

Aberystwyth University

Voluntary code of ethics and best practice for research involving invertebrate animals not covered under the Animal Scientific Procedures Act 1986 (revised 2012).

At Aberystwyth University we recognise our moral and ethical responsibility to care and respect all research animals, even when there is no clear evidence that they are sentient or experience pain and suffering in the same way that higher, protected organisms (like vertebrates) do. We also recognise the necessity to conduct our research and teaching in the most rigorous, reproducible, and open way.**

Background

The use of animals in scientific research is regulated by UK law under the Animal Scientific Procedures Act (1986, amended in 2012, ASPA)*. In brief, all vertebrate animals (those with a backbone) and a few species of invertebrate animals (including squids, octopus and cuttlefish) are protected under ASPA and researchers must have special license to conduct scientific work with them. ASPA protects animals by minimising pain, suffering, distress or lasting harm that may be caused by scientific procedures and licenses are granted only where robust justification for the work is provided. All work must be recorded and reported to the UK Home Office inspectorate for regular monitoring and auditing. In contrast to vertebrate animals, the majority of invertebrate species are not protected under the ASPA and researchers are unrestricted and unregulated by law when using them in scientific research. There has been comparatively little work done to pain and suffering in invertebrates but there is some evidence that some species exhibit behavioural responses to noxious, stressful, or damaging stimuli that may indicate pain perception and avoidance (nociception). This has been demonstrated mostly in decapod crustaceans such as crabs and lobsters (1-5). There is published evidence that the lay public believe invertebrates should receive oversight if used for scientific experiments, and that proper consideration of this risks “social licence” to conduct scientific research on these animals (6).

Aberystwyth University’s guidelines for use of non-protected species in scientific research.

In order that AU staff and students conduct animal research, whether it is observational or experimental, in an ethical and scientifically robust way, we adhere to our voluntary code of best practice and ethics regarding our use of non-protected animal species. This is detailed below. We include here a list of the animals we routinely use in our research and teaching and outline methods we currently employ to anaesthetise and euthanise these animals.

The University mandates that any research involving the use of non-protected animals will comply with the equivalent animal welfare and ethical standards that apply to those under ASPA.

- 1) Researchers at Aberystwyth University must, wherever possible adopt procedures and techniques that avoid the use of animals in their research.
- 2) Animal housing and husbandry must be done in consultation with the relevant Named Animal Care and Welfare Officer (NACWO) to ensure highest possible standards are met for each given species.

*The Animals (Scientific Procedures) Act 1986 Amendment Regulations were approved in 2012. These Regulations amend the Animals (Scientific Procedures) Act 1986 to transpose the European Directive 2010/63/EU into UK law. The current UK Act is known as the Animals (Scientific Procedures) Act 1986 (as amended).

** As a general rule of thumb this document covers invertebrate species that possess a ganglionated, or organised nervous system, and/or cephalisation which are most likely to have capacity to feel pain and suffer from experimental insult.

- 3) In the case of research on non-protected invertebrates (i.e. not under Home Office License), this *must* be declared in full on the [Ethical Review System](#) for consideration by Aberystwyth University Animal Welfare and Ethical Review Body, AWERB (or designated authority under AWERB) against the **3Rs framework**; the 3Rs refer to principles aimed at reducing animal use and improving the welfare of animals in scientific research:
 - i. **Replacement**: Finding alternatives to animal testing wherever possible, such as *in vitro* or *in silico* studies.
 - ii. **Reduction**: Minimizing the number of animals used in experiments to the minimum necessary to obtain meaningful results, through improved experimental design, data analysis, and sharing of information.
 - iii. **Refinement**: Enhancing the welfare and care of animals involved in research, by improving living conditions, handling, and procedures to minimize pain, distress, and suffering, i.e. appropriate anaesthesia, analgesia and humane end points should be used to minimize any pain and suffering.
- 4) Researchers planning to work with animals need to be aware of their responsibilities and ensure they have appropriate training.
- 5) Even though the use of non-protected invertebrates is not governed by ASPA, AU researchers must demonstrate best practice and refer to and follow guidance from relevant professional bodies and societies e.g.,
 - i) Association for the Study of Animal Behavior: <https://www.asab.org/ethics>
 - ii) Royal Society of Biology: <https://www.rsb.org.uk/policy/policy-issues/biomedical-sciences/animal-research>
 - iii) UKRI: <https://www.ukri.org/manage-your-award/good-research-resource-hub/use-of-animals-in-research/>
 - iv) American Veterinary Medical Association (AMVA). <https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf>
- 6) Researchers will submit an application for Use of Non-Protected Animals in Research form which considers aspects of animal welfare, experimental design and staff training and expertise.

NB The experimenter or research advisor (supervisor or principal investigator) should ensure all legal requirements for the use, transportation and/or importation and keeping (where appropriate) are satisfied.

Further guidance

The following text is a guide for AU researchers. It refers to additional relevant literature that should be consulted when designing experiments to ensure animal welfare and sound experimental practice is built into our work and that adhere to the principles of the 3R's that underpin the use of vertebrate species (Reduction, Replacement and Refinement):

Given the enormous biological diversity in invertebrate species and the variety of scientific questions addressed for which these animals are used, a comprehensive, species-specific list of procedures for anaesthesia and humane killing is unfeasible and will also depend on the specific needs of the experimenter (for example, tissues to be sampled and the downstream processing of those samples). Therefore, AU staff must consult with existing literature and learned body guidelines for their study model for best practice. A sample of relevant sources is provided below and detailed under bullet point 5(i-iii) above. When designing research with non-protected species and prior to submission to AWERB for ethical consideration, we refer staff to the guidance paper published by the Association for the Study of Animal Behaviour (ASAB) (ref 7 and link above in bullet 5i) for detailed guidance on the ethical use of animals in scientific research, which includes reference to non-protected invertebrates and cites relevant literature and sources of information. In all cases, our staff must prioritise animal welfare and reduction of lasting harm or suffering and make a considered cost-benefits analysis of the experiment against the harm to the invertebrate animal. For example, in accord with the ASPA guidelines, and where possible, power analysis and rigorous experimental design should be done to ensure sufficient, but minimum numbers of animals are used to yield statistically reliable outcomes. Staff are directed also to

<https://eda.nc3rs.org.uk/experimental-design> for advice and help with experimental design.

Experimenters should consider where procedures might be refined to reduce potential stress, harm and suffering and replacement (e.g. the use of non-living tissue samples, *in silico* analyses etc).

Staff must ensure that all animals used are recorded with sufficient metadata detail for periodic reporting in accordance with the [Concordat for Openness on Animal Research](#) for which Aberystwyth is a signatory. For example, Experimenter ID, Animal species, date, numbers used, procedure, anaesthesia and analgesia as appropriate, sex, developmental stage, follow-up observations and end-point (euthanasia/release).

Further information

As at August 2023 the following invertebrate (non-protected) species utilised in our research at AU include:

- Flies (*Drosophila melanogaster* and *Lipoptena* sp. *Stomoxys calcitrans*, *Musca domestica*, *Calliphora* spp., *Lucilia* spp)
- Crickets (*Gryllus bimaculata*)
- Butterflies (Lepidoptera)
- Locusts (*Schistocerca gregaria* and *Schistocerca migratoria*)
- Amphipod crustaceans (*Gammarus zaddachi*, *Gammarus pulex*, *Gammarus duebeni*, *Dikergammarus villosus*, *Talitrus saltator* and *Parhyale hawaiiensis*)
- Isopod crustaceans (*Eurydice pulchra*)
- Decapod crustaceans (*Carcinus maenas*, *Homarus gammarus*, *Palaemon serratus*, *Pagurus bernhardus*).
- Beetles and spiders (Coleoptera and Araneae- various species caught in natural situations using pit-fall traps).
- Bumble bees (*Bombus* sp.)

Anaesthesia protocol† (for guidance only- please check literature for species specifics)

Species	Anaesthesia	Euthansia (or humane killing)
Flies	'Fly-nap' (triethylamine) or gaseous CO ₂	Prolonged chilling (>1h)*, or decapitation
Crickets, locusts and lepidoptera	Chilling‡ (5 mins, or as appropriate for species)	Prolonged chilling (>1h)*, or decapitation for dissection
Amphipods	Carbonated water and chilling‡, or clove oil (0.5ml L ⁻¹); MS222 at 0.5–1.0 g L ⁻¹	Chilling (to frozen)* or decapitation for dissection
Decapods (crabs, lobsters)	MgCl ₂ , chilling‡, MS222 (0.5 g L ⁻¹), or 5% EtOH	Decerebration, prolonged chilling (>2h)*, injection of absolute ethanol.
Insects in pitfall traps	N/A	Drowning in 50% ethylene glycol
Nematode and Trematode worms	N/A	Prolonged chilling (>1h)*
Bees	Chilling‡ or gaseous CO ₂	Prolonged chilling (>1h)*

† The following resources identify relaxants (anaesthetics) and killing agents for aquatic invertebrates, including suggested doses:

<http://comm.archive.mbl.edu/BiologicalBulletin/ANESCOMP/AnesComp-Intro.html>

<https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf>

Anaesthetic and Sedative Techniques for Aquatic Animals Edited by Lindsay G. Ross and Barbara Ross © 2008 L.G. Ross and B. Ross, ISBN: 978-1-4051-4938-9

‡Chilling should be done on ice or at 4°C as appropriate for species and ideally in combination with other chemical agents (inhaled or dissolved)

*Prolonged chilling for killing should be done at -20°C or lower and after chemical anaesthesia.

NB- All solutions used should be made in appropriate media for the species being used (e.g. seawater for marine species etc.)

References

1. Elwood RW. Assessing the Potential for Pain in Crustaceans and Other Invertebrates. In: Carere C, Mather J, editors. The Welfare of

1. Elwood, R. W. Assessing the Potential for Pain in Crustaceans and Other Invertebrates. In: Carere C, Mather J, editors. The Welfare of Invertebrate Animals. Animal Welfare, Vol. 18. Cham: Springer (2019). doi: [10.1007/978-3-030-13947-6_7](https://doi.org/10.1007/978-3-030-13947-6_7)

2. Elwood, R. W. Pain and suffering in invertebrates? ILAR J. (2011) 52:175–84. doi: [10.1093/ilar.52.2.175](https://doi.org/10.1093/ilar.52.2.175)

3. Diggles, B. K. Review of some scientific issues related to crustacean welfare. ICES J Mar Sci. (2019) 76:66–81. doi: [10.1093/icesjms/fsy058](https://doi.org/10.1093/icesjms/fsy058)

4. Bergmann, M., Taylor, A. C., & Geoffrey Moore, P. Physiological stress in decapod crustaceans (*Munida rugosa* and *Liocarcinus depurator*) discarded in the Clyde *Nephrops* fishery. J Exp Mar Bio Ecol. (2001) 259:215–29. doi: [10.1016/S0022-0981(01)00231-3](https://doi.org/10.1016/S0022-0981(01)00231-3)

5. Chang, E. S. Stressed-out lobsters: crustacean hyperglycemic hormone and stress proteins. *Integr Comp Biol.* (2005) 45:43–50. doi: [10.1093/icb/45.1.43](https://doi.org/10.1093/icb/45.1.43)
6. Brunt, M.W., Kreiberg, H. & von Keyserlingk, M.A.G. Invertebrate research without ethical or regulatory oversight reduces public confidence and trust. *Humanit Soc Sci Commun* 9, 250 (2022). <https://doi.org/10.1057/s41599-022-01272-8>
7. Guidelines for the ethical treatment of nonhuman animals in behavioral research and teaching. *Animal Behaviour*, Volume 195 (2023), Pages I-XI. ISSN 0003-3472. doi: [10.1016/j.anbehav.2022.09.006](https://doi.org/10.1016/j.anbehav.2022.09.006).