National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

Key points from the UK's 2024 Amphibian Welfare Workshop

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The UK National Centre for the 3Rs

- The NC3Rs is an independent scientific organisation.
- Established by UK Government in 2004, to accelerate the development and uptake of the 3Rs.
- Work across the bioscience sector, with industry, academia, regulators and funders – UK, and collaborators in Europe, North America and Asia.
- Track record of influencing policy and practice internationally.

NC 3Rs

www.nc3rs.org.uk

2024 Workshop

RSPCA/NC3Rs Workshop on Amphibian Care and Welfare

- In person workshop on amphibian care and welfare.
- Key stakeholders in the UK amphibian research sector.
- Aims of the workshop:
  - Define welfare hazards for amphibians used in research.
  - Identify where empirical evidence is lacking on the degree of effect these welfare hazards have.
  - Discuss current knowledge on amphibian welfare assessments.
  - Discuss current husbandry guidelines for *Xenopus* species used in laboratory research and agree priorities to further improve best practice guidance.
  - Produce a summary report to disseminate the outcomes of discussions.

Who attended the workshop?

Participants

- Participants were from laboratory and zoo settings

- Many career stages:
  - PhD students
  - Early Career Scientists
  - Senior professors
  - Veterinary surgeons
  - Technical staff

- Fields of study ranged from biomedical to conservation research.

2024 Workshop

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What was discussed?

Questions for *Xenopus* discussions

- What are the key elements of *Xenopus* husbandry and care that we have some certainty about?
- What are the main gaps in our knowledge of *Xenopus* husbandry and care?
- Can we prioritise which of these gaps we need to fill most urgently to improve *Xenopus* care and welfare in biomedical research?

Why research animal welfare matters to science

Stressed, unfit, obese animals <u>do not</u> make good models*

- Housing conditions and interactions with humans can cause stress to animals before scientific procedures even begin = altered physiology, behaviour and immunology

- Stress as an unintended variable can confound studies and lead to uncontrolled variation in experimental results that impairs reliability and repeatability = reduced ability to make reliable conclusions

**when this is not what you are intending to study*

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Common themes in all discussions

- Lack of empirical evidence on best practice for *Xenopus* housing, husbandry and care.
- Lack of available funding allowing *Xenopus* researchers to fill these gaps.
- Consensus through collective experiences of the community can be valuable – the wisdom of crowds.

2024 Workshop

Case study

- Gas bubble disease arose in a *X. laevis* colony while the technician with specific *Xenopus* expertise was on leave.
- The covering staff did not have the expertise to catch the problem in the early stages.
- The returning expert technician diagnosed the disease and found the cause (a cracked pipe).

How could this be prevented in future?

- Greater knowledge of *Xenopus* by more staff to enable all to pick up when frogs are not healthy.
- Clearly described behavioural or clinical signs to monitor.

Existing guidance

Guidance on *Xenopus* housing, husbandry and care – **resource centres**

- European Xenopus Resource Centre (UK)
  - https://xenopusresource.org/husbandry
  - https://xenopusresource.org/animal-health-and-general-protocols

National Xenopus Resource (USA)

- https://www.mbl.edu/research/resources-research-facilities/national-xenopus-resource/protocols

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Existing guidance

Guidance on *Xenopus* housing, husbandry and care – **textbooks**

- *Xenopus* methods and protocols (part of the Methods in Molecular Biology book series, Springer Protocols). 2018. Ed: *Kris Vleminckx*
- *Xenopus*: A Laboratory Manual (Cold Spring Harbour Laboratory Press). 2023. Ed: *Hazel L. Sive*
- The Laboratory *Xenopus* sp. (Taylor and Francis). 2010. Ed: *Sherril L. Green*
- Sourcebook for models of biomedical research (Springer Nature). 2008. Ed: *P. Michael Conn*
- The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals (Wiley). 2024. Eds: *Huw Golledge and Claire Richardson*
- Handbook of Genetics (Springer Nature). 1975. Ed: *Robert C King*

2024 Workshop

Areas of consensus and least concern

- Basic husbandry protocols that keep the animals alive – we know what is acceptable but not what is ideal.
- A lot of our information/guidance was established years ago and we have not checked if they still represent best practice.

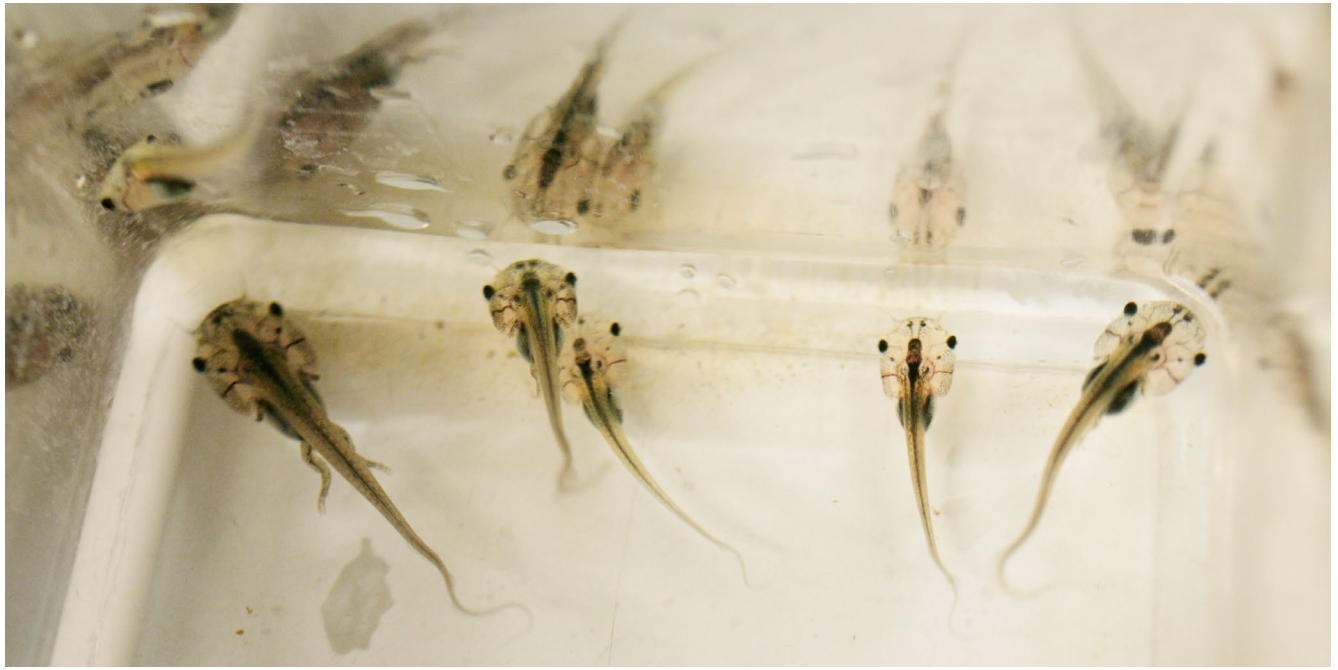


Photo courtesy of the EXRC

2024 Workshop

Knowledge gaps identified

Discussions during the workshop revolved around knowledge gaps in four key areas:

- Environment
- Husbandry
- Welfare indicators
- Moving/transporting

Photo courtesy of the EXRC

2024 Workshop

Knowledge gaps – **environment**

- Tank type – colour, dimensions including depth
- Tank system type – which is best? static, recirculating, flow-through
- Water chemistry parameters
- Lighting type – dawn and dusk vs on/off
- Lighting timing
- Cleaning regime – how often is too often?
- Vibrations

Photo courtesy of the EXRC

2024 Workshop

Knowledge gaps – **husbandry**

- Feed type
- Feed frequency
- Anaesthetics
- Analgesics
- Humane euthanasia

Photo courtesy of the EXRC

2024 Workshop

Knowledge gaps – **welfare indicators**

- Natural behaviours of *Xenopus* in the wild
- Clinical signs

Knowledge gaps – **moving/transport**

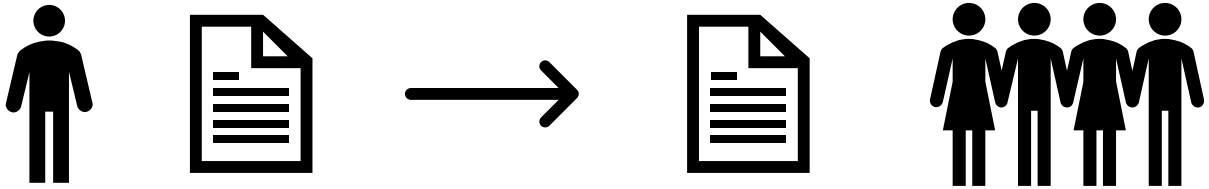
- Moving individuals for procedures – net vs hand, gloves vs no gloves
- Transporting groups of frogs
- Acclimatisation time after moving or transport

Photo courtesy of the EXRC

2024 Workshop

Priority – a mechanism for sharing best practice and sharing good methods

- Zoos tend to collaborate and have taxonomic working groups that share living documents that are updated as best practice evolves – could the *Xenopus* community do something similar?



Existing evidence - publications

Factors affecting oocyte quality

- Tank water flow – static vs recirculating (Delpire et al., 2011).
- Water hardness (Godfrey and Sanders 2004).
- Water chemistry and enrichment (Heyworth and Owens, 2019).

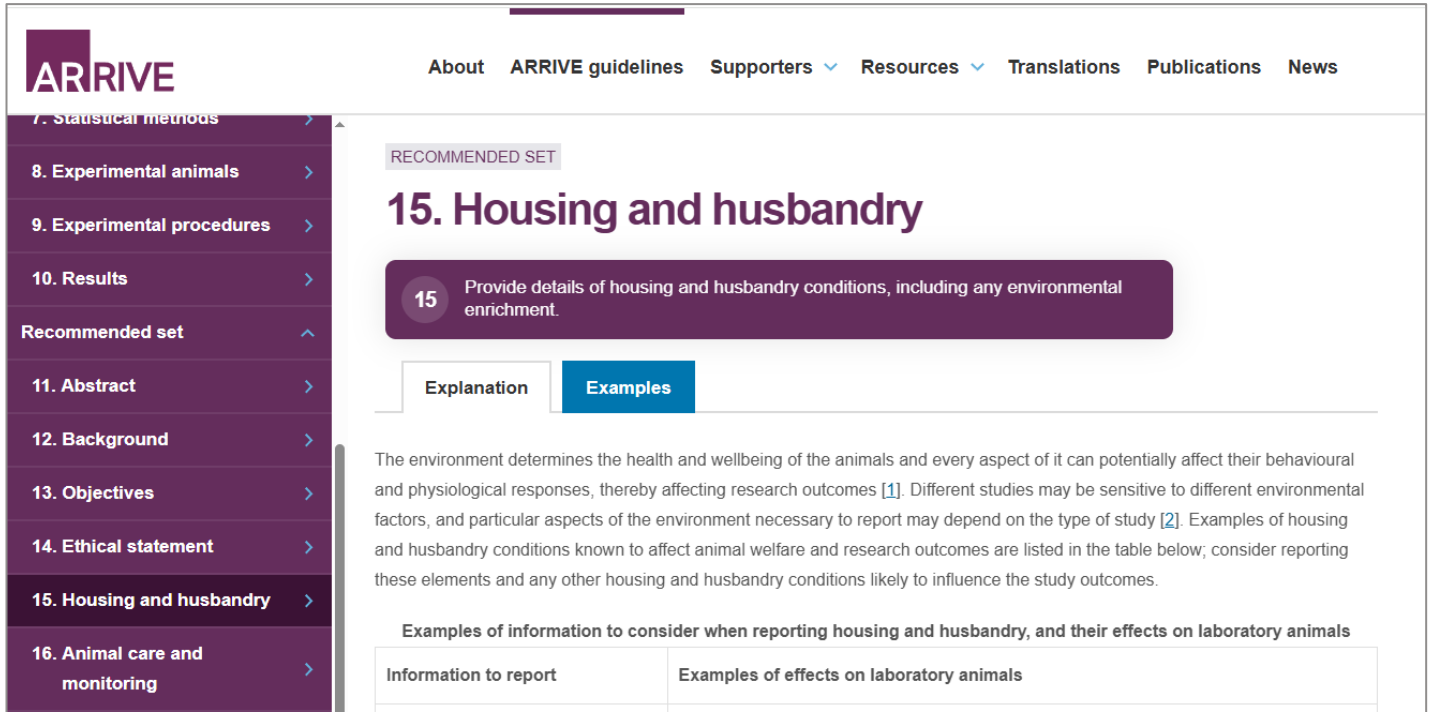
Stress in *Xenopus*

- Effect of tank colour (Holmes et al., 2016).
- Effects of transport (Holmes et al., 2018).
- Effect of ovulation frequency (Moss et al., 2024).
- Validating water corticosterone measurements (Smith et al., 2025).

Building our evidence base

Assessing the welfare impact of enrichment – **reporting housing and husbandry in detail**

- Most papers don't contain enough housing and husbandry detail.
- This makes it hard to get a consensus from the community.
- The ARRIVE guidelines, and associated Explanation and Elaboration document can help remind us what to include in publications



RECOMMENDED SET

15. Housing and husbandry

15 Provide details of housing and husbandry conditions, including any environmental enrichment.

Explanation | Examples

The environment determines the health and wellbeing of the animals and every aspect of it can potentially affect their behavioural and physiological responses, thereby affecting research outcomes [1]. Different studies may be sensitive to different environmental factors, and particular aspects of the environment necessary to report may depend on the type of study [2]. Examples of housing and husbandry conditions known to affect animal welfare and research outcomes are listed in the table below; consider reporting these elements and any other housing and husbandry conditions likely to influence the study outcomes.

Examples of information to consider when reporting housing and husbandry, and their effects on laboratory animals

Information to report	Examples of effects on laboratory animals
Cage/tank/housing system (type and dimensions)	Affects behaviour [3] and fear learning [4]. Tank colour affects stress in aquatic species [5,6].
Food and water (type, composition, supplier and access)	Affects body weight, tumour development, nephropathy severity [7], and the threshold for developing Parkinsonian symptoms [8]. Maternal diet affects offspring body weight [9].
Bedding and nesting material	Affects behavioural responses to stress [10] and pain [1
Temperature and humidity	Modifies tumour progression [12]. Regulates sexual differentiation in zebrafish [13].

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Sanitation (frequency of cage/tank water changes, material transferred, water quality)	Affects blood pressure, heart rate, behaviour [14]. Adds an additional source of variation [15,16].
Social environment (group size and composition/stocking density)	Compromises animal welfare [17]. Induces aggressive behaviour [18,19] and stress [6].
Biosecurity (level)	The microbiological status of animals causes variation in systemic disease parameters [20].
Lighting (type, schedule and intensity)	Modifies immune and stress responses [21].
Environmental enrichment	Reduces anxiety [22,23], stress [22,23] and abnormal repetitive behaviour [24-26]. Reduces susceptibility to epilepsy [27] and osteoarthritis [28] and modifies the pathology of neurological disorders [29]. Increases foraging behaviour in fish [30].
Sex of the experimenter	Affects physiological stress and pain behaviour [31].

Environment, either deprived or enriched, can affect a wide range of physiological and behavioural responses [32]. Specific details to report include, but are not limited to, structural enrichment (e.g. elevated surfaces, dividers), resources for species-typical activities (e.g. nesting material, shelters or gnawing sticks for rodents; plants or gravel for aquatic species), toys or other tools used to stimulate exploration, exercise (e.g. running wheel), and novelty. If no environmental enrichment was provided, this should be clearly stated with justification. Similarly, scientific justification needs to be reported for withholding food and water [33], and for singly housing animals [34,35].

If space is an issue, relevant housing and husbandry details can be provided in the form of a link to the information in a public repository, or as supplementary information.

References

Building our evidence base

Compiled by the NC3Rs, RSPCA and Institute of Animal Technology

www.nc3rs.org.uk/EEE

NC 3Rs

Assessing the welfare impact of enrichment – **the Evaluating Environmental Enrichment resource**

- An **introduction** to evaluating environmental enrichment.
- Advice on **choosing appropriate enrichment.**
- **Different approaches** to evaluation.
- Example **protocols.**
- Lots more useful **advice and information.**
- There is also a **webinar recording** available which showcases some of the resources available and provides practical guidance from a technician's perspective nc3rs.org.uk/webinar-evaluations-environmental-enrichment

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Thank you!

For more information

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