Insect Welfare Research Society

Updated: January 2024

Guidelines for Protecting and Promoting Insect Welfare in Research

Background and Intended Use

These guidelines were established in 2023, and are updated annually, by the leadership of the Insect Welfare Research Society (IWRS). They are intended to provide guidance to individuals researching insects in laboratory, field, education, and industry contexts. The guidelines are informed by standard principles of animal care and use: the 3Rs (Russell and Burch 1959). The 3Rs encourage researchers to *replace* animals with non-animal models, to *reduce* the number of animals used in research, and to *refine* research, housing, and husbandry methods to minimize stress (Crump et al. 2023). In light of these principles, the guidelines provide strategies for protecting and promoting insect welfare.

Replacement is motivated by recognizing that research often involves compromising the welfare of individuals. Where it is possible to answer a research question without using live insects, such alternative methods are preferred. However, many research questions clearly require the use of insects and, if those questions are to be answered, reduction and refinement become relevant. Reduction involves minimizing the number of insects used for a given research aim (e.g., by using power analyses to determine the required sample size) and maximizing the amount of information gained from each individual (preventing the need for additional individuals). The IWRS offers a power analysis guide for researchers interested in learning statistical techniques to reduce animal use in their work (Perl 2023). Refinement involves minimizing stress vectors and providing beneficial conditions, which often requires some research into the natural history of the species in question. These guidelines focus on research that requires the use of live insects, and therefore emphasize the practical implications of reduction and refinement.

These guidelines provide principled suggestions about best practices, none of which are meant to replace the professional judgment of researchers about how to meet the needs and interests of individual animals. Any specific recommendations are tentative for two reasons. First, insect species and research contexts are both many and varied. So, while some general principles may hold across them, specific recommendations may not. Second, there are significant uncertainties about insect welfare, even given a particular species and research context. So, best practices will evolve as knowledge advances.

These guidelines focus on the welfare of individual insects. They are not, therefore, comprehensive guidelines for ethical research. For instance, they presuppose the importance of complying with applicable federal, state, and local laws and regulations, institutional policies, and international conventions. Likewise, they presuppose the importance of environmental responsibility, the integrity of scientific results, non-discrimination, and managing conflicts of interest. While such ethical issues are significant, they have been addressed capably elsewhere.

Insect Welfare Research Society

Updated: January 2024

Those seeking more information about these ethical issues should consult other community resources (such as the ESA Ethics Statement) and the appropriate authorities.

Questions about these guidelines should be directed to the IWRS (director@insectwelfare.com).

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Sampling

Researchers should try to reduce the number of insects sampled, maximize the amount of information obtained from specimens, use sampling methods that minimize bycatch, and use sampling methods that minimize the duration and severity of harm. This includes when sampling insects from maintained stock populations, as well as collecting insects from wild populations.

Researchers can reduce the number of insects sampled by, first, considering alternative ways of collecting comparable data (see Montero-Castaño et al. 2022 and Lövei and Ferrante 2024 for lists of non- or less-destructive methods). They can also perform statistical analyses to assess the required number of individuals needed for their experiments, adjusting their sampling and statistical methods and stock population maintenance accordingly (Perl 2023). Researchers can maximize the information obtained from samples by preparing to collect multiple kinds of data from each individual, using one individual (instead of multiple individuals) per data point where scientifically appropriate, and making all data (including, potentially, samples and bycatch themselves) and associated metadata freely/publicly available to other researchers, such as in online repositories (Cuff et al. 2023).

To minimize bycatch in collection, researchers can change the methods they use: they can switch from passive to active sampling strategies; they can adjust the size, location, and coloration of the traps; and they can take traps down more quickly (and more; Lövei and Ferrante 2024). Researchers can minimize the duration of stress during collection by using faster-acting methods for euthanasia (e.g., replacing ethyl acetate with potassium cyanide in field-deployed kill jars).

Transportation

Transportation can be stressful for insects, sometimes resulting in mortality. Accordingly, researchers should reduce the number of live insects transported (as sourced from breeders, farms, or the field) via the methods mentioned in 'Sampling' and by transporting eggs instead of live juveniles or adults where possible. In addition, researchers should minimize the general and species-specific stressors that transportation involves (e.g., by providing appropriate nutrition and hydration, selecting an appropriate density, or providing dark containers for photophobic insects).

Insect Welfare Research Society

Updated: January 2024

Housing

Most insects are non-domesticated animals that may find aspects of captivity stressful. Housing should be designed with that concern in mind. Insects should be housed in species-appropriate environments (to include, e.g., temperature, moisture, pH, photoperiods, spatial constraints, etc.) that promote physical health (e.g., cleanliness). Stock population density should be managed to promote health and avoid both injury and resource limitation. Insects should also be provided with environmental enrichments that allow for species-specific behaviors (e.g., nest construction, opportunities to hide from predators, etc.). Enrichment may include conspecific insects, where species are expected to benefit from intraspecific interaction. Researchers maintaining stock populations should use a management plan to ensure that adequately trained, responsible caretakers maintain appropriate housing conditions and densities in the population, with check-ins at regular intervals and suitable record-keeping to ensure that welfare-relevant trends can be identified (e.g., Free and Wolfensohn, 2023).

Creative housing design that minimizes disruption to insects when cleaning/managing their rearing containers is encouraged. For example, including a second room between the hive and flight-arena for lab-reared bumblebees provides a space for bees to dump their waste, which can be easily cleaned without disrupting the hive. Management can also occur during periods of natural inactivity (e.g., under red light conditions during the 'dark' part of the photoperiod for diurnal insects) to minimize stress.

Unfortunately, there may sometimes be tensions between various housing goals: for example, preferred humidity levels may also increase the risk of fungal disease. Accordingly, researchers must balance these objectives as best they can, using proxies like changes in activity levels, physical appearance, and mortality rates as evidence of inadequate housing arrangements.

Nutrition and Water

At a minimum, researchers should avoid food and water deprivation (where relevant), providing fresh and safe nutrition and water (e.g., free of toxins) at reasonable intervals for the species, according to their stock management plan. Ideally, food should be of a kind that members of the species prefer; changes in activity levels, physical appearance, and mortality rates as evidence of inadequate nutrition or hydration. For some species, this may be live prey. To minimize both stress on prey insects and the risk of injury for predators, it is preferable to euthanize prey insects prior to feeding when this does not affect predators' consumption. Either way, the care of prey insects should otherwise follow these guidelines.

Disease Management

Insects may experience both latent and acute disease outbreaks when reared in stock populations. Many insect diseases and their symptoms are poorly characterized; latent/chronic disease may be particularly difficult to detect as it may not result in mass mortality. Researchers should familiarize themselves with the literature on diseases known to affect their study organism (as well as species-specific disease prevention strategies) before beginning to rear them.

Researchers maintaining stock populations should be trained in maintaining appropriate hygiene standards—e.g., cleaning facilities regularly (while employing strategies to minimize stress from disturbance or handling), sterilizing equipment used with multiple insect stock populations, monitoring activity levels, physical appearance, and mortality rates for indicators of disease, etc. Additionally, researchers should consider providing an environment where insects can manage their own disease risk, when appropriate for their species—e.g., by providing space for infected individuals to avoid others, or by providing access to materials with prophylactic properties that reduce risks of infection (de Roode and Lefèvre, 2012).

If diseases are detected, it may sometimes be possible to pursue non-lethal management options that allow for recovery, such as providing appropriate abiotic conditions for inducing behavioral fever (de Roode and Lefèvre, 2012). However, recovery may not always be possible and researchers will often need to euthanize some or all members of the population to avoid spread or prolonged suffering. This decision involves balancing two goals: reducing the risk of harm due to disease and avoiding killing insects unnecessarily. Researchers will need to use their best judgment to determine the most appropriate course of action.

Invasive Methods

When possible, researchers should use non-invasive methods over invasive ones (e.g., using thermal imaging instead of a "grab and stab" method to measure insect body temperatures). When it is not possible to use non-invasive methods, researchers should consider, where appropriate, anesthetizing insects prior to such procedures and/or euthanizing them immediately afterward. When using invasive methods, extra care should be given to the planning of the experiment and the training of the experimenter, to avoid unnecessary delays, suffering, or death.

Anesthesia/Analgesia

Insects should be anesthetized before procedures that they could find aversive (e.g., dissection) and non-instantaneous sacrifice, whenever possible. *There are significant uncertainties about the best methods of anesthesia at this time and further research is needed*. The IWRS currently recommends cold or a suitable chemical agent (e.g., CO_2, isoflurane; MacMillan et al. 2017; Tucker et al. 2023). Gamma-aminobutyric acid B (GABA) receptor agonists are known analgesics for fruit flies when delivered via injection (Manev and Dimitrijevic 2004); recent research suggests NSAIDs could be further explored as analgesics, but there is less support as the mechanisms underpinning their action are currently unknown (Jang et al. 2023).

Providing GABA agonists in food or water sources will likely generate unreliable dosing and thus efficacy across individuals, but may be the only feasible option given the difficulty of injecting individual insects. Researchers should consider species-specific biology, such as cold or hypoxia tolerance, when choosing an anesthetic (Cooper 2011). Researchers should allow some buffer in the anesthetization procedure, increasing the probability that insects are fully anesthetized and not simply immobilized before beginning procedures. Extra care should be taken to train experimenters in anesthesia/analgesia delivery, to avoid unnecessary delays, suffering, or death.

Insect Welfare Research Society

Updated: January 2024

Release

When it is in the best interest of the individual insects and local populations, and complies with all relevant laws and institutional policies, insects should be released back into the wild rather than euthanized.

Euthanasia

Insects should be euthanized when they are subject to unmanageable and significant stressors. When inducing such a stressor is part of an experimental design, researchers should identify the earliest point when insects can be euthanized to avoid unnecessary harm.

Insects should be killed humanely (e.g., in a way that they do not find aversive). The best way to achieve this is to kill insects instantly (in less than a second) or while under the influence of an anesthetic like isoflurane (e.g., Tucker et al. 2023). However, as that is not always possible, other strategies must be used that are appropriate given the constraints imposed by species, life stage, and experimental design. The best option available may also vary based on context and the needs of the experiment; researchers must use their best judgment when deciding on a method of euthanasia for their study. *There are significant uncertainties about the best methods of euthanasia at this time and further research is needed*. The IWRS currently recommends:

- the injection of suitable chemical agents (e.g., potassium chloride; Bennie et al. 2012),
- freezing (rapid freezing in liquid nitrogen is preferred over slower cooling in air, though either method may be employed),
- immersion in 70% isopropyl alcohol *following* isoflurane anesthesia or 24 hours in isoflurane (Tucker et al. 2023),
- crushing (e.g., complete and instant crushing between hard surfaces) or grinding to a very small particle size (Barrett et al. 2023).

Researchers should consider species-specific biology, such as cold or hypoxia tolerance, when choosing a euthanasia method. When groups of insects are to be euthanized instead of a few individuals, batch-killing methods may be used to reduce handling time and associated stresses. However, as it can be more difficult to kill many insects instantaneously, anesthetic agents may be necessary to ensure a humane death for each individual. Extra care should be taken to train experimenters in each method of sacrifice to avoid unnecessary delays or suffering.

Disposal

While euthanized insects cannot be harmed themselves, they can harm other insects as disease vectors. Euthanized insects should be disposed of properly to avoid harm to stock or wild populations (e.g., incineration of diseased honey bees and comb; Mutinelli 2023). Facilities with separate disposal procedures for biohazardous material should employ them, even if not required by institutional policy.

Insect Welfare Research Society

Updated: January 2024

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