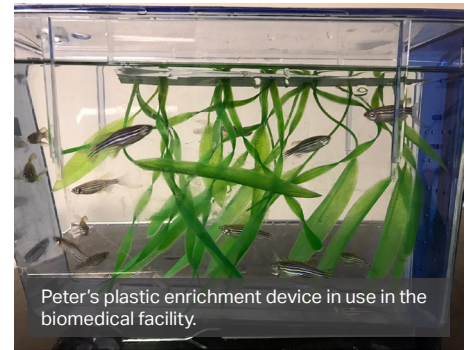
My colleagues and I also felt that housing the fish in a less artificial environment would result in the collection of more ecologically valid, and thus higher quality, research data. Our fish are used in behavioural studies and we believe that by providing more naturalistic housing conditions we increase the likelihood that behaviours will be expressed in a more natural way.

### **How did you develop this project?**

Within my unit I am lucky to work with researchers who are enthusiastic about making refinements to the housing and husbandry of our fish. This has given me the freedom to introduce new enrichment. I began by adding sand and gravel substrate to the tanks, as there is strong evidence that fish prefer tanks that contain substrate [1]. I went on to replace the plastic plants with live ones and

now most of our fish are housed with live plants. Organic plants are more than just physical enrichment. They improve water quality by removing build-up of carbon dioxide, ammonia, nitrites and nitrates; they also release oxygen. I quarantine and sterilise live plants with bleach before thoroughly soaking in clean water and introducing them into the aquarium.

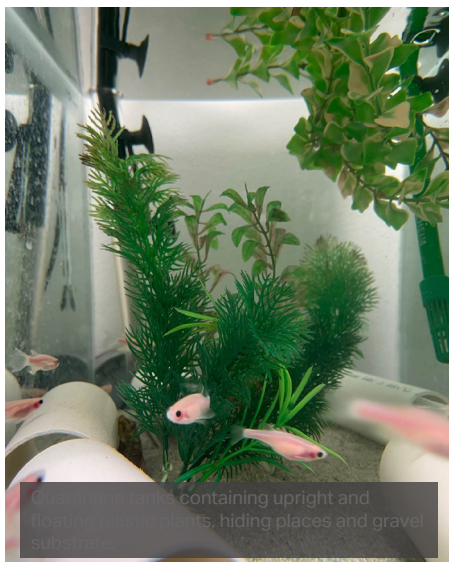
When you have a combination of floating and upright plants and refuges, the fish have an abundant variety of hiding places that give them choices as to how they use the space. This increased environmental complexity also means that monopolisation by dominant fish isn't possible (although aggression between our zebrafish has never been an issue). I have found that shy species (e.g. *Danio erythromicron*) are more likely to use the entirety of the tank and are less likely to startle when they are housed in well-enriched tanks. I have also noticed that all *Danio* species shoal in a more cohesive way in enriched environments than they do in barren tanks, which indicates improved welfare. The performance of 'stereotypies' (repetitive and apparently functionless behaviours) is a negative welfare indicator observed in captive animals, including fish. From my personal observations, stereotypies (e.g. repetitively swimming in circles) occur much less frequently in well-enriched tanks.



### **What are your future plans?**

Previously I worked in our biomedical facility and have maintained links with my colleagues there. Based on my observations of how our zebrafish have responded to environmental enrichment, I was inspired to create an enrichment device that mimics a floating plant with multiple fronds for use in the biomedical facility aquarium.

I am enthusiastic to organise some events for aquatic technicians, in both life sciences and biomedical research, focused on environmental enrichment. I would love to run a workshop where technicians can use readily available materials, knowledge of the species they work with, and their own imagination to create enrichment devices, like I have done. I am also planning some presentations on ways that aquatic enrichment can be implemented and would like to create a hub of enrichment ideas that can be accessed by others for inspiration.



**If you have an aquatic enrichment idea that you would like to discuss with Peter, email us at [tech3rs@nc3rs.org.uk](mailto:tech3rs@nc3rs.org.uk) and we can connect you with him.**

**The RSPCA has created posters that can be used to inform your choice of zebrafish environmental enrichment: <https://bit.ly/3J4aMk5>**

**Would you like to be featured in our next issue? Please email [tech3rs@nc3rs.org.uk](mailto:tech3rs@nc3rs.org.uk)**

[1] Stevens CH et al. (2021). Enrichment for laboratory zebrafish—a review of the evidence and the challenges. *Animals* 11(3): 698. doi: [10.3390/ani11030698](https://doi.org/10.3390/ani11030698)

# Upcoming events



RSPCA Refining severe disease models and procedures  
24 – 25 August, Stockholm, Sweden

In association with the Karolinska Institute, the RSPCA is hosting an international meeting as part of the Focus on Severe Suffering initiative. This free in-person event is relevant to those directly involved in the care and use of laboratory animals. Over two days, attendees will be invited to consider opportunities and approaches to minimise and avoid the severe suffering of research animals. The programme covers a range of animal models used in basic and applied research and regulatory testing, including rodents, fish and non-human primates.

Dr Elliot Lilley (NC3Rs) will be speaking about opportunities to implement the 3Rs principles in the quality control and batch release testing of vaccines and other biological therapeutics.

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**View the full programme and register at**  
[www.focusonseveresuffering.co.uk](http://www.focusonseveresuffering.co.uk)



LASA/UFAW Stitching the 3Rs into Surgical Procedures  
27 September, GSK Stevenage, UK

The Laboratory Animal Science Association (LASA) and Universities Federation for Animal Welfare (UFAW) are hosting a joint meeting focusing on refining the peri-operative period. Speakers at this single-day event will cover topics such as new or under-utilised surgical refinements, minipig safety pharmacology and how to provide additional warmth to mice in IVC cages.

Dr Esther Pearl (NC3Rs) will provide guidance on how to use blinding (also known as masking) at different stages of an experiment. Technical staff can play an important role in supporting researchers to achieve blinding, helping to improve the reliability of the data collected from research animals.

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**Read the full programme and register at**  
[www.lasa.co.uk/lasameetings](http://www.lasa.co.uk/lasameetings)



73<sup>rd</sup> AALAS National Meeting

22 – 27 October, Louisville, USA

Featuring in-person workshops, lectures, poster sessions and exhibits, with varied topics relating to the production, care and use of laboratory animals. This year's meeting theme is oncology, with a focus on managing animal care, husbandry procedures and the health and welfare for humane endpoints in animal models for cancer.

The NC3Rs is co-sponsoring an one-day workshop on teaching monkeys to cooperate with restraint, using positive reinforcement and temperament testing methods. Staff will also be presenting the benefits of housing non-human primates in European-style pen enclosures, participating in a panel discussion on refined mouse handling and highlighting how the NC3Rs works to support technicians to improve animal welfare.

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**Register at**  
[www.aalas.org/national-meeting](http://www.aalas.org/national-meeting)

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**Webinar: Microsampling in Toxicology**  
Thursday 15 September 2022, 14.00 – 16.00 (BST)

The use of smaller sample volumes (microsamples) to assess drug and chemical exposure in blood is now possible. There are a number of animal welfare benefits to using microsamples, including reducing restraint time and blood loss. Join experts to hear how microsampling is being implemented and opportunities for adoption.

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**Register now at**  
[www.nc3rs.org.uk/microsampling-workshop-2022](http://www.nc3rs.org.uk/microsampling-workshop-2022)

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**NC3Rs Primate Welfare Meeting**  
Tuesday 8 November, London, UK

Bringing together animal techs, vets, researchers and others, nationally and internationally, to share the latest best practice in the care and use of non-human primates. The meeting will be held as a hybrid event with live streaming. Delegates are encouraged to attend in person to make use of the networking opportunities.

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**Registration, abstract submission and travel bursary at**  
[www.nc3rs.org.uk/PWM2022](http://www.nc3rs.org.uk/PWM2022)