Insect Welfare Research Society

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Guidelines for Protecting and Promoting Decapod Crustacean Welfare in Research

Background and Intended Use

The decapod order contains ~15,000 species, including crabs, lobsters, crayfish, prawns, and shrimps. The idea that these animals might experience pain was long dismissed because they were thought to respond to noxious stimuli and tissue damage purely by nociceptive reflex—a position questioned by Sherwin (2001). Later experimental studies demonstrated that their responses were complex, long-lasting, and largely consistent with criteria for pain that had been produced initially for vertebrates (Elwood 2019). While the nature and extent of potential sentience in decapods remains an active area of research, these animals were recently recognized as sentient for the purposes of UK law, suggesting that the evidence for this conclusion is far from negligible (Crump et al. 2022).

The present guidelines were developed at the behest of the leadership of the Insect Welfare Research Society (IWRS) and modified from Fischer et al.'s (2023) guidelines on insect welfare in research. They are intended to help individuals studying decapod crustaceans in laboratory, field, education, and industry contexts. The guidelines are informed by standard principles of research animal care and use: the 3Rs (Russell and Burch 1959). The 3Rs encourage researchers to *replace* animals with non-animal models, *reduce* the number of animals used in research, and *refine* research, housing, and husbandry methods to minimize stress. Given these principles, the guidelines provide strategies for protecting and promoting decapod crustacean welfare.

Replacement is motivated by recognizing that research often involves compromising the welfare of individuals. Where a research question can be answered without using live decapods, such alternative methods are preferred. Replacement could involve using existing data sets, as well as *in vitro*, organoid, and *in silico* approaches.

However, many research questions clearly require decapod crustaceans and, if that knowledge is sought, reduction and refinement become relevant. Reduction involves minimizing the number of decapods used for a given research aim (e.g., by using power analyses to determine the required sample size for statistical validity; Perl 2023) and maximizing the amount of information gained from each individual (thus preventing excessive sampling). Refinement involves minimizing stress vectors and providing environmental conditions that are sensitive to the species' natural history. These guidelines focus on research that requires live decapod crustaceans. They emphasize practical methods for reducing the number of animals used as well as refining research and husbandry practices.

The guidelines provide principled suggestions about best practices, none of which replace the professional judgment of researchers or technicians about how to meet the needs and interests of individual animals. Specific recommendations are tentative for two reasons. First, decapod species and research contexts are many and varied. While some general principles hold across them, specific recommendations may not. Second, there are significant uncertainties about decapod welfare and stress. Best practice will, therefore, evolve as knowledge advances.

These guidelines focus on the welfare of individual decapod crustaceans. They are not, therefore, comprehensive guidelines for ethical research. For instance, they presuppose compliance with federal, state, and local laws and regulations, institutional policies, and international conventions. Likewise, they presuppose environmental responsibility, the integrity of scientific results and academic conduct, non-discrimination, and managing conflicts of interest. While such ethical issues are important, they have been addressed elsewhere (e.g., in standard RCR training, typically available to all university-affiliated researchers). Those seeking more information about these ethical issues should consult other community resources and the appropriate authorities.

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

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Sampling and Experimental Design

Researchers should try to reduce the number of decapod crustaceans sampled, maximize the amount of information obtained from specimens, and use sampling methods that minimize bycatch and the duration of harm. This applies both when sampling decapods from maintained stock populations and when collecting them from wild populations.

Researchers can reduce the number of decapod crustaceans sampled by, first, considering alternative ways of collecting comparable data (e.g., technological advances such as eDNA; Garlapati et al. 2019). Researchers can maximize the information obtained from samples by collecting multiple kinds of data from each individual, using one individual (instead of multiple individuals) per datum point where scientifically appropriate, and making all data (including, potentially, samples and bycatch themselves) and associated metadata freely available to other researchers (e.g., in online repositories; Percie du Sert et al. 2020).

To minimize bycatch, researchers can modify the methods they use: they can switch from passive to active sampling strategies; they can adjust the size or location of traps/pots, trawls, or nets; and they can check sampling equipment more frequently or use remote monitoring devices where appropriate.

In line with best practice for terrestrial traps, and particularly in the tidal zone, traps and nets should be checked at least once per day, although we realize this is not always possible due to practical constraints. Where dead specimens are necessary, either by project design or local laws, researchers can minimize the duration of stress during collection by using faster-acting methods for killing (see "Killing" section). When catching wild decapods, any harmful effects on the local target population or habitat should also be considered.

Transportation

Transport can be stressful for decapods (Powell et al. 2017), sometimes resulting in injury and mortality (Conneely and Coates, 2024). Accordingly, researchers should consider the methods mentioned in 'Sampling' to reduce the number of live animals collected and transported (as sourced from breeders, aquaculture, or the field). In addition, researchers should minimize the general and species-specific stressors that transportation involves (e.g., by providing an appropriate temperature, aeration, and hydration, selecting an appropriate holding density, and transporting photophobic species in dark conditions).

Gills must be kept wet for maintaining oxygen (O_2) supply and waste excretion – some species are more vulnerable (e.g., velvet swimming crabs) to emersion than others (e.g., lobsters). Equally, transport in a small volume of water can lead to rapid DO depletion. Where a large water volume is not practical for short collection trips on rocky shores, animals should be kept wet (e.g. by placing them under wet seaweed) rather than immersed in a small volume of seawater. This is especially true of intertidal species that naturally experience daily emersion and seek refuge under wet seaweed.

As many decapods are aggressive and can injure or attempt to cannibalize each other, transport should be designed to minimize this. For example, placing individuals in sealed tubes or open cups or providing cover such as seaweed. Newly molted (soft) individuals and gravid females are in a weakened state and are particularly vulnerable. This creates a presumption against transporting them, using them in experiments, or confining them with crustaceans with hard exoskeletons.

For longer periods of transport, or where individual housing is impossible, rubber bands may be appropriate to restrain the claws of larger decapods to reduce aggressive encounters. Alternatively, decapods can be sedated (Spoors, et al. 2023) where this would not interfere with experimental practice.

Handling

When handling decapod crustaceans, researchers may wish to restrain claws with a rubber band to prevent injury to both themselves and the decapods. Handling methods differ depending on the species and overall size, but a general rule is to never carry by tails, legs or claws due to the risk of carapace damage or autotomy (casting off a body-part).

Most decapods (excluding Brachyura and many Anomura) should be held by the cephalothorax, ensuring eyes are not covered. Thumb and fingers should touch the lateral edge of the carapace. For large individuals (>1.5kg), additional support can be provided by cradling the ventral surface. This reduces the risk of dropping the individual, limb damage or autotomy, as well as minimizing the risk of injury to the handler. Nephropidae, in particular, flip their tails to escape; so, a firm grip is required to prevent escape or injury.

Hold Brachyura (true crabs) by the midline of the cephalothorax, ensuring thumb and fingers are facing the eyes, and firmly holding both the dorsal and ventral surfaces. This method works well for both small and large crabs and prevents crabs from pinching the handler, though banding the claws may still be appropriate. Another technique for smaller crabs is to hold them by the carapace, with the thumb and index or middle finger at each edge of the widest part of the carapace.

Anomurans (e.g., hermit crabs, squat lobsters, porcelain crabs) have various body forms, ranging from crab-like through to typical elongate decapod forms where the abdomen is free of the cephalothorax. They are often small and delicate; so, they must be handled carefully to avoid damage. This is especially true of hermit crabs, which are weakly calcified, and special care is needed when handling out-of-shell individuals to avoid damaging the membranous abdomen.

Emersion

For aquatic species, emersion (air exposure) is extremely stressful (Bergmann et al. 2001; Simonik & Henry, 2014) and should be minimized as much as possible. Intertidal species manage emersion challenges by finding shelter in microhabitats that mitigate changes in the physical conditions (Simonik & Henry, 2014). In line with this, decapods that must be out of their tanks for extended periods should be misted or covered with wet newspaper, wet macroalgae, or similar items to prevent dehydration, thermal stress, and hypoxia.

Housing and Water Quality

Most decapod crustaceans are non-domesticated animals that may find aspects of captivity stressful. Housing should be designed with this in mind. Decapods should be kept in species-appropriate environments (considering, e.g., temperature, pH, photoperiods, shelter, spatial constraints) that promote physical health (e.g., cleanliness and hygiene). Stock population density should be managed to promote health and avoid stress, injury, and resource limitation. Decapod crustaceans should also be given environmental enrichment that allows for species-specific behaviors (e.g., shelters, tank substrate that allows burrowing, or seaweed as refugia). Enrichment may include conspecifics, where species are expected to benefit from intraspecific interaction (although some species are likely to engage in competition and aggressive behavior, which could harm welfare). Researchers maintaining stock populations should use a management plan to ensure that adequately trained, responsible caretakers maintain appropriate housing conditions and densities in laboratory populations, with check-ins at regular intervals and suitable record-keeping to identify welfare-relevant trends.

A particular consideration for decapod crustaceans is water quality. Most decapods are aquatic and require either seawater or freshwater, although some can cope with brackish water. Water quality management is key to successful maintenance. Low levels of ammonia are important, but nitrites and nitrates should also be monitored (Romano & Zeng 2013). Calcium is important to consider for freshwater species but seawater usually has enough for marine decapods. Bioavailability of dissolved oxygen decreases as salinity and temperature rise and low oxygen is also caused by bacterial decomposition of uneaten food. Further, high temperatures (i.e., higher than season-specific temperatures typical of the natural environment) should be avoided (Elwood & Ingle in press).

Strategies for maintaining optimal water quality depend on the type of aquarium system. A key consideration is adequate water flow rate or volume for a given biomass of housed animals. Stock-maintenance systems can be of open flow-through and run-to-waste designs, semi-open recirculating designs, or closed systems based on tanks containing an essentially static body of water. In closed systems, constant aeration is essential and filtration pumps of appropriate capacity for the water volume are also recommended. Without filtration, regular water changes are required. Even when filter systems are used, regular partial water changes may be necessary when routine testing shows low water quality. Note that aeration via 'air-stones' causes substantial loss of water via aerosol spray from air bubbles bursting at the water surface. This leads to reduced volume and, in the case of seawater, increased salinity, making regular monitoring required with partial water changes or top-ups as needed. While constant aeration and filtration should be present in stock tanks and during long-term experiments (e.g., in mesocosms), these may be impractical during shorter periods of behavioral observation. Observation containers are usually small relative to stock tanks, facilitating cleaning and complete water changes between observations to maintain adequate water quality.

We encourage creative housing design that minimizes disruption when cleaning/managing decapods' environments. Management can also occur during periods of natural inactivity to minimize stress. Various designs for specific species and stages of development are described in Elwood & Ingle (in press). An often-overlooked requirement is an escape-proof lid, as many decapods are remarkably good at climbing out. Lids will also prevent aerosol spray from aeration where 'air stones' are used, preventing cross-contamination between tanks and reducing corrosion to laboratory equipment.

Many decapod crustaceans are covered with mechanoreceptors that are sensitive to noise (see Tidau & Briffa 2016), so housing should attempt to mitigate facility noise and vibration, which may induce stress (Powell et al. 2017, Aimon et al. 2021; Conneely and Coates, 2024). Noisy environments (e.g., from air and water pumps, filtration systems, and maintenance machinery) significantly reduce growth and reproduction rates and may increase aggression (cannibalism) and mortality (Regnault & Lagardere, 1983). Mitigation strategies, such as noise dampening insulation under the tanks, may be appropriate. Similarly, crustaceans are light sensitive, and major deviations from their usual light regime can disrupt circadian rhythms, leading to abnormal behavior and affecting resting metabolic rate (Velasque et al. 2023). Therefore, natural light-dark cycles should be mimicked for laboratory populations.

There are sometimes tensions between various housing goals. Researchers must use their best judgment to balance these objectives as best they can.

Nutrition

At a minimum, researchers should avoid food deprivation (where relevant), providing fresh and safe food sources at reasonable intervals for the species according to their stock management plan. Ideally, food supplies should mimic the species' wild diet. Changes in decapods' activity levels or behavior, physical appearance, and mortality rates can indicate inadequate nutrition (see Elwood & Ingle (in press) for further comments on feeding). *Ad libitum* feeding regimes provide constant food but require close monitoring as decomposing food can reduce water quality. The nutrition plan should be designed with this in mind.

Some species may require live prey. To minimize both stress on prey decapods and the risk of injury for predators, it is preferable to kill prey decapods before offering them as food when this does not affect predators' consumption. Either way, the care of prey decapods should otherwise follow these guidelines.

Molt and Cannibalism

Captive animals may undergo ecdysis (molting of the exoskeleton). This poses two problems. First, it requires massive energy expenditure and may fatally exacerbate stress. Second, the newly molted animal is vulnerable to disease and attack by conspecifics, which tend to be cannibalistic. Animals should be closely monitored for signs of impending molt and separated from other individuals until they are no longer vulnerable.

Disease Management

When reared in stock populations, decapod crustaceans may experience both latent and acute disease outbreaks. Many decapod diseases and their clinical signs are not fully characterized (Rowley, Coates & Whitten, 2022); latent/chronic/idiopathic disease may be particularly difficult to detect as it might not cause mass mortality, or alterations in individual behavior and physical appearance. Researchers should familiarize themselves with the literature on diseases that affect their study organism (as well as species-specific disease prevention strategies) before rearing 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 decapod stock populations, as well as appropriate monitoring of disease indicators (e.g., activity levels, physical appearance, and mortality rates).

If diseases are detected, non-lethal management options may be possible (see Elwood & Ingle (in press) for examples). However, recovery may not always be possible and researchers will often need to kill some or all 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 decapods unnecessarily. Researchers will need to use their best judgment to determine the most appropriate course.

Invasive Methods

Invasive methods are often—though not always—more stressful for animals than non-invasive methods. So, given the importance of minimizing stress, researchers should consider using non-invasive methods over invasive ones (e.g., measuring decapod body temperatures using thermal imaging instead of needle temperature probes). When it is impossible to use non-invasive methods, researchers should consider, where appropriate, anesthetizing decapod crustaceans before invasive procedures (see discussion below) and/or killing them immediately afterward where the procedure is expected to have ongoing negative welfare impacts. When using invasive methods, extra care should be given to planning the experiment and experimenter training to avoid unnecessary delays, suffering, or death (e.g., cleaning areas with ethanol before an injection).

Anesthesia/Analgesia

Whenever possible, decapod crustaceans should be anesthetized before highly aversive procedures (e.g., dissection) and non-instantaneous killing. *There are significant uncertainties about the best methods of anesthesia at this time and further research is needed*. In particular, it is important to ensure that methods of anesthesia are causing loss of consciousness and not merely 'waking' immobilization, where the animal may still be able to experience pain and suffering.

There are some promising methods of chemical anesthesia for decapods. In particular, eugenol and isoeugenol products (e.g., clove oil and its derivatives and AQUI-S) temporarily immobilize a range of decapods (see reviews in de Souza Valente 2022, Rotllant et al. 2023, Spoors et al. 2023), though induction takes time and it is still uncertain whether there is true anesthesia or merely temporary paralysis. Recent work in European lobsters found a reduced heart rate commensurate with dosage, indicating true anesthesia (Khan et al, unpublished). There are currently no established methods of analgesia for use in decapods, though the local anesthetic lidocaine has been successful in shrimp (Taylor et al. 2004, Diarte-Plata et al. 2012).

Methods for administering eugenol and isoeugenol-based anesthetics vary, though the least physically invasive method is administration in a water bath (aqueous route). Appropriate concentrations will depend on species, individual mass, environmental conditions (e.g., temperature and salinity) and required time to insensibility.

Researchers should allow some buffer in the anesthetization procedure before beginning procedures, increasing the probability that decapods are fully anesthetized and not simply immobilized. Insensibility can be identified using physiological measures, such as heart rate, or behavioral responses, such as loss of mouthpart, pereiopod response to stimulation, or righting behavior. Extra care should be taken to train experimenters in anesthesia/analgesia delivery to avoid unnecessary delays, suffering, or death.

Release

When it is in the best interest of the individual decapod and local populations and complies with all relevant laws and institutional policies (e.g., invasive species), wild-caught decapods should be released near their capture site rather than killed. If release is appropriate, researchers should minimize the duration that individual decapods are kept.

Killing

Decapod crustaceans should be killed when they are subject to extreme stressors with which they do not have the capacity to cope. When an experiment requires inducing such a stressor, researchers should identify the earliest point when decapods can be killed to avoid unnecessary harm (i.e., a "humane endpoint"; see Morton 2000).

Decapod crustaceans should be killed humanely. The best way to achieve this is to kill them instantly (in less than a second) or while they are under anesthetic. However, when that is not possible, alternative strategies must be used that are appropriate given the constraints imposed by species, life stage, and experimental design. The best option may also vary based on context and the needs of the experiment; researchers must use their best judgment when deciding on a method of killing for their study. *There are significant uncertainties about the best methods of killing and further research is needed*. Details of various methods and the welfare implications can be found in Conte et al. (2021).

The IWRS currently recommends either terminal overdose of anesthetic or electrical stunning at species-appropriate voltage and duration. These can then be followed by spiking or instantaneous maceration to ensure loss of life. Mechanical dispatch (e.g. 'double spiking' crabs or 'splitting' lobsters), even when performed by competent trained operators, can still take several seconds to complete and is recommended to take place under anesthesia. Chilling, boiling, and freshwater immersion are highly likely to cause suffering (unless performed under anesthesia) and are not recommended.

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

Disposal

After death, the remains of decapod crustaceans can harm other decapods as disease reservoirs. Dead decapods should be disposed of properly to avoid harm to stock or wild populations. Facilities with separate disposal procedures for biohazardous material should employ them as a matter of good practice even if not required by institutional policy.

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