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The welfare of invertebrate animals in research: Can science's next generation improve their lot?

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Abstract

Invertebrates have a long history of use in scientific research but there has been little concern for their welfare until very recently. Unlike vertebrate research animals, whose uses are closely regulated, invertebrate animals are minimally protected. In some countries regulations extend to a few species, but the vast majority of invertebrate animals can be used in research with no oversight, protections or legal regulation. Whether this is cause for concern depends on the ability of invertebrate animals to experience pain, suffering or distress as a result of husbandry and experimental procedures. To date there is minimal evidence that invertebrate animals are capable of experiencing such affective states, but this is largely due to very little experimental effort devoted to testing such hypotheses. In this article I review current regulatory guidelines for use of invertebrate animals and their relevance to species of varying neural and behavioural complexity. I define some simple steps that postdoctoral and other young researchers can take to improve the welfare of research animals within their own laboratories, and provide a framework for contributing more broadly to the field of welfare-based research by publishing their observations when improvements have had a positive effect on their animals and their research.

Introduction

Almost all researchers who use experimental animals will encounter questions at some point in their careers regarding the ethics of animal research. For researchers working with invertebrate animals these questions can be difficult to answer; the welfare of invertebrate animals has received comparably little study, and there are few if any regulations on their use (Harvey-Clark, 2011). But this is beginning to change, and within the past decade concerted efforts have been made to extend regulatory protection to invertebrate animals (see below). It is likely that within the coming years legislative efforts will expand both their geographic and phylogenetic reach, ultimately regulating research on multiple species of invertebrates in most countries in which scientific research occurs.

Young scientists - postdocs, graduate students and junior technicians – form the bulk of the hands-on workers in most laboratories. It is these researchers who perform most of the day-to-day tasks involving research animals, provide training to new lab members, and who will eventually become leaders of their own research programs and laboratories, within which they will preside over the use of their own research animals. Post-doctoral researchers are often the most senior personnel involved day-to-day in research, and we are therefore well placed to direct, influence, and ultimately improve the way we utilize research animals.

How can postdoctoral researchers and other young scientists contribute to the effort to improve welfare of invertebrates used in scientific research? In this article I define a dual approach that includes efforts both at the individual level to change and improve their own laboratories' practices, and in the larger arena of welfare-based research, identifying which invertebrates have the capacity to suffer pain or distress, how this can be minimized, and how regulations governing the use of these species might be best formulated.

Invertebrates in scientific research
Invertebrates have a long history of scientific use. The most numerous invertebrates in modern laboratories are certainly *Drosophila melanogaster*, an arthropod, and *Caenorhabditis elegans*, a nematode, but many other species contained in most invertebrate phyla are represented to lesser degrees in a wide variety of scientific enterprises (Wilson-Sanders, 2011).

Along with Drosophila and *C. elegans*, which are used widely, developmental biologists utilize many insects, molluscs, turnicates and echinoderms. Neurobiologists work extensively with gastropods and cephalopods (molluscs), but also insects, spiders and crustaceans (arthropods), worms (annelids) and flatworms (planarians). Immunologists utilize the self-recognition systems of colonial invertebrates such as ascidians (chordates) and corals and anemones (cnidarians) to understand fundamental mechanisms of immune functions, and study parasitism and infectious disease processes using insects, marine shrimp (arthropods) and roundworms (nematodes). Biomedical studies using drosophila and *C. elegans* have yielded important insights into diseases of addiction, cancer and genetic diseases. Sensory biologists use many invertebrate species in behavioural and physiological studies, including crickets, lobsters, crabs, flies and cockroaches (arthropods), and octopuses, cuttlefish, squid and snails (molluscs). Ethologists use many insect species, including honeybees, ants, roaches and moths, along with crustaceans and, to a lesser degree, molluscs such as gastropods and cephalopods (Wilson-Sanders, 2011).

Among these species (and the many not included among the examples above) there is a wide range of neural and behavioural complexity, and for those animals with very small, primitive or loosely organised nervous systems (e.g. cnidarians, planarians, nematodes), there is minimal concern about welfare. Animals with more complex sensory abilities and larger nervous systems deserve more extensive consideration; complexity and size of the nervous system is probably the most appropriate measure by which we define those invertebrate animals on which efforts to improve welfare should focus (Mather, 2011).

The taxa that generate the most concern therefore are the arthropods (e.g. crustaceans, arachnids, insects, etc.), and molluscs (e.g., snails, slugs and cephalopods), because these invertebrates have typically the most complex brains and behaviors (although there is considerable variations among them). My own experimental efforts are confined within these two phyla, so many of the examples given in this article come from experience with crustaceans and cephalopods, but I would nonetheless urge researchers to consider welfare beyond these two phyla alone.

The current regulatory environment
Welfare of research animals is not a new concern; the first act of a parliament governing the rightful uses of animals in scientific experimentation was passed in 1876 (the UK Cruelty to Animals Act), and since then many countries have enacted their own legislation that seeks to protect animals used in research from unreasonable harm, distress and pain. But in each case the term ‘animal’ is either implicitly or explicitly defined as a vertebrate animal (and in some cases, only subsets thereof). The idea that invertebrate animals may also deserve similar consideration is a rather newer concept.

Vertebrate animals used in scientific research are protected by strict regulation governing their housing, handling, breeding and all experimental procedures, including analgesia and euthanasia (for example, the Animal Welfare Act, USA, 1966). The primary ethos underpinning such regulation is that vertebrate animals have similar (although not necessarily identical) capacity to feel distress and pain that we have as humans, and therefore for our research to be ethically acceptable, we must conduct our studies in such a way that minimizes these negative experiences (Allen, 2004; Carbone, 2004; Kaliste, 2004; Baumans, 2005).

The use of invertebrate animals, by contrast, is still very minimally regulated. Only recently have there been efforts in a few countries to extend some of the same protections given to vertebrate animals to some invertebrates, but these cover only a few species, and in some cases are grounded on minimal knowledge of the animals' physiology. This general exclusion of invertebrate animals from legal protection is predicated on the assumption that invertebrates, being in the majority simpler, behaviourally and neuroantomically, than vertebrates, cannot experience pain or distress (Eisemann et al., 1984; Fiorito, 1986; Kavaliers, 1988; Mather, 2001; Sømme, 2005) and therefore our relatively minimal concern for them is justified (Kellert, 1993). This same assumption had previously been applied to various 'lower' vertebrates – reptiles, fish and amphibians. For each of these, careful research has provided evidence that these omissions were unwarranted (Stevens, 1988; Sneddon et al., 2003; Sneddon, 2004; Ross and Ross, 2008; Mosley, 2011), and legislation has generally been amended to include them (e.g., the US Public Health Service's Policy on Humane Care and Use of Laboratory Animals has been amended to include both adult and larval amphibians and fish). As we begin to consider whether invertebrates too encompass capacity to experience emotive and affective states, this same slow change will almost certainly proceed further into the invertebrate realm.

Despite scarce experimental evidence currently for affective states, necessary to enable the attachment of emotive value onto physical sensations, in any invertebrate animal (Mason, 2011; Mendl et al., 2011) , restrictions on the use of selected species are already in place in the UK (Animals (Scientific Procedures) Act, 1993), Canada (Canadian Council on Animal Care (199) and in the EU (Directive 2010/63/EU, 2010). The UK law requires approval only for the use of the common octopus, *Octopus vulgaris.* Canada's CCAC expressly differentiates between 'invertebrates', which are placed in the tier of lowest concern for invasive procedures, and 'cephalopods and other higher invertebrates' which may be included in the higher tiers, but does not specify what these 'higher invertebrates' might be. The EU's new directive, implemented in 2012, includes all cephalopod molluscs but no other invertebrates, despite originally also considering crustaceans. Although there is no published evidence to date that cephalopod molluscs are more capable of experiencing negative affective states than any other invertebrate, or that their emotive capacity is similar to that of vertebrate animals', their large brains and complex behavioural repertories are used as supporting evidence for this possibility (Mather, 2001, 2011). However, the lack of solid evidence underlying the current guidelines must be addressed in the near future if we expect regulations to be functional in the long term.

What is important when considering welfare of invertebrates?

Welfare considerations for invertebrates in research are largely similar to those for vertebrate animals, although there is often far less information readily available to inform those concerns. Concerns about appropriate housing, feeding, social interactions and handling are valid, but by far the most common concern is that of pain, distress and suffering during procedures.

Nociception or pain?

Most animals having a nervous system can detect noxious or damaging stimuli, and will make avoidance or withdrawal motions when stimulated. This is nociception - a capacity to react to tissue damage or impending damage with activation of sensory and motor pathways, with or without conscious awareness. Activity in nociceptive sensory pathways usually results in reflexive behavioral responses and may or may not also produce other, higher-order responses. Reflexive withdrawal responses tend to be mediated by very simple sensorimotor circuits optimized for speed and reliability and can occur without input from higher processing centers, although more complex escape and avoidance behaviors involve more complex neural circuits (Walters, 1994; Chase, 2002). Even in humans the initial reflexive response to a noxious stimulus is sometimes faster than can be consciously perceived, and nociceptors can sometimes be activated without conscious sensation (Adriaensen et al., 1980). Invertebrates that lack appropriate higher processing centers in their central brains may be capable of only this rapid, unconscious processing.

The definition of pain widely accepted by scientific investigators is “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (Merskey and Bogduk, 1994). The emotional component required by this definition of pain makes its identification in other species, and especially in species quite different from humans, extremely difficult, if not impossible. Emotion is usually defined in terms of conscious experience (e.g., Izard, 2009), and while evidence of consciousness in some animals is available, proof of consciousness is not (Allen, 2004). It is therefore important to distinguish between nociception as detection of a noxious stimulus (which can be recognized scientifically by unambiguous behavioral and neural responses), and pain as the unpleasant feeling associated with that stimulus (and inferred by behavioral and neural responses of uncertain relation to consciousness). Pain requires neural circuitry that incorporates additional functions, some of which might entail highly complex processing by very large numbers of neurons. Whether any invertebrate species might meet these minimal criteria for experiencing the emotive aspect of pain is debatable, and there have been only a few studies aimed at testing this directly (Elwood and Appel, 2009; Elwood, 2011).

Nociception and nociceptive sensory neurons have been described in many invertebrate species (Smith and Lewin, 2009) and it is likely that they occur in many others, but no invertebrate has been shown to experience pain. Because nociception does not necessarily involve any negative ‘feeling’ about the sensation, and is by definition not aversive, alone it need not be grounds for concern. However, for any animal, including invertebrate animals, with brains even plausibly capable of attaching emotive value to nociceptive experiences (i.e., can suffer and feel pain), preventing activation of nociceptive pathways during experimental procedures should be paramount.

Improving welfare of research invertebrates: A two-pronged approach for young investigators

In the remainder of this article I describe a two-pronged approach for young investigators aiming to improve the welfare of invertebrate animals used in research. The first involves efforts at the local level to make changes to husbandry and experimental procedures for animals in our direct care, and the second encompasses wider efforts in welfare-focused research.

In the laboratory

For postdocs and other young investigators, changing an existing laboratory culture can be rather difficult. However, there are a number of relatively small and easy steps a researcher can take to make a substantial difference to the lives and deaths of their research animals. There are two primary aspects of welfare within the laboratory environment – husbandry and experimental procedures – both of which provide scope for welfare-related improvements.

Husbandry

The first step to improving husbandry procedures (such as housing and feeding) is to understand the biology of the species you are using. The natural behaviours and habits of the animals should form the basis for husbandry regimens in the lab. Assembling a checklist of simple questions about current husbandry procedures is a useful means of identifying potential deficiencies, and I provide some general suggestions here; species-specific lists should be developed wherever possible.

Is the housing arrangement appropriate?

Housing arrangements must balance comfort of animals against convenience for experimenters; completely naturalistic housing is rarely appropriate or feasible for experimental animals. However, some considerations for naturalistic habitat and abiotic features for each species can be incorporated into artificial housing.

If your animals are primarily nocturnal or prefer low light, is the standard flourescent laboratory lighting too strong for them? A simple shield around the housing area might be all that is needed to approximate more ecologically relevant conditions. Aside from a correct day:night cycle, which is usually in place to retain appropriate circadian rhythms, other aspects of lighting could be considered. If animals are startled by sudden bright light, the sudden lights-on in the morning might be improved by installing a dimmer switch or timer that increases light levels slowly. Aquatic species, such as freshwater crayfish and molluscs, may find bright lighting stressful or aversive (Cook and Carew, 1986; Barr and Elwood, 2011), a concern when we consider that chronically stressed animals may yield quite different experimental results to those without such stressors. Other lighting aspects such as spectrum, intensity and providing light-sheltered areas may be worthwhile to consider.

Stocking density of holding spaces should be based upon reasonable encounter rates in natural environments. Animals that school, swarm or form social groups should be permitted to maintain similar structure if at all possible, with this need balanced against the need to individually identify members of a group; if marking or labeling involves a stressful or potentially painful procedure, on balance individual housing may be more appropriate.

Are your animals normally solitary or territorial? If so, group housing may be inappropriate even though it might be more convenient. For example, octopuses can be highly aggressive towards conspecifics both in the wild and in captivity (Bradley, 1974; Hanlon and Forsythe, 1985; Huffard et al., 2010). Housing multiple octopuses in a closed system is unavoidable for labs without access to running seawater, thus maintaining olfactory isolation is all but impossible. In the case where water must be recirculated, including visual if not olfactory barriers between animals may still significantly enhance welfare and reduce stress. In octopuses stress levels can be monitored noninvasively to assess the efficacy of changed housing conditions (Malham et al., 2002; Larson and Anderson, 2010). Alternatively, group-tolerant cuttlefish may be a better alternative. For crustaceans that form loose dominance hierarchies based on resource holding potential (Gherardi and Daniels, 2003; Fero et al., 2007; Irvin and Williams, 2009), providing more shelters than individuals in group housing tanks can reduce stress and injury associated with territorial conflicts.

Promoting natural feeding behaviours

Feeding methods also provide opportunities for enrichment and otherwise improved welfare outcomes. For animals that are normally predatory, is offering live food an option? If live food cannot be offered such that welfare of both predator and prey species is not compromised unreasonably (non-lethal injury of either, for example, is rare) or regular consumption rates cannot be assured, is there another way of proving naturalistic foraging opportunity to the animals? For cephalopods trained to accept dead prey, frozen food can be suspended in water currents to provide additional visual and olfactory stimulation, which may enhance consumption rates (Hanley et al., 1998; Domingues et al., 2003). For terrestrial species, food that is buried or otherwise concealed behind barriers and must be sought actively can simulate naturalistic foraging behaviours. For grazing herbivores (e.g., locusts, marine and terrestrial snails), consider replacing or supplementing dried or artificial diets with live plant material (Hinks and Erlandson, 1994; Capo et al., 2009), which most likely better represents the nutritional profile the animal requires (although extreme care should be taken to avoid foods contaminated with pesticides or other chemicals). For all species, a varied diet is almost certainly better than a homogenous one, and any opportunity to stimulate naturalistic foraging behaviours should be considered as worthwhile.

Experimental procedures

Even more so than changing husbandry procedures, making changes to experimental procedures that have in the past yielded good results can be challenging, even though improving animal welfare should not adversely affect experimental outcomes. Experimental procedures here are divided into two phases; restraint (involving handling, noninvasive examinations and noninvasive marking) and intervention (covering invasive procedures, surgery, anesthesia, analgesia, and euthanasia).

Restraint

Restraint techniques should always aim to protect both the experimenter and subject. Many invertebrates have defensive weapons, some of which present genuine dangers to experimenters. Toxic and venomous animals should be handled with extreme care, but appropriate restraint that reduce stress on the animal can also prevent deployment of defenses, improving outcomes for everybody involved.

Procedures involving handling and immobility of invertebrates should be optimised to be as brief as possible, but balanced against causing minimal disturbance and distress to the animal. Direct handling, although often faster, may be more stressful to the animal than secondary containment (allowing the animal to enter a small, sealable transfer container, then moving animal and container together). This technique is useful for arthropods such as stinging insects, spiders and scorpions, venomous molluscs such as blue-ringed octopuses, stinging cnidarians, or any animal where acclimation to handling is unfeasible or presents a considerable risk to experimenters (Dombrowski and De Voe, 2007; Bennie et al., 2011; Harvey-Clark, 2011).

During transfer from one environment to another, shielding the animal from unnecessary visual and tactile disturbance should be a priority, even if the animal has limited sensory capacities in some modalities. Cover transfer containers if possible, use careful handling to avoid jostling your animals about, and if animals are prone to aggressive behaviours when stressed (crayfish and squid, for example, will attack familiar conspecifics in transfer tanks when they had previously cohabited in housing without concern), always transfer animals individually rather than in groups.

Intervention

Intervention is considered any procedure that is invasive (from injections to major surgery), and experimental procedures where the animal performs a task that is not part of its normal activity. Probably the most pressing and controversial issue related to intervention is pain: How can we tell if the animal experiences pain, and how can it be prevented? Because invertebrate animals are phylogenetically, morphologically and behaviourally far removed from ourselves, these are difficult questions to answer.

Anesthesia, analgesia and euthanasia

Many of the same anesthetic agents appropriate for invertebrates are also effective for euthanasia, although this is not universally true. Analgesia is a more difficult topic, both because it is difficult to assess whether it might be necessary (does the animal feel pain or is capable only of nociceptive reflexes?), and efficacy in animals where pain is a valid concern is equally difficult to discern. Maintenance analgesia is rarely employed for invertebrates, and analgesia during procedures is typically accomplished by either local or general anesthetics (Cooper, 2001, 2011).

Methods for euthanasia should be appropriate for each animal's physiology. The primary aim of any euthanasia method is to kill the animal rapidly and without undue distress, while keeping tissue in a state that is suitable for use in the given experiment (AVMA, 2007). In some cases where unaltered physiology is particularly important, anesthetic agents are not desirable and a physical method of euthanasia is preferred. In all cases ensuring death has occurred prior to sample collection should be paramount. Many techniques that are reliable in vertebrate species are ineffective in invertebrates, so a means of confirming death should be established.

A good example for improved euthanasia methods can be found in the use of the squid *Loligo pealei* for studies on the giant axon. The giant axon and synapse have yielded many fundamental insights into the functioning of the nervous system, and are a common model for studying action potential dynamics (Cole and Curtis, 1939; Hodgkin and Huxley, 1939, 1952; Hodgkin and Katz, 1949a, 1949b; Takeuchi and Takeuchi, 1962). The axon and its synapse are located in the mantle of the animal, so the chosen method of killing is decapitation, removing the head at the point where it joins the mantle. This method is fast, requires minimal training and does not require an anaesthetic agent that might disrupt the physiology of the nervous system. However, anyone who has witnessed this preparation will be struck by the continued, co-ordinated movements of the head-end of the squid. The arms continue to move in sequence, are responsive to touch and the mantle-less animal will crawl about. The animal appears to still be conscious; while animal bodies will make reflexive movements even after brain death, the coordinated nature of the squid movements and the attempts to escape suggest perhaps something more. The brain of the animal is in the head, of course, and is not damaged by decapitation. In contrast to vertebrates, there is also a large tissue volume above the neck, containing approximately 25% of the animals' total blood volume. The hemostatic mechanism in molluscs is muscle contraction (Spurling, 1981; Krontiris-Litowitz et al., 1989), meaning that immediate contraction at the decapitation site closes the wound: these factors suggest that the squid's brain may be functional for minutes after decapitation.

Of course, we do not know whether the animal suffers during this death; there is no evidence that cephalopods feel pain, despite their brains being highly complex (Crook and Walters, 2011; Crook et al., 2011). Nonetheless, this method of killing can be greatly improved with very little additional time and effort. Simply decerebrating (using a scalpel blade to make 4-6 slices through the cranium, slicing the brain into multiple small pieces) immediately after decapitating ensures that the brain is immediately dead, rather than allowing slow hypoxia to achieve brain death. Death is clear from the immediately relaxation of the chromatophores, giving a uniform pale appearance, and the cessation of arm movement. This simple procedural change, which takes no more than an extra 10 seconds, dramatically alters the death experience for squid used in such experiments.

Because physical euthanasia typically requires close handling and experimenters must be trained to ensure good technique, chemical euthanasia methods are preferable if they do not interfere with the intended use of the animal tissue. For aquatic species, bath immersion is a noninvasive method for delivering drugs, while inhalants or injectables are typical for terrestrial species (Cooper, 1980, 2011). Whatever the method used, animals should be monitored throughout the procedure and signs of distress should indicate changes to the technique may be necessary.

Isoflurane is a highly effective volatile anesthetic that is used on vertebrates and terrestrial invertebrates alike. It is typically fast acting and readily reversible, and is used commonly for anesthesia of terrestrial arthropods such as spiders (Cooper, 2011). However, its appropriate use requires specialized equipment and training, and its efficacy in humans after accidental exposure is well documented (Langley et al., 1995; Wenker, 1998; Epp and Waldner, 2012). Carbon dioxide gas is effective for euthanasia (but not analgesia) in most terrestrial invertebrates (Cooper, 2011), and is a cheap and effective means of euthanizing multiple animals simultaneously.

Cold euthanasia (freezing) is a decreasingly common method of anesthetizing or killing invertebrates, as it has several notable drawbacks. Cold may not produce antinociception in invertebrates, and is thus inappropriate for surgical procedures (Zachariah, 2011). Primary euthanasia by freezing can make ascertaining death extremely difficult, as poikilothermic animals can withstand low temperatures by entering torpid or static states that are difficult to distinguish from death. Additionally, many vertebrate animals show nociceptive responses to extreme cold, and homologous ion channels that transduce noxious cold are also found in some invertebrates, suggesting that cold may also be noxious for invertebrates (Vriens et al., 2004). A primary goal of euthanasia should be to avoid sustained activation of nociceptive circuitry, thus cold anesthesia/euthanasia may not meet this criterion.

Irrespective of the technique used, ensuring death has occurred within a reasonable timeframe can be difficult. The use of typical endpoint indicators such as cessation of respiration, absence of reflexive responses to strong stimulation, or cessation of heartbeat may not be accurate indicators of death. A secondary method of euthanasia should be used in ambiguous cases. Once animals are insensible (not just immobile; selecting the correct anesthetic agent is vital to ensure anesthesia and not only paralysis), extensive tissue harvest, decerebration by pithing, removal of the brain or injection of chemicals directly to ganglia (Battison et al., 2000; Bennie et al., 2012), rapid freezing or immersion into fixative or ethanol are all acceptable methods for ensuring animals will not revive spontaneously (Cooper, 2011).

Studies on invertebrate animal welfare; informing regulation, disseminating the message.

In addition to efforts made directly at the level of individual laboratories, postdoctoral researchers and other young scientists can aim to improve animal welfare by making it a focus of their own studies. There is relatively little literature on welfare of invertebrate animal in research, and plenty of scope for additions to it with careful study. However, in a funding-poor environment (where many of us find ourselves), extending or changing our research focus away from funded areas is risky, and the funding available for welfare-based research on invertebrate animals is minimal. Despite these limitations, if you have made improvements in your lab practices or if improved welfare has improved your experimental outcomes, publishing your observations could be of considerable personal and scientific benefit – a brief communication to a welfare-focused journal, a poster at a conference, or simply mentioning your findings in your talks are all relatively low-investment ways of disseminating your own findings to other researchers.

If you have invested time and effort into learning about the naturalistic behaviours of your lab animals, publishing an ethogram of normal and aberrant behaviours observed under different housing or experimental conditions would be a useful contribution, as such information can be difficult to find. If during characterization of behaviour or physiology you find evidence for nociception, affective states or something perhaps indicative of pain, a controlled anesthesia or analgesia test might yield information valuable both for your own research but also for the broader field of animal welfare.

In talking with well-established investigators using invertebrate models, I find that there is often an extensive base of unpublished observations on behavior and physiology, which may not be of particular relevance to their major research question but is nonetheless informative for others focused specifically on the wellbeing of research animals. While there is great value in improving the lives and deaths of the animals in our direct care, a far greater effect could be achieved by sharing our scattered observations through publication in peer-reviewed journals. I find most post-doctoral researchers are quite strongly motivated by opportunities for additional publications, so this is a mutualistic goal.

For researchers whose experimental species is either already, or likely to be, regulated, establishing a productive, open relationship with your institutional vets and animal welfare committee members should be a priority. If there are not established guidelines in place already for husbandry or procedures, find out if they have voluntary standards or are willing to work with you on establishing them. If regulations are likely to be imposed upon you in future, having a good working relationship with your veterinary staff and animal welfare committees will be advantageous; in these cases it is the researchers who are typically far more familiar with the biology of their particular species than committee members and veterinary staff who may have very minimal previous exposure to invertebrate animals. Being included in discussions during initial efforts to develop guidelines will almost certainly mean a better outcome for researchers and animals alike.

Finally, for those of us working with invertebrates in currently unregulated research environments (in the US, for example), taking an active role in future discussion of national regulation or legislation will be vital. Be proactive about establishing your message and position – which animals should be protected and on what grounds your opinion is based, what form protections should take, and if there are animals or procedures you feel confident should not be restricted (non-invasive or transiently invasive procedures such as injections, or all procedures for animals whose nervous systems are clearly too simple to experience suffering or distress, for example), be willing to advocate for their exclusion, too.

A word about activist organisations

Scientific knowledge on invertebrate animal welfare should not be shared only with other scientists. Animal rights and activist groups seeking a scientific view on issues of concern to them occasionally contact researchers working on aspects of welfare (although these are most commonly about commercial harvest and food preparation rather than animal research). The relationship between activists and scientists is typically, and often justifiably, characterized as adversarial, but I nonetheless encourage colleagues to engage these queries if they can provide an empirically supported view on issues of welfare. While such interactions can be frustrating, they should be considered as important as interactions with other scientists. Activists have a powerful and well-defined public presence, and their role in shaping past policies on animal welfare is unquestioned. In any effort to develop regulations of invertebrates in research, activist organisations will almost certainly have a role. However, certain organisations do target scientists using animals (and sometimes aim deliberately at graduate students and post-doctoral researchers), thus it is essential to confer with your direct supervisors and the public information office at your institution before responding to any such requests.

Conclusions

In the coming years it is likely that at least some invertebrate species in all major research countries will be included in legislation protecting animals used in scientific research. In Europe, Britain and Canada these changes are either already established or will take effect in the coming several years. While the appetite for legislation in the US is probably likely to remain low, US researchers seeking to publish in journals based in these regulated nations may find themselves asked to comply with rules from these nations for their work to appear in these journals. Thus restrictions on invertebrate animal research are likely to be felt broadly, even in countries without their own, domestic legislation.

If we permit that some regulation is largely inevitable, it is young investigators at the start of their careers who therefore have the most incentive to ensure that such regulation is reasonable; well grounded scientifically and appropriate to the biology of each species. Young scientists should thus make a concerted, organized effort to establish their positions on what constitutes acceptable practice. If we ignore the opportunities we have now, to make changes to our own lab practices and to conduct research to support our positions prior to further restrictions being enacted, we are at risk of being governed by restrictions implemented without scientific grounding.

This is the real 'danger' of regulation of invertebrate use – that without a body of evidence that we ourselves have gathered, any new legislation will be poorly informed, with little relation to our animals' biology and physiology. The science of invertebrate animal welfare is one that that all young investigators should consider as worthy and important, and only our continued non-engagement with these issues will ensure that we find ourselves bound by regulations within which we cannot work effectively. Therefore I hope to see many more publications from post-docs, graduate students and young faculty in the coming years on these important topics.

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