A recent report, published in *Lab Animal*, identifies key issues and important but unresolved questions on amphibian welfare. Knowledge on amphibian husbandry and welfare remains limited, with little established guidance or evidence-based refinements for their captive care.

The report provides a summary of a workshop jointly hosted by the NC3Rs and ZSL (Zoological Society of London) to discuss the welfare of amphibians in research. The event brought together experts from across academic and zoo research communities to identify the welfare challenges facing amphibians in research and to discuss the steps that need to be taken to improve conditions for this diverse group of animals.

A few of the unresolved questions are summarised below. The full set of questions can be found in the report.

- Can *Xenopus* superovulation / general breeding protocols be refined?
- What is the ideal nutritional composition of a regulated amphibian diet?
- Is live food a necessity in an amphibian diet?
- How does water quality and composition affect amphibian welfare?

If you work with amphibians, we invite you to review and consider these unanswered welfare questions and share any insights you may have to finding the answers. Please send any correspondence to Dr Samuel Brod (samuel.brod@nc3rs.org.uk).
3Rs champions

James Ward is a Departmental Colony Manager at the University of Oxford. Here he describes the approach he takes to reduce the number of mice used for breeding.

What 3Rs ideas are you developing?

Our idea has been to be more active in keeping breeding colonies under tight control through several related services and it is my job to help make that happen. When we audited our mouse numbers for 2015, we found that 57.5% of mice culled had been surplus to breeding. While some surplus is unfortunately inevitable, we wanted to reduce this number.

This helps to reduce breeding numbers

I help researchers set up enough breeders for their needs through our breeding calculation service and conduct a review of each colony every three weeks to ensure that this remains at the right level. I also run the training on colony management as well as genetics, covering topics like backcrossing and when the breeding stock needs refreshment. This helps to reduce breeding numbers and surplus animals without affecting the science being conducted. In our annual audit for 2017, we found that we had got the surplus down to 48.1%. While this is still high, we want to get it even lower, this already represents a reduction of thousands of animals, so it has been a real success.

What are your future plans?

We are hoping to start offering training in colony management to researchers as well as the animal care staff so that those academics who want to have a more active role can do so in a productive way. I have also started advertising these services so that any groups that are not aware of them have the chance to use them, so there might be more work in the future!

Kiya Robinson is an Associate Licensee at Covance, Harrogate.

In this interview, Kiya shares her experiences of implementing refined handling techniques.

What 3Rs idea are you developing?

In recent years there has been a wealth of scientific information to support the use of refined handling techniques in laboratory mice. The use of tunnel or cup handling methods versus tail handling is a large focus, and so I was intrigued to see how these methods would work in our institution. I undertook a small trial and the difference in behaviour with the mice surprised me.

How did you develop your idea?

Covance is deeply committed to animal welfare and continuous improvement. As part of this commitment, I looked through the information on the NC3Rs website and then attended the mouse handling workshop in 2017. I was sceptical at first but hearing the experience of other technicians at the workshop was convincing. There is strong scientific data to support these techniques, I initially handled some health screen mice for two weeks. We then identified a four-week repeat dose study, obtained our client's approval and selected a team of 10 technicians that would work in the room and offer feedback. This study demonstrated its value when future technicians would talk to me about their concerns, because we had already considered these concerns and found solutions on that initial study. We have around 160 technicians currently working with mice, so the next step was to pick a long-term study. Our training officer helped me to develop a training plan consisting of a presentation and a theory test, followed by a practical assessment. We wanted to make sure that everyone understood why we were introducing the cupping technique and its intended benefits. The majority of our technicians are now trained and our standard operating procedures have been updated to reflect the new handling techniques. We are still gaining experience with different strains and ages of mice. However, whenever possible, new mouse studies will now be cup handled.

What are your future plans?

A few more technicians still need to complete their initial training. Then our focus will shift to improving their handling techniques with experience. We are also evaluating how to help technicians stay engaged with this method whilst it is still being rolled out. Monitoring the impact on welfare and the unit will be very important.

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So far, our measurement has mainly taken the form of technician feedback. Once technicians are used to the method they confirm that the mice are easier to handle, appear less anxious, and that it does not require a large increase on their time. Once we have a standard and measured approach, an important future goal of the project is to share the refined handling techniques with our colleagues around the world.

Please email tech3rs@nc3rs.org.uk to be featured in our next issue.

3Rs papers of interest

Each issue we feature recent 3Rs publications of interest, providing summaries and links to the full articles for further information. This issue we focus on the welfare of fish in the laboratory, in particular the commonly used zebrafish.

Is heightened-shoaling a good candidate for positive emotional behavior in zebrafish?

(Franks et al., 2018)

- Research on positive emotions in laboratory animals is becoming increasingly recognised as fundamental to research animal behavior and welfare. Good welfare is not simply the absence of negative experience (e.g. pain and suffering) but also the presence of positive emotions (e.g. pleasure).

- The aim of this study was to characterise and evaluate heightened-shoaling in zebrafish and its potential as an indicator of positive emotional behavior.

- Heightened-shoaling is defined as discrete periods of tight group cohesion and increased behavioral synchrony in groups of fish. The authors characterise and describe this behavior and provide supplementary videos to help demonstrate.

- The study extends research on the social behavior of zebrafish and heightened-shoaling appears to be a good candidate for future research into positive emotional behavior in zebrafish.

This article featured in a special issue on Animal Emotion. Further articles about current progress in understanding animal emotions to help improve animal welfare are available at www.mdp.com/journal/animals/special-issues/Animal_Emotion


Effects of environmental enrichment on survivorship, growth, sex ratio and behaviour in laboratory maintained zebrafish Danio rerio

(Lee et al., 2018)

- Environmental enrichment can help increase the complexity of a fish's environment and improve their welfare. Despite this, the majority of zebrafish used in research are usually kept in barren tanks due to a lack of clear evidence in this area.

- The aim of this study was to investigate the effects of environmental enrichment in the form of aquarium gravel and aquatic plants on the health and welfare of zebrafish.

- This study showed that tank environmental enrichment had positive effects on larvae survivorship, growth and behaviour in zebrafish. The study also found monopolisation of resources by dominant fish and associated aggression in enriched tanks, which may have negative effects on welfare.

- The authors showed that fish from enriched tanks displayed lower levels of anxiety behaviour compared with fish from barren tanks. The challenge is to design enrichment that provides measurable welfare benefits and can also be practically adopted without compromising husbandry procedures or the research question of interest.


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Welfare challenges influence the complexity of movement: fractal analysis of behaviour in zebrafish (Deakin et al., 2019) – NC3Rs funded research

- Despite being the second most commonly used species in research, welfare assessment in fish is still a challenge, especially for fish kept for behind roosters. Many routine procedures, such as fin clipping, are carried out in zebrafish with no clear indication of the pain the fish are subjected to.

- The aim of this study was to determine if fractal analysis, a method to study complex patterns, could be used to analyse zebrafish movement behaviour following painful procedures.

- Fractal analysis showed less complex movement patterns in fish following painful procedures (e.g. fin clipping). The administration of the local anaesthetic lidocaine reversed the changes in movement patterns associated with fin clipping, providing further evidence that fin clipping is a painful procedure requiring pain relief.

- The authors also demonstrated a decrease in movement complexity with increasing strengths of acetic acid (a standard pain test), which allowed the development of a scale of pain intensity which could be adopted as a new tool for assessment and monitoring of zebrafish welfare.


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The NC3Rs has a broad range of projects and resources directly relevant to the work of animal technicians in laboratories around the world. Whether it's the latest refinements in mouse handling, or practical advice on blood sampling, these resources can help support animal technicians to apply best practice in their work.

This poster highlights the relevant web resources available on the hub. Further information can be found here: [www.nc3rs.org.uk/animaltech](http://www.nc3rs.org.uk/animaltech)

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<th>Animal Technician Hub</th>
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<tr>
<td><strong>Tech3Rs:</strong> A newsletter for animal technicians</td>
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<td><strong>Laboratory mouse aggression study</strong></td>
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<td>Crowdsourcing data project for technicians to identify patterns and triggers of aggression in laboratory mice.</td>
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<td>A dedicated website of online tutorials and guidance to support the adoption of best practice for commonly used procedures in animal research.</td>
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<td><strong>Events calendar</strong></td>
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<tr>
<td>The latest workshops, conferences and events from the NC3Rs and other related organisations, many of which will be relevant for animal technicians.</td>
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<td>Video presentations on refinement opportunities and approaches to improving the welfare of a range of laboratory animal species.</td>
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<td>Free online tutorials designed to support the training of technicians and others working with laboratory animals.</td>
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<td><strong>Contemporary training on the 3Rs</strong></td>
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<td>A video presentation demonstrating the 3Rs in principle and practice.</td>
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<tr>
<td><strong>3 Minute 3Rs podcast</strong></td>
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<tr>
<td>A monthly podcast produced with LabAnimal and the NA3RsC summarising the latest research and news in 3Rs science and technology.</td>
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We recommend that you pull out this poster and display it in your animal unit to highlight the resources available to all.
Solutions

New Solution – Assessing aquatic organism health status non-invasively

Researchers at the University of Naples Federico II have developed a non-invasive method to detect and identify potential pathogens (viruses, bacteria, protozoa, metazoa, parasites) in aquatic facilities. The technology uses environmental DNA (eDNA) extracted from water samples, coupled with DNA sequencing technology (metabarcoding). The improved identification of pathogens in local and imported animals, prevents potential pathogens spreading to healthy animals, and reduces the number of sentinel fish used.

The Solution provider is keen to work with animal care staff interested in providing water samples to help assess and validate this eDNA metabarcoding technology against current practice.

Further information can be found here: www.crackit.org.uk/aquaticassessment

Listen to the 3 Minute 3Rs Podcast

We’ve teamed up with LabAnimal and the North American 3Rs Collaborative to bring you 3 Minute 3Rs, a monthly podcast that gives you the lowdown on the latest 3Rs research in just a few short minutes.

You can find 3 Minutes 3Rs anywhere you get podcasts by searching for LabAnimal or visit www.nc3rs.org.uk/podcast to listen to all of our past episodes.

Webinar – NC3Rs mouse aggression study

Did you participate in the NC3Rs Mouse Aggression Study?

If you did and would like to find out more about the results, a recording of the webinar hosted by the NC3Rs on 11 December 2018 is available upon request. Please email mouseaggressionstudy@nc3rs.org.uk for further information.

Highlight 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 a new approach to training macaques.

Welfare-friendly, tablet-based method for training macaques

Training non-human primates (NHPs) to perform cognitive tasks in the laboratory is a time-consuming process with significant welfare and experimental limitations. To refine the practice, Drs James Butler and Steve Kennerley from University College London developed Mymou, a novel system for automated training of NHPs within their home cage rather than in the laboratory. The Mymou system is a wireless tablet training system attached to the home enclosure that overcomes many of the disadvantages linked to the traditional approaches.

For further information about automated training systems and a video demonstration, visit: www.nc3rs.org.uk/mymou

The monkeys we’ve trained using the system have learnt to use it quickly and we are excited at the prospect of other labs using the system”

Drs James Butler and Steve Kennerley

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Spotlight on guinea pig husbandry

We have updated our housing and husbandry page for guinea pigs.

Check out the new page for information on social behaviour, considerations for enclosures and environmental enrichment. If you have any ideas or photos of guinea pig enrichment you would like to share with us. Please email enquiries@nc3rs.org.uk

Further information: www.nc3rs.org.uk/guinea-pigs
NC3Rs workshop, IAT Congress
Tuesday 9 - Wednesday 10 April, North West England

NC3Rs staff will be leading a workshop on ‘Fundamental concepts in experimental design: randomisation and blinding’ at IAT Congress.

Recent studies have highlighted that the results from animal experiments are often not as reliable as they should be. The role of an animal technician in improving experimental design is often underestimated. During this two-hour workshop, we will cover some approaches that are proven to improve experimental quality and which animal technicians are likely to be involved in – randomisation and blinding. We will explain what they are, why they are important and when they are needed. The workshop will also provide practical advice and guidance on how to blind and randomise.

This workshop is for technicians involved in supporting and running experiments (e.g. setting up experiments, performing procedures, preparing animals for studies). Whilst we will focus on common species such as rodents and fish, the principles will apply across all species. This interactive workshop will include talks, voting, group work and hands-on activities.

Other events at IAT Congress:
- We will have a stand in the congress exhibition hall; please come and say hello.
- Dr Katie Lidster will be presenting an update on the NC3Rs mouse aggression study on Thursday 11 April.
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Register at [www.iat.org.uk/congress](http://www.iat.org.uk/congress) by 22 March.

Animal Tech Month
March 2019

As part of the IAT’s Animal Technologist Month in March to celebrate the hard work of technicians, the NC3Rs will be tweeting about how technicians contribute to the success of 3Rs science. Follow us [@NC3Rs](https://twitter.com/NC3Rs) and look out for the hashtag #animaltechmonth – why not join in the conversation and share your own experiences?

NC3Rs/IAT Animal Technicians’ Symposium 2019
Wednesday, 2 October 2019, central London

Save the date for this year’s NC3Rs/IAT Animal Technicians; Symposium. A free, informative meeting to provide animal technicians with an opportunity to find out more about the latest advances in the 3Rs. More details to follow.