

NEWS

Implementing the 3Rs: improving experimental approaches in animal biology

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The pages of this journal are full of remarkable stories of scientists working with extraordinary and commonplace creatures to understand how these species survive and interact with their environment. None of these studies could have been undertaken without the contributions of the central protagonists themselves, and in the fields of biomedical research and ecotoxicology, large numbers of vertebrate model organisms contribute to our understanding of disease and the impact of pollution in the environment. With recent rapid changes in climate and the degradation of many environments, animal research has never been more significant. However, this expansion has been accompanied by increasing awareness of the welfare of the animals that contribute to our growing knowledge and a rising appreciation of the three Rs (3Rs) – replacement, reduction and refinement – which underpin the ethical use of animals in research. Having previously organised a successful meeting in 2012 dedicated to the implementation of the 3Rs with the National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs), Lynne Sneddon from the University of Liverpool, UK, says that she and Nic Bury from King's College London, UK, felt that it was time for the Society for Experimental Biology (SEB) 'to showcase the state of the art in 3Rs science and to educate our membership to give them opportunities to employ the 3Rs in their research'. Collaborating with the NC3Rs, Sneddon and co-organiser Bury coordinated the 'Improving Experimental Approaches in Animal Biology: Implementing the 3Rs' meeting at the SEB headquarters in London over three days in late June 2016.

Inviting speakers from a broad spectrum of laboratories to present examples of best practice and to discuss the promotion and education of the 3Rs, Sneddon and Bury structured the first day of the meeting around case studies dedicated to the refinement of procedures involving animals. 'The whole reason for the 3Rs is to improve welfare, make animals healthier and fitter, to use fewer of them, thereby improving the quality of the data', says Sneddon, emphasising that the purpose of refinement is to develop and use the most humane and non-invasive methods, where traditional methods may have previously been more intrusive. With some approaches focusing on improving the welfare of more traditional laboratory animals, such as zebrafish, mice and *Xenopus*, other presentations reviewed the assessment of pain in ball pythons and the effects of morphine on the South American rattlesnake.

In her Association for the Study of Animal Behaviour prize-winning talk, Lottie Hosie from the University of Chester, UK, reported on refinements in laboratory husbandry in African clawed frogs (*Xenopus*). Although *Xenopus* is one of the major model animals and has been reared in laboratories since the 1940s, little is known about the best conditions for keeping this species in



Fig. 1. Craig Franklin with the ASAB prize winner, Lottie Hosie. Photo credit: SEB.

captivity. In their native environment, *Xenopus* inhabit dark, muddy lakes and rivers, in contrast to the barren environments that they occupy in laboratories, and Hosie has been investigating ways of enriching the animals' environment to reduce stress. 'We have developed non-invasive tools, one of which is an assay for corticosterone, which is the amphibian stress hormone... and alongside that we are developing behavioural tools so that we can also assess behaviour as a welfare monitoring tool', says Hosie. She adds that providing plastic refuge tubes and shelters leads to measurable welfare improvements for the animals. In a separate presentation, Matthew Leach, from Newcastle University, UK, reported his assessment of the expression of pain in animals through observation of facial grimaces, which he has developed as an alternative to other more costly and time-consuming approaches that have traditionally been used. Focusing on the three main rodent models – rat, mouse and rabbit – Leach's observations have been rolled out in a series of posters (<https://www.nc3rs.org.uk/grimacescales>) distributed by the NC3Rs to raise awareness of the grimace scales and their utility for assessing the degree of pain experienced by an animal, allowing researchers to deliver pain relief more rapidly to improve welfare.

While refinement has clear and direct benefits for animals used in research, the replacement of vertebrates with alternative systems – from cell culture to invertebrate models – offers great potential to reduce the numbers used in experimentation. With a broad range of presentations – from the use of the social amoeba *Dictyostelium discoideum* to assess the health benefits of dietary flavonoids, to the use of paired-down memory circuits in the pond snail *Lymnaea*

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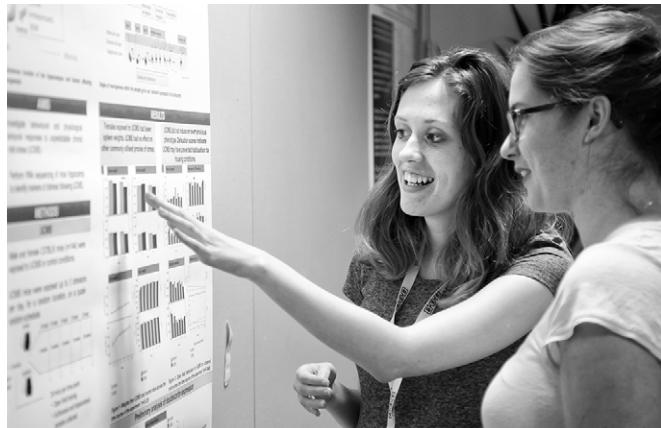


Fig. 2. Poster discussion at the meeting at Charles Darwin House, London.
Photo credit: SEB.

stagnalis and the implementation of gill cell cultures to assess effluent toxicity – the session addressing animal replacement presented a glimpse of some of the creative approaches that are being taken by laboratories to reduce their reliance on traditional vertebrate model organisms. ‘Invertebrates present a much simpler system to work with without the difficulties and confounding factors of the very complex neurobiology of vertebrates’, says Sneddon, adding that other strategies include replacing adult animals with earlier life stages that are not protected under legislation, such as zebrafish up to 5 days post-fertilization.

Although embracing all of the 3Rs can be tricky for scientists that adopt a comparative approach, the Chair of the SEB Animal Section – Craig Franklin, from the University of Queensland, Australia – recommends that scientists that are unable to replace study animals with alternative species focus on reducing the numbers of animals used in trials. In the session detailing examples of reduction, Manasi Nandi, from King’s College London, UK, presented an exciting collaboration inspired by an earlier NC3Rs workshop – which brought mathematicians and biologists together – that allowed Nandi and her collaborator, Philip Aston, from the University of Surrey, UK, to develop a mathematical method that can detect changes in cardiovascular diseases more sensitively and earlier than traditional measures. Together they have applied this to sepsis and found that fewer animals are needed to detect a clear change. Several discussions also highlighted the importance of efficient experimental design to avoid overuse of animals and cautious selection of animal models to ensure the relevance of observations to specific biomedical scenarios. Continuing the theme of experimental design, Nathalie Percie du Sert, from the NC3Rs, described several online tools developed by the centre to improve experimental design and optimise animal use, including the Experimental Design Assistant (<https://eda.nc3rs.org.uk/>) and the ARRIVE Guidelines (<http://www.nc3rs.org.uk/arrive-guidelines>), to improve the communication of animal-based research.

After the discussions of successful examples of 3Rs-based approaches, UK research funding bodies – including the NC3Rs and the Biotechnology and Biological Sciences Research Council (BBSRC) – reviewed funding opportunities for scientists that wish to advance laboratory animal welfare and the 3Rs (<http://www.nc3rs.org.uk/funding>), before representatives of the Laboratory Animal Science Association and Understanding Animal Research discussed the roles of their organisations in animal welfare. Wendy Jarrett also reviewed the launch of the Concordat on Openness on Animal Research in 2014 (<http://www.understandinganimalresearch.org.uk/policy/concordat-openness-animal-research/>), which is intended to dispel public misconceptions about the use of animals in research and is currently supported by 100 signatory organisations, including the SEB. The practicalities of obtaining permission to work with animals were also addressed during a mini-workshop coordinated by Penny Hawkins, from the Royal Society for the Protection of Animals (RSPCA), who encouraged researchers to become involved with their local ethical review body. Presentations from a regulator, a laboratory animal veterinarian, a lay member and a scientist explained how scientists can both contribute to, and benefit from, the process of local ethical review.

However, Sneddon warns that ensuring that the 3Rs are well instilled in the young scientists that will be the principal investigators of tomorrow is a priority, and she is concerned that current training at the early research career stage is already too late. ‘I would like to see a shift of emphasis that students at undergraduate level should really be getting training in 3Rs that takes them into postgrad studies with a good sound base so that [the 3Rs] really becomes part of their psyche’, she says. To address their concerns, Sneddon and Bury invited educators and scientists John Bryant, Chris Willmott and Teresa Valencak to discuss their experiences of training the next generation of scientists, using innovative teaching approaches to develop the enquiring strategies essential to address the ethical concerns surrounding animal experimentation.

Reflecting on the impact of the 3Rs on his own career, Franklin says, ‘The 3Rs [approach] has affected my research enormously; it has allowed me to think in a different way’, describing how the approach has led him to transfer his research focus from the lab into the field. ‘I view the 3Rs as beneficial; I think you can do better science if you embrace them’, says Franklin, encouraging other physiologists and animal researchers to enshrine the 3Rs at the heart of their research programs. In addition, Mark Prescott from the NC3Rs is optimistic that organisations such as the SEB can foster a wider appreciation of the 3Rs in the research community through communication and the promotion of advocates and best practice, while offering support and training to practising scientists. And Sneddon is clear that high quality ethical research is essential in an era of accountability when research is government funded. ‘The application of the 3Rs is driving more ethical experimentation and shows that we are accountable. We are funded by public money and we are showing that we are doing the public a good service; we are doing right by them and by the animals’, she says.