Animal protection legislation for scientific purposes and the non-inclusion of invertebrates – a bioethical analysis

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Abstract

Animal welfare legislation in Western countries follows similar principles, differing mainly in complexity and scope. The legislation basically protects vertebrates, since it considers them capable of having subjective experiences such as pain and suffering. Studies utilizing physiological, neuroanatomical and behavioral parameters indicate that invertebrates like cephalopods and decapods are sentient beings, and thus eligible for legal protection in some countries. We suggest an approach to sentience that uses basic requirements, not restricted to vertebrates. Social and economic factors as well as species seem to influence the non-attribution of sentience to invertebrates. Thus, it is evident the need for a bioethical analysis to substantiate their inclusion in the legislation. The "benefit of doubt" is supported by the arguments of "evolutionary continuity" and "principle of precaution". In the mean time we suggest that the use of invertebrates for scientific purposes should be cautious and sensible.

Key words: Invertebrates. Legislation. Sentience. Animal experimentation. Specism.

Resumo

Legislação de proteção animal para fins científicos e a não inclusão dos invertebrados – análise bioética

A legislação que regulamenta o uso de animais para fins científicos nos países ocidentais segue princípios semelhantes, diferindo apenas em complexidade e extensão. Em geral, a lei protege apenas os vertebrados por considerá-los dotados da capacidade de ter experiências subjetivas como dor e sofrimento. Estudos utilizando parâmetros fisiológicos, neuroanatômicos e comportamentais evidenciam a senciência em invertebrados como cefalópodes e decápodes, dando-lhes o direito de proteção legal em alguns países. Sugerimos uma abordagem de senciência com requisitos elementares, não restritos apenas aos vertebrados. Fatores socioeconômicos e o especismo parecem influenciar a não atribuição de senciência aos invertebrados. Portanto, fica evidente a necessidade de uma análise bioética para fundamentar a inclusão desses animais na legislação. O "benefício da dúvida" é corroborado pelos argumentos da "continuidade evolutiva" e do "princípio da precaução". Nesse ínterim sugerimos que a utilização dos invertebrados para fins científicos seja criteriosa e responsável.

Palavras-chave: Invertebrados. Legislação. Senciência. Experimentação animal. Especismo.

Resumen

La legislación de protección animal para fines científicos y la no inclusión de los invertebrados – un análisis bioético

La legislación reguladora del uso de animales para fines científicos en países occidentales sigue principios similares, distinguiéndose en complejidad y extensión. En general, la ley protege apenas a los vertebrados, considerándolos dotados de capacidad de tener experiencias subjetivas como dolor y sufrimiento. Estudios utilizando parámetros fisiológicos, neuroanatómicos y comportamentales demuestran sintiencia en invertebrados como cefalópodos y decápodos, dándoles el derecho de protección legal en algunos países. Sugerimos un enfoque de sintiencia con requisitos elementales, que no se restrinjan únicamente a vertebrados. Factores socio-económicos y el especisismo parecen influir en la no atribución de sintiencia a invertebrados. Así, se hace evidente la necesidad de un análisis bioético para fundamentar la inclusión de esos animales en la legislación. El "beneficio de la duda" es corroborado por los argumentos de "continuidad evolutiva" y "principio de precaución". En ese ínterin, sugerimos que la utilización de los invertebrados para fines científicos sea criteriosa y responsable.

Palabras-clave: Invertebrados. Legislación. Sintinencia. Experimentación animal. Especisismo.

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Animals are used for scientific purposes since Antiquity. However, since the eighteenth century, the morality of this practice has been in question [1]. The controversy generated by the dilemma between the advancement of biomedical sciences and damage to animals has persisted until nowadays.

There is disagreement among different philosophical reflections on the relationship between humans and animals, notably as regards the obligations and the recognition of their potential rights. There are organizations that resort to violent attitudes and practices against those who work with animal experiments [2], but they are isolated cases and, in contrast, there is an increasing interaction between the scientific community and civil society to respect the sensitivity of animals used in research [3].

From the combined efforts of politicians, scientists, philosophers, activist organizations, keepers and others involved in animal experimentation, many countries have developed a legal system of animal protection. The comprehensiveness of the inclusion of groups of animals protected by law has been expanded gradually – as described in the following sections of this article.

Although vertebrates – such as mice, rabbits, among others – are the most studied in biomedical research, animals with lower visibility, such as the invertebrates are also equally used for scientific purposes since the nineteenth century.

In Western countries, the animal protection legislation primarily aims at the protection of vertebrate animals considered sentient due to ability to feel pain and experience suffering. The lack of evidence of sentience in invertebrate animals excludes them from the scope of protection.

In this article we will analyze three possible factors to explain the non-inclusion of invertebrates in animal protection legislation: no sentience, political and economic interests, and speciesism.

Legislation that protects vertebrate animals in science

The first legislation aiming to regulate the use of animals for scientific purposes was established in England in 1876, as an amendment to Against Animal Cruelty Act, dated of 1835. Later, many countries have created specific legislation to that effect.

Laws regulating the use of animals for scientific purposes in Western countries have been subject to successive changes, showing progress in animal care and establishing specific guidelines for the use of these organisms in research and teaching.

The animal protection legislation in different countries follows the basic principles that underlie the use of animals in research. The difference lies in the degree of complexity and detail, in particular with respect to its implementation and execution [4].

In the European Union (EU) legislation is based on the balance between scientific progress and animal welfare. These two aspects were important for the review that resulted in Directive 2010/63/EU, which reinforce the standards of animal welfare in comparison with the earlier Directive (86/609/EEC). One of the main objectives of the new policy was to harmonize the standards of animal welfare and conditions for research among the member states.

A guideline adopted by the EU requires transposition into national law of each member state; this process currently underway is carefully monitored and followed up with a view to developing a common understanding [5].

In North America, the main regulatory system of animal testing is not Federal law. The United States of America (USA) have an extensive system of institutions and institutional committees committed to establishing guidelines for use and care, appointed Institutional Animal Care and Use Committees (IACUC). In Canada, this activity works by a system of self-regulation by the Canadian Council on Animal Care (CCAC), whose mission is to formulate guidelines for animal experiments and control research facilities. In each research center, a Committee on Animal Care (ACC) is responsible for the evaluation of animal experiments [6].

In Australia, a similar system of animal ethics committees was implemented and established under state law, but operating under the code of conduct proposed by the National Health and Medical Research Council [4].

In Brazil, the first animal protection legislation was created in 1934 (Decree 24.645/34) [7] and marks the prohibition of inflicting physical ill-treatment of animals during the execution of teaching methodologies. In 1941, Decree Law 3,688 predicted punishment for those who, even for educational and scientific purposes, perform in public place or exposed to audience painful or cruel experiments on live animals [8]. Though these decrees were concerned about the care of animals, yet they were very large and were not intended to regulate the use of animals in experimental studies.

Only in 1979, the first Vivisection Act (6.638/79) emerges in Brazil [9]. In 1988, with the promulgation of the Federal Constitution, discussions about animal protection were generated again. In that year the National Health Council (CNS) has established standards for animal research under preclinical medical research context (Resolution 1/88) [10]. In 1996, to replace 1988 Resolution, CNS has adopted Resolution 196/96 [11], which establishes guidelines and rules for research involving humans.

Ten years later the Environmental Crimes Law (9.605/98) was enacted, according to which *painful or cruel experiments on living animals, even for educational or scientific purposes, are considered crimes when there are alternative resources* [12].

Nowadays, Arouca Law is in force in Brazil [13], which regulates animal testing, repealing the Vivisection Act of 1979. This law regulates the creation and use of animals for teaching and research, and provides for the establishment of the National Council for the Control of Animal Experimentation (CONCEA) and the formation of the Ethics Committee on Animal Use (Ceua).

Oftentimes, the institutional committees of the countries that have legislation to protect animals use methods of self-regulation involving scientists with expertise in the area and veterinary staff. In some cases, these committees have greater representation, also including non-technical staff of the institution and representatives of civil society activists for animal welfare [4].

With regard to the implementation of the work involving animals, the law is generally implemented through review of research projects applying the premise of so-called 3Rs: replacement of live and conscious animals by any scientific method employing material without sensitivity; reduction of the number of animals used to the minimum necessary to obtain information from a sample accurately, and refinement of procedures applied to animals, in order to minimize their suffering [14].

However, the Brazilian legislation, as in the other countries mentioned, defines as *animal* in way that excludes invertebrates: their first rule defined that *animal is every irrational being, quadruped or biped, domestic or wild, excluding the harmful ones* [7].

Current legislation defines as "animal" only individuals of the phylum Chordata, subphylum Vertebrata. *It is understood by: Phylum Chordata: animals that have as unique features, at least in the early stage, the presence of notochord, gill slits in the pharynx and single dorsal nerve tube; Subphylum Vertebrata: chordate animals that have as unique features a large brain enclosed in a skull and a backbone*... [13].

Invertebrates in research

The invertebrates

The phylum Chordata, which is known as vertebrates, corresponds to the minority species of animals described, with approximately five thousand species, approximately 5% of all known fauna. The remaining 34 phyla of the Animal Kingdom comprise more than a million species and they are characterized as "invertebrates" [15].

Although the phylogenetic systematics has progressed in the classification of animals, the dichotomous terminology based on the presence/absence of vertebrae in the classification of animals with vertebrae is still used: "vertebrates"; with no vertebrae, they are called as "invertebrates".

This classification goes back to Aristotle's categorization, which separated the animals according to the similarity with humans: "animals with blood and "animals without blood" [16], and it remains in effect in the school system both in the subjects of the courses in biological sciences and related as in textbooks.

However, the classification does not reflect the natural order of kinship among species, which is considered artificial by phylogenetic systematics, given that, as a unique feature, present in a single phylum, is used to separate the whole animal kingdom. This same logic could be used to separate groups of animals with larger number of species from that ones with fewer species, such as "arthropods" and "non-arthropods", since Arthropoda group has 85% of all known animals.

The artificiality of the concept of "invertebrates" is evident by the high heterogeneity of forms and habits of animals, and it is not possible to establish a single common feature among all. Annually, approximately 10-13 thousand new animal species are described worldwide (in its greatest part invertebrates), with the caveat that there may still be 10 to 30 to 100 to 200 million species to be discovered [15].

Based on this imprecise definition of animal, laws protect only 5% of the fauna. The remaining 34 phyla collected in the group of invertebrates are not listed, except for a few species. Coincidentally, the invertebrate species with evidence of sentience and which are protected by law in some countries are part of two major groups of animals: phylum Ar-

thropoda (1,097,289 species) and phylum Mollusca (93,195 species) [15].

Scientific use

Wilson-Sanders [17], by analyzing the electronic database PubMed, identified the increasing use of invertebrate animals in experimentation. From 1800 to 1900 only three studies used experimentally such animals; already in the period 2008-2010, 44,000 works were identified. Another indicator is the Nobel Prize. According to the author, 18 between 74 awards were intended for researchers whose animal models included invertebrates.

The species most commonly used in biomedical research are the fruit fly (*Drosophila melanogaster*) and the nematode worm (*Caenorhabditis elegans*). The fruit fly is one of the most widely studied animals, with particular contribution to the development of research in Cytogenetics – genetics of human diseases. As for the nematode worm, its body structure is relatively simple, facilitating the development of biological and biomedical studies.

Although they are structurally simple, these invertebrates have molecular and cellular mechanisms similar to those of human beings, allowing the study of many diseases [17]. They also stand out for having been the first organisms to have the genome fully described [18].

Recently, other invertebrates such as snails and insects are being studied for the understanding of more complex animals, including human beings. Indeed, these animals are being used in various biological models, such as in developmental biology, cell biology, immunology, learning and behavior, muscle skeletal disorders, neural and neuromuscular system and its diseases, among others [4,17].

Legislation and invertebrates

The European Commission, in considering the possible need to expand the scope of animals to be protected by law, invited the European Food Safety Authority to produce an opinion on the revision of Directive 86/609/EEC on the protection of animals used for experimental or scientific purposes.

This opinion was adopted from the procedure described by the Scientific Board of Health and Welfare, which was entitled "Question about the sentience of invertebrate species, embryonic and fetal forms of both vertebrates and invertebrates" [19]. The board recommended the protection of two groups of invertebrates: Cephalopoda (squid and octopus etc.) and Decapoda (lobster, shrimp, crabs etc.). However, in the final version of the Directive 2010/63/EU only cephalopods (squid and octopus etc.) are included.

Prior to the inclusion of some invertebrates by the EU, some species groups of cephalopods and decapods were already protected by national legislation in countries such as the UK and the Scandinavian countries such as Australia and New Zealand [19]. In Canada, there is stratification between lower invertebrates, which require no concern in scientific procedures, and higher invertebrates, which should receive special care when used in research. In this last category cephalopods and decapods are included, among other species not clearly mentioned [6].

Why not including invertebrates in the legislation?

The sentience issue

Over the years, the concept of sentience has changed and now many studies believe that animals are provided with emotional states capable of experiencing positive and negative experiences [20-23]. The Cambridge Declaration on Consciousness (2012), dedicated to animal consciousness, states: *The absence of a neocortex does not seem to prevent an organism from experiencing affective states. Convergent evidence suggests that non-human animals have neuroanatomical, neurochemical and neurophysiological substrate of state of consciousness, along with the ability to display intentional behavior* [24].

The acceptance of animal sentience of vertebrates is in the process of consensus, unlike the sentience of invertebrates, which does not arouse sufficient interest yet. However, this debate is legitimate and not by chance Cambridge Declaration on Consciousness mentions evolutionary indication that some invertebrates, such as insects and mollusks, have neural circuits and behavioral/electrophysiological states of attention, sleep and decision.

Although there is almost consensus on the sentience of vertebrates, this concept is not well circumscribed or precise, whether of humans, vertebrates or invertebrates. The theme has considerable gaps for science and the main reason is because sentience involves idiosyncratic aspects inaccessible to the observer [25,26].

Accessing such intrinsic and subjective information in animals is a great challenge for neurobiology, since the argument for assigning sentience must be founded on evidence collected systematically [27].

Several definitions regarding animal sentience can be found in the literature, encompassing aspects from morphophysiological to cognitive-behavioral aspects.

The best-known argument advocating sentience as the basis for moral consideration of animals was given by the jurist Jeremy Bentham, who, in 1789, warned: *The question is they cannot reason, or even speak, but rather, can they suffer?* [28] For Bentham there is no difference if the animal has vertebrae or not, or if they have two or many legs; but only the possible ability to feel.

A behavioral variation of the concept is that an individual is sentient when they demonstrate some ability to evaluate the actions of others, of themselves and others; they are able to remember some of their actions and consequences; they are capable of assessing risks, have some feelings and have some degree of awareness [29]. In these different settings, sentience or consciousness of the bodies would be described in five levels: unconscious beings; beings capable of perception; beings capable of cognition; beings with the ability to assess and beings capable of performance [29].

The ability to perceive or feel is one of several kinds of consciousness. Sentience refers to the response of the central nervous system to activate the peripheral sensory system. Sentient is one that has its own life experience. Sentience is sometimes also called 'phenomenon of conscious awareness' [25].

According to Dawkins [21], sentience is an attribute of consciousness. It is an ability to adapt according to Darwinian terms, thus evolved from natural selection. In other words, consciousness evolves due to the advantage it gives to organisms that have it: experience subjectively makes the animal fitter to survive and reproduce than those who only act or react automatically without going through conscious subjective experiences. For this author, consciousness refers to broad spectrum of states in which there is immediate awareness of thought, memory or feelings.

Regarding cognition, it is the process by which animals perceive, process and store information. The author mentions three kinds of consciousness: 1.) phenomenal consciousness (experience of seeing, hearing, feeling pain etc.); 2) access consciousness (experience of being able to think about or report a mental state, both now and in the past) and; 3) awareness and self-monitoring (experience of thinking about their own actions and their effects and, if necessary, modify them).

One of the underlying reasons to different views on animal sentience may be related to different types of consciousness. Here, we classify the categories of Dawkins from the terminology of Sant'Ana-Magalhães [30], who calls "phenomenal consciousness" as sentience itself; "access consciousness" as cognition and "consciousness of self-awareness and monitoring" as self-consciousness.

From the three categories of consciousness proposed by Dawkins, the "phenomenal consciousness" is the most basic and relatively simple to be evaluated in animals. In this sense, we believe it is the best definition for sentience, because it can be assigned to any animal that has awareness of phenomena, presenting identity of oneself and the other, avoiding negative states and increasing positive states.

The other categories of consciousness are variations of this primordial consciousness that varies according to the specialization of each taxonomic group, but that does not determine whether or not an animal is sentient.

Given the diversity of species in the Animal Kingdom, it would be extensive task trying to categorize animals according to three types of consciousness described by Dawkins. However, it is necessary to standardize the concept of sentience to serve as a foundation for outlining the regulations for animal protection.

Methods to infer sentience

There's a new vision in which to promote animal welfare it is necessary not only to avoid suffering, but also provide them favorable conditions that may provide pleasure. As it is not possible to confirm directly that organisms are sentient, due to the subjectivity of their feelings, it is necessary to use indirect methods or indicators to assess how positive or negative is the feeling of the animal.

It has been suggested that states of suffering and pleasure are involved with the resolution, respectively, of "necessary conditions", such as reproduction, and "situations of opportunity", as the so-called games, for example. While it is most desirable and efficient to promote the increase of the positive experiences than negative [23], the main studies about animal welfare arise from analysis of negative emotions, such as stress level, which is considered a reliable indicator of well-being [20].

Duncan [20] believes that we gathered some information regarding the state of suffering, such as pain, fear, frustration and deprivation. From these negative experiences, the pain seems to be the sim-

plest and objective state, and it is the most widely tested. The argument for assigning sentience must be based on evidence collected systematically. However, it is not possible to use the main instrument used to access subjective experiences in humans, which is the verbal report. Determining different aspects of consciousness in non-verbal animals is therefore a challenge for neurobiology.

According to certain philosophical theories, consciousness is intimately intertwined with the ability of speech or thought, so that no animal would be endowed with sentience [31]. Meanwhile, cognitive biology disagrees with this view and says it is possible to take that other people are sentient based on the behavior and physical similarities to humans [25].

Due to the subjective nature, it is not possible to directly measure sentience, and the use of safe and sufficiently subtle methods are required to access as much as possible and indirectly the idiosyncratic states of the animals tested. The design of the methodology and choice of parameters to be evaluated to infer sentience are crucial for obtaining reliable and objective results, i.e., free of too subjective interpretation by the observer.

Dawkins [21] evokes 'traps' that can influence the results of the study of consciousness: use of wrong terminology to indicate mental state of the animal; use of comparison through analogies among animals of different taxonomic levels; presumption of consciousness in responses derived from purely innate mechanisms and with no requirement for learning; interpretation of automatic responses as being conscious; assumption that behavioral complexity implies cognition; assigning the capacity of cognition exclusively to complex organisms.

Wemelsfelder [22] postulates that behavior is the surest expression of the mental state of the animal, indicating that parameter as a tool that, when used properly, it can access the subjective aspects of individuals without incurring the trap of anthropocentrism. Due to the absence of language, especially in the case of invertebrates, the descriptive method without comparison is not widely used.

The most widely used tool to infer the sentience of these animals is the analogy, obtained by comparing the responses of the 'lower' (baseline) animals and the highest (derivatives) [32]. According to Elwood [33], the pain and the emotional and mental state that generate suffering are assigned only to those animals with skill learning and anticipation, able to avoid noxious stimuli. The presence of pain and suffering requires that the animal which is able to experience such sensations is endowed with complex morphofunctional systems.

However, animals with simplified systems would respond involuntarily to noxious stimulation, through mechanisms of nociception. This reflex response would allow the animal to escape the noxious stimulus, without establishing association or experiencing emotional and cognitive involvement [33].

It is certainly based on this premise that the animal protection legislation operates, protecting vertebrates under the argument that they can feel pain and are therefore sentient, whereas invertebrates are considered only possessors of unconscious mechanisms of nociception.

Evidence of sentience in invertebrates

Octopus, squid, crabs and hermit crabs are invertebrate animals that, thanks to recent studies, acquired the status of sentient beings, which is only the prerogative of vertebrates. The scientific evidence of the emotional states of this group of animals assures them legal protection in some countries. As follows, we highlight other groups of invertebrates poorly studied, but they also seem to have the same potential to sentience.

Sherwin [32], when he was questioning whether invertebrates may or may not suffer, mentions that popularly this broad category of animals is regarded as having reduced capacity to experience pain. Arguments used as justification are invertebrates that show simple forms of learning; have low memory capacity; do not show behavioral stimuli that indicate pain responses, and have physiological differences that exclude the possibility of experiencing suffering.

However, more detailed studies that examine these mechanisms indicate that the responses of invertebrates can be considered similar to those of vertebrates. Indeed, the analysis of invertebrates such as cockroaches, flies and snails shows evidence of sentience from observations that have the ability to show short and long term memory; memory capacity affected by age of the individual; complex spatial sense; capacity for social and associative learning; and behavioral and physiological responses that indicate pain [32].

Sherwin [32] has self-assessed his work by questioning whether observations obtained from the analogy method can be considered inaccurate and worthy of further assessment, if, indeed, we can consider that some invertebrates are capable of suf-

fering in a similar way (which does not mean identical) to vertebrates.

Studies on nociceptors and nociception models in different organisms show that they are similar from invertebrates to humans. Such similarities that cut across all phyla of the Animal Kingdom especially occur at the molecular level of ion channels involved in the detection of noxious stimuli [34]. The animals protect themselves from possible tissue damage through activation of the mechanisms of nociception; however, these are not directly related to the experience of pain, as previously seen.

Elwood [33] examined criteria that can distinguish nociception and pain in the phyla Cnidaria, Nematoda, Arthropoda and Mollusca from multiple parameters: presence of nerve receptors, presence of central nervous system, capacity of responsiveness, and response to opioid analgesics, physiological changes, learning to avoid noxious stimuli, motor responses protection, trade-off between avoiding stimuli and other activities, and cognitive ability of sentience.

Additionally, he describes that the investigation of the central nervous system provides limited clues about the potential of experiencing pain. The responses to opioids and other analgesics do not allow a clear discrimination between nociception and pain, but the behavior may provide more information in this regard. Finally, it is concluded that the available data are consistent with the idea of pain in some invertebrates, because they go beyond the idea of nociception [33].

The panel that prepared the documentation of sentience of invertebrates and fetal animal forms to the revision of the European legislation [19] established measures of invertebrate sentience through four research approaches: 1) cognitive ability; 2) number of brain cells; 3) nociception and pain; 4) evidence against non-sentience of invertebrates. The indicators used were: long-term memory, plasticity of behavior, complex learning and ability to feel pain.

Species had the following evidence of sentience: short and long term memory; complex learning, such as social learning; spatial awareness and cognitive maps; analyzes to gain reinforcement or avoid punishment; receptors sensitive to noxious stimuli linked by nerve pathways to a central nervous system and the centers of the brain; receptors for opioid substances; from analgesics they modify the response to stimuli that would be painful to humans; respond to stimuli that would be painful to humans functionally similar to the human response form, and they respond to resist and re-undergo painful procedure again [19].

Based on data collected systematically from 13 invertebrate groups provided by the scientific literature, the panel concluded that 60% of the analyzed animals responded to the requirements that infer sentience. This study distributed invertebrates tested in three categories:

Category 1 – There is scientific evidence that they are capable of feeling pain and distress: cephalopods and decapods;

Category 2 – There is no evidence that they can feel pain and distress: Hemichordata, terrestrial gastropods, Tectibranchia and Nudibranchia mollusks, non-social insects, Isopoda, and echinoderms, annelids, flatworms and nematodes;

Category 3 – There is some scientific evidence that they are capable of feeling pain and suffering, but not sufficient to evaluate the reasonable risk of their sentience or of their non-sentience: tunicates, social insects and spiders [19].

Regarding the complexity of the brain, it was found the existence of simpler nervous systems, if they are compared to those of vertebrates. The cerebral cortex, which in humans is probably where awareness is processed, is not present in these animals. But the absence of this structure does not necessarily mean that invertebrates do not feel pain. This is due, because the areas of nervous tissue of invertebrates possibly have evolved similar brain skills to the mammalian, which may also give these animals the ability to suffer [19].

Arguments that invertebrates do not have the ability to feel pain are based on two observations: 1) absence of similar behavioral responses of vertebrates and 2) lack of complex central nervous system. Both are inconclusive and do not prove the non-sentience of invertebrates, which indicates the need for studies in which the analyzed parameters are intrinsic to the groups in question, and do not take as comparison criteria only responses of different organisms such as vertebrates.

The first recommendation of the panel was to include some invertebrates (category 1) in the Guideline, due to the fact there is scientific evidence demonstrating that the tested animals feel pain and suffering. The second recommendation relates to those organisms for which there is still not enough knowledge (category 3): the panel recommended the protection of these animals until it is possible to demonstrate that, in fact, they do not experience pain and suffering.

Pragmatic political-economic issue

Although sentience is the main justification for the exclusion of invertebrates, other issues also influence decision-making about which groups should be included in animal protection legislation.

For economic reasons, invertebrates are animals with great potential for conducting biomedical research. The reason is that a large amount of invertebrates can be created at a fraction of the resources needed to create vertebrates such as mice and rats, among others [35].

The need for reduced space reduces spending on facilities, plus the cost of other processes involved in the creation and deployment of individuals. Other features of the biology of the species, such as short life cycle, rapid reproduction and maturation of offspring, are also directly reflected in low the cost of maintaining these animals compared to the vertebrates.

The genetic homogeneity of the descendants and the possibility to use large sample of individuals increase the reliability of the research, setting high statistical significance tests [35]. The greater consideration of invertebrates and their inclusion in legislation – and therefore in the review of protocols – will bring practical consequences for the scientific community and the regulatory and supervisory system.

The establishment of protocols of procedures requires specialized involvement for the development of specific methodologies for each invertebrate concerned. Furthermore, it is also necessary to install suitable laboratories for studies. The administrative involvement is another important aspect to be considered, because there would be a consequent increase in the workload of ethics committees, research centers and government agencies.

From all the aspects mentioned above, it is possible that the issue of control is the most dominant point of the whole process of inclusion, due to the need for strengthening the means of supervision of existing relevant bodies.

A matter of speciesism

When Aristotle [16] makes the assertion *plants are for the welfare of the animals and these are for the welfare of humans*, the idea of what is today known as speciesism is emerged.

This concept was termed by Ryder [36] only in the 70s, and since then it has been used by animal rights supporters to demonstrate prejudice against animals. According to that author, speciesism is similar to racism or sexism, where treatments offered to certain individuals are dictated by morally irrelevant physical differences.

The way we deal with animals is socially defined according to the cultural values of society. The hierarchization established from the moral status that animals take is called sociozoological scale [37]. In this scale, the animals are not ranked based on biological characteristics, but according to their use or how well they can perform functions for humans.

"Good" animals are put at the top of the scale and "bad" animals in the lower positions [37]. "Good" are those that can be controlled by humans and offer some kind of advantage, such as pets, edible or laboratory ones. Animals that cannot be controlled, such as pests and disease agents, are considered as "bad" ones.

It also seems to be no regularity in moral values between and among individuals within a same society. Studies with two populations of Portugal and Guinea-Bissau demonstrate how culture can shape different sociozoological scales. Factors such as religion, gender, age and intellectual level of the individuals appear to change the moral status attributed to animals [38].

Another ambivalence in sociozoological scales is that a species is not necessarily fixed in a certain position; it may be present in higher or lower positions according to the degree of moral values. As an example, dogs for company can occupy high position when compared to dogs used in experimental tests.

The variables that determine the sociozoological scale seem to have multi-causal origin, but they appear to be directly related to speciesism. Indeed, in the West sociozoological scale puts pets such as dogs and cats, large carnivores and nonhuman primates at the top of the scale.

In a middle position, large animals used in slaughterhouses, such as oxen, pigs etc. Below, there are the animals considered as harmful, such as rats and mice, followed by fish, which, because of being cold and slippery, are also in a lower position on the scale [39]. Probably the fact that invertebrates do not present physical or behavioral similarities with the human may place them in the lower positions of the scale [39].

Another unfavorable factor is that many of these animals are called "pests", such as caterpillars that feed themselves with cultivated plants, or

"harmful" such as cutting ants, or even considered "disgusting", such as cockroaches.

The inclusion of animals in the sentience conception follows a timescale: initially, only pet mammals were considered sentient beings; later, the primates were included (due to their similarity to humans), large mammals, mammals in general; warm blooded animals and more recently, all vertebrates [29].

In summary, speciesism associated with no evidence of sentience in invertebrates seems to contribute to justify the inclusion of these animals in protection laws.

Ethics and (non) inclusion of invertebrates in protection legislation

Morally, the only reason why invertebrates are not included in the regulation of animal protection is nonscientific proof of sentience. Although some researchers disagree on the possibility of invertebrates have such quality, there is a general recommendation that they also receive humanitarian assistance [40-42]. Considering that scientific rigor in the treatment of animals subjected to experimental tests depends on the proof of how sensitive they can be; the theme of inclusion of invertebrates needs to be debated.

The issue requires urgent attention as a measure of improvement and promotion of well-being used in scientific research. The inability to access emotional stages, as well as accurately map the mechanisms of pain and suffering, should not exclude these animals from moral consideration to provide them with care and wellbeing.

Meanwhile, in that sentience requires systematic evidence about sentience, we cannot say that all invertebrates are able to experience positive and negative emotional states, but, on the other hand, we cannot say that they are inherently insensitive to external stimuli.

Before circumstances, we advocate the principle of "benefit of the doubt" in favor of invertebrates. From the perspective of "evolutionary continuity", Darwin showed that all organisms are related and that this continuity is not only anatomical and physiological, but it can also be mental [43]. Accordingly, the dichotomy between vertebrates and invertebrates and the premise that only vertebrates can feel pain, suffer, have intelligence or perception of themselves seems unfounded.

Ethics can also be sought in debates about the inclusion of invertebrates in the regulation of animal protection, based on the "precautionary principle" [44], defined as a security measure that prevents potentially hazardous activities. In this sense, even with no agreement regarding emotional states of invertebrates and considering that the legislation does not consider them sentient, using indiscriminately the scientific use can be considered a risky activity, when we do not ponder the physical and emotional integrity of the studied individual.

Fortunately, the "benefit of doubt" is already being used in the implementation of some methodological proposals with invertebrates. Studies are being conducted on euthanasia and analgesia [41,45] and welfare in creation [3]. It is also notable the activity of the CCAC of Canada to review the case studies with the objective of delineating desirable methodology for standardization of procedures protocols with invertebrates. As an example, the measurement of ideal temperature for transporting lobsters for consumption and anesthesia procedures in spiders used for the study of neurobiology [6].

We agree that, on the one hand, it is desirable to provide invertebrates the "benefit of the doubt", on the other; it is not desirable to make use of a non-inclusive perspective, as seen in the case of speciesism. The speciesist attitude may explain underprivileged positions of invertebrates in sociozoological scales. These animals are not charismatic and therefore they cause no empathy in population, leaving them outside the scope.

A way to overcome this tendency is to use scientific knowledge to the extent that knowledge on sentience progresses. An example is the case of *Octopus vulgaris*, which in the UK the species is protected in legislation since 1993. With Policy of the European Union of 2010, all species of Cephalopoda group were included.

Thus, not only the neurobiology, but also the phylogeny of taxa, may be a strategy of inclusion. Take phylogenetic data from monophyletic groups (individuals with common ancestor) as parameter can provide greater breadth of protection without the risk of considering only one species, but all species of a group that shares the same characteristics.

Political and economic reasons should also be pointed out in the ethical debate on the inclusion of invertebrates. Replacing vertebrates by invertebrates can be economically advantageous, but the indiscriminate use may compromise the physical and emotional integrity of the individuals studied,

likewise as experimentation with vertebrates was dealt in the past.

Final considerations and future studies

Reflecting on the non-inclusion of invertebrates in protection regulation allows expanding the animal care beyond vertebrates. This expansion should be careful and requires further investigation regarding which taxonomic groups are priority in terms of sentience. Studies with sociozoological scales may also contribute to the understanding of the perception of society about these animals. Non-speciesist attitudes may extend our appreciation for this multitude of animals.

Although these animals are not covered by the legislation, we suggest that the use of individuals for scientific purposes is analyzed by the researcher in responsible way. The establishment of protocols and careful procedures that take into account the possible sentience of invertebrates will contribute to the development of humanitarian scientific research with respect to appropriate ethical and moral values.

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