Discussing the future of amphibians in research

Report of the NC3Rs/ZSL Workshop on Amphibian Welfare

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Abstract

On 5 October 2017 The NC3Rs in collaboration with the Zoological Society of London (ZSL), jointly hosted a workshop to discuss the welfare of amphibians in research. Chaired by Trenton Garner of ZSL the workshop was designed to promote discussion on approaches that can be taken to help improve conditions for amphibians in research, and take initial steps in establishing common principles for their housing and care. The following report summarises the key outcomes of the day and recommendations for research priorities in this area.

Introduction

Amphibians have been used as experimental organisms for centuries, and recent years have seen a growth in their use. Driving this is a renewed interest in amphibians as models of human development and disease and an urgent need to understand and mitigate the impacts of the chytridiomycotan and ranaviral pathogens that pose a global threat to these animals. Alongside this increase in laboratory use comes an increased responsibility to conduct research using amphibians with proper consideration made of the unique welfare requirements of this diverse vertebrate class. Despite this, knowledge of the welfare needs of amphibians remains limited, with little scientifically justified guidance or evidence-based refinements for their captive care [1-4]. Held on 5th October 2017 and hosted and supported by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) in collaboration with the Zoological Society of London (ZSL), this workshop sought to bring together experts from various fields of academic endeavour and the zoo community to identify the key topics in amphibian welfare and identify the next steps that can be taken to help monitor and improve conditions for amphibians in research.

Amphibians are widely used in science as both model and non-model organisms. This was encapsulated by the varied research backgrounds of the 72 participants, with specialisations ranging

from oncology and developmental biology to conservation and epidemiology. The cross-disciplinary and international importance of amphibian welfare was further exemplified by the events' talks.

Talks

A growing demand for *Xenopus laevis* and *X. tropicalis* as models for disease, development and genetic manipulation has led to a surge in their use. The coordinator of the European Xenopus Resource Centre (EXRC; https://xenopusresource.org/), Professor Matthew Guile (University of Portsmouth, UK), discussed the welfare and biosecurity challenges created by the storage and distribution of hundreds of genetically modified *Xenopus* lines. By improving current methods of sperm recovery and cryopreservation, his centre has significantly reduced the number of male frogs required for research, as well as refining their husbandry and potentially eliminating the need for transporting male frogs [5]. Complimenting Professor Guile's talk, Professor Jacques Roberts (University of Rochester Medical Center, US) described how the rapid increase in *Xenopus* use in laboratories has highlighted the absence of realistic, scientifically-based welfare standards for these animals. Helping to address this shortage, Professor Roberts is currently leading the update and standardisation of Cold Spring Harbour Protocols guidelines for *Xenopus* husbandry.

Assessing the welfare of captive amphibians, when considered at all, has proven notoriously difficult. Few behavioural or physiological indicators of welfare have been developed or shown to be effective or consistently applied across amphibian species. Dr Lottie Hosie (University of Chester, UK) reported team efforts to validate such methods, again focussing on *Xenopus*. She demonstrated that a combination of behavioural (such as activity levels and tank butting) and endocrine measures of stress (waterborne corticosterone) may prove useful in gauging amphibian welfare, but that greater effort and collaboration by researchers was needed to confirm their more general application and to develop additional indicators [6, 7].

Professor Richard Griffiths (University of Kent, UK) outlined issues around reconciling effective research on amphibians in the field, the limited and sometimes limiting welfare guidelines that govern their use in scientific procedures (for examples see [8, 9]). With such a biodiverse set of morphologies, habitats and physiological needs, a one size fits all approach for field work with amphibians seems untenable. Building on this topic, Professor Caren Helbing (University of Victoria, Canada) presented her work developing non-lethal and non-invasive "omics" focused sampling methodologies applicable to multiple amphibian species and able to provide information on the relationships between early development and health [10]. Validation of such methodologies may pave the way for better assessment of the impact of environment on amphibian welfare in both the laboratory and field as well as helping to implement the reduction and refinement principles of the 3Rs.

Live infection studies are currently the only effective means of studying the Ranavirus epidemic threatening amphibian populations worldwide. Dr Stephen Price (University College London, UK) presented an alternative *in vitro* model using amphibian cell culture systems to study host-pathogen

interactions [11]. Such research stands to significantly reduce animal as well as helping combat this serious disease.

Perhaps the greatest practical challenge to increasing scientifically supported amphibian welfare is securing the funding to support the research. Dr Mark Prescott (NC3Rs, UK) closed off the talks by drawing on NC3Rs' experience of funding 3Rs-relevant science, including the aforementioned work of Guille and Hosie, and highlighting how to write a successful grant application to the NC3Rs and integrate an interest in welfare with wider research aims.

Talks were followed by afternoon breakout sessions that identified subjects of broad importance for improving amphibian welfare in a research setting:

1. The dual problem of multiple species and life history stages.

In contrast to many other vertebrate classes, dozens of species of amphibians are currently used in research, with a diversity of specific housing and husbandry requirements. Compounding this issue most amphibians have complex life histories, and the housing and husbandry needs of embryo to larvae to juvenile to adult are each significantly different [2]. These issues were regarded as the major impediment to devising a basic, transferrable set of welfare and husbandry standards for amphibians. One proposed alternative was to select a "flagship species" to serve as the platform for initiating a concerted effort to optimise amphibian welfare. The obvious choice for this species would be the most commonly used amphibian in laboratory research, *Xenopus laevis*. However, others commented that such a course would only serve to recapitulate the welfare limitations, as the ecology and natural history of *Xenopus* species do not represent the majority of frog or toad species; even less so the newts, salamanders and caecilians.

2. The need for basic, relevant guidelines on amphibian care and husbandry

Despite the acceptance that the requirements of different species and life history stages cannot be harmonized into a single set of guidelines, the participants still recognized that fundamental guidance on the housing and husbandry of amphibians is markedly limited compared to that of other taxa. Many representatives regarded the evidence base for existing guidance to be dubious, dated or not truly applicable to amphibians. It was suggested that regulators can lack the appropriate knowledge base to decide the suitability of housing and husbandry and validated measures of welfare and humane endpoints for amphibians. The distinction between different jurisdictional oversights for zoo, laboratory and conservation-based research also proved a point of contention, with some researchers feeling unfairly restricted in the work they may carry out while others fell outside the lines of governance [12]. Participants also acknowledged a lack of interaction amongst the different sectors, which impairs the collaborative development of husbandry protocols.

There was a consensus that current guidelines and best practice documents on amphibian housing and husbandry need to be revised and expanded and that existing training resources specific to amphibian welfare should be updated, utilised in regulator-approved training courses and delivered by more

training centres. However, there was also some uncertainty regarding how widely and to whom these new guidelines should apply. This hesitation again derived from current uncertainties regarding jurisdictional oversight for amphibian research in the field and in zoos and conservation centres.

3. The need for basic measures of welfare across the amphibian research community

Workshop participants generally agreed that current means of measuring welfare in amphibians are poor and sometimes contradictory across research groups and countries. Many of the measures in use are inefficient, not validated or indiscriminately applied across amphibian species that exhibit divergent behaviours and responses to stress and poor health. These issues were attributed at least in part to a perceived poor understanding of the need to apply the 3Rs in amphibian research, poor communication between amphibian research groups, a lack of funding for welfare research and the challenges created by using multiple species of amphibians in research.

4. A call for an active community on amphibian welfare in research

The theme discussed regularly across the workshop and breakout discussions was the need for better communication on welfare between amphibian researchers. The creation of a community actively discussing the topic of, and sharing findings on, amphibian welfare would help to harmonise best practice in husbandry, drive new research and bring to the fore any new developments in the field.

Alongside this call for better communication came a desire for the creation of a centralised resource hub on amphibian care, husbandry and breeding. Some of the suggested purposes for this hub would be to:

- Create an outlet to publish husbandry and welfare information
- Build a centralised resource on welfare for building consensus
- Aid in cross sector learning
- Improve information sharing and knowledge exchange

5. Securing funding for research on amphibian welfare

Amphibian research appears in something of a transitionary period with some optimistic that that current innovations in genome editing will drive a continued interest in the species, while others felt such work (along with current wild populations of amphibians) to be in decline. The reticence of zoos and other conservation organisations to be associated with research that involves invasive regulated procedures on animals the invasive animal research currently restricts access to a knowledgeable community and funding, and limits the progress of amphibian research. At the breakout group focused on securing funding for welfare research, it was suggested that this issue stems partly from a lack of cohesion between amphibian researchers, which limits their ability to construct project proposals of the necessary size and scope to attract large funders. It was also agreed that greater efforts to integrate welfare research into grants ostensibly focused on basic research as a source of “added value” would likely aid in getting more welfare research published.

While much of the day focused on highlighting the shortcomings of amphibian welfare in laboratories, the consensus was that the workshop had been productive. There was clear enthusiasm for greater

research on amphibian welfare and agreement that the workshop had revealed key challenges that must be faced for the field to progress. In addition to these issues, several important unanswered questions facing amphibian researchers were identified throughout the day, which are summarised in Table 1. Together these five challenges and thirteen questions could serve as the stepping stones towards greater engagement with this topic and the advancement of amphibian welfare.

	Table 1: Some important but unresolved questions on amphibian welfare
1	Is MS222 an appropriate chemical for euthanasia of amphibians?
2	What biomarkers of welfare are applicable to amphibians?
3	What is the efficacy of current analgesia and anaesthesia?
4	Can *Xenopus* superovulation / general breeding protocols be refined?
5	Are corticosterone readouts valid measures of welfare?
6	Can established preference tests be created for amphibians?
7	What non-invasive monitoring methodologies exist for these animals?
8	Have species-specific baseline behaviours been documented?
9	What is the ideal nutritional composition of a regulated amphibian diet?
10	Is live food a necessity in an amphibian diet?
11	How does water quality and composition affect amphibian welfare?
12	What (if any) are the health benefits of ultraviolet light for amphibians?
13	Does providing a gradient in the physical captive environment of amphibians (to enable choice of microenvironment) improve health and welfare?

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Declarations

Samuel Brod is a member of the NC3Rs, the organisation that funded and co-hosted the event reported upon.

Lola Brookes and Trenton WJ Garner are members of the Zoological Society of London, the organisation that co-hosted the event reported upon.

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