Crespi-Abril, A.C., & Rubilar, T. (2021). Moving forward in the ethical consideration of invertebrates in experimentation: Beyond the Three R's Principle. *Revista de Biología Tropical, 69*(S1), 346-357. DOI 10.15517/rbt.v69iSuppl.1.46366

DOI 10.15517/rbt.v69iSuppl.1.46366

Moving forward in the ethical consideration of invertebrates in experimentation: Beyond the Three R's Principle

Augusto C. Crespi-Abril[1,3]*
Tamara Rubilar[2,3]

1. Instituto Patagónico del Mar, Universidad Nacional del a Patagonia San Juan Bosco, Boulevard Brown, Puerto Madryn, Argentina; crespi@cenpat-conicet.gob.ar (*Correspondence).
2. Laboratorio de Química de Organismos Marinos, Instituto Patagónico del Mar. Facultad de Ciencias Naturales y Ciencias de la Salud. Universidad Nacional de la Patagonia San Juan Bosco. Bv. Brown, Puerto Madryn, Chubut, Argentina; rubilar@cenpat-conicet.gob.ar
3. Laboratorio de Oceanografía Biológica, Centro para el Estudio de Sistemas Marinos. Boulevard Brown, Puerto Madryn, Argentina.

Received 11-VII-2020. Corrected 24-X-2020. Accepted 06-XI-2020.

ABSTRACT

Introduction: The Three R´s Principle (Refinement, Reduction, and Replacement), postulated more than 60 years ago, is the main ethical framework currently applied for conducting animal research. This principle has never been reviewed applying a philosophical reflection during all of these years, even though a variety of animal ethics studies have presented new insights. The Three R´s Principle was designed to be used as a policy tool to ameliorate the suffering of animals and to reduce the use of animals in research, but has failed in achieving these goals. This principle is only applied when using sentience vertebrates, and fails to consider invertebrates as their capacity to sentience is still disputed. In this way, invertebrates are reified, which has been determined to be detrimental as their suffering has been consistently denied. As a consequence, new insights are necessary to improve scientific practices. Epistemology and ethics have always been viewed as opposing approaches. ´Epistemology-based Ethics' subordinate ethical concern to scientific facts and 'Ethics-based Epistemology' purports that ethical practice should guide epistemological practices. **Objective:** In this paper, we maintain that unifying both approaches under a broader conceptual framework may result in the view that these are not, actually, opposite approaches. We propose to progress beyond the Three R's Principle and extend it to a position equal to the level of the ethical and epistemological approaches. We also propose to use the Precautionary Principle as it is always better to be safe than sorry, and to include two more Rs. **Methods:** This paper is based on the analysis of different ethical frameworks used in biology and ecology that can be implemented in invertebrates experimentation. **Results:** The analysis revealed that different ethical approaches are frequently used in biological research, but not all of them are implemented in experimental research that involves invertebrates. We argue that the ethical considerations used in any research field can be implemented in invertebrate research. **Conclusion:** We propose a Five R´s Principle: the traditional Refinement, Reduction, and Replacement, used along with Respect and Responsibility (a respectful relationship with every living being regardless of its complexity and personal commitment to conscientiously apply ethics concepts).

Key words: five R´s Principle; responsibility; respect; precautionary principle; animal ethics.

Non-human animals (hereinafter referred to as animals) have been used in experiments for more than two thousand years. The majority of these experiments were done on vertebrate animals (and some in cases on invertebrates) as they were considered adequate models of human anatomy and physiology (von Staden, 1989). During many years, an anthropocentric view dominated and animals were not considered subjects of ethical concern. However, the relationship between humans and animals has changed through time and ethical concern regarding animals started to increase due to the evident suffering animals were experiencing in order to satisfy scientific enquiry. In this sense, Bentham (1823) stated: "The question is not, Can they reason? Nor, Can they talk? But, Can they suffer?". Although animal research was acceptable under his utilitarian ideology (i.e., a moral action is the one that results in the highest overall wellbeing for all stakeholders) as benefits to mankind justified it, two hundreds years later Bentham's statement continues to exert a great deal of influence in the debate on animal use in the life sciences in general. The recognition of animals' sentience (capable of experiencing suffering) was an important starting point for the new philosophical thought that would be crucial for the development of new ethical perspectives concerning the moral status of animals. Singer (1975) published his work claiming for animal rights. This works is a breakthrough since it provides an exhaustive philosophical framework about ethics and animal suffering. In spite of its solid philosophical ground, Singer's ideas were controversial, but they inspired a rising public concern about animal rights that influenced animal experiments (Horta, 2011). Currently, debate on animals rights are on the table (Regan, 2003; Regan, 2004) and significant progress has been made at the normative level with the inclusion of some groups of invertebrates (European Parliament, 2010).

In 1959, Russell & Burch published their work "The Principles of Human Experimental Technique" where they postulated the Three R's Principle (Refinement, Reduction, and Replacement) (Russell & Burch, 1959). However, this principle was largely ignored for more than 20 years, until Smyth (1978) reinforced the utilization of the three R's by postulating "all procedures which can completely replace the need for animal experiments, reduce the numbers of animals required, or diminish the amount of pain or distress suffered by animals in meeting the essential needs of man and other animals". This definition is not only a restatement of the Three R's, it also places the responsibility onto researchers to provide robust evidence that can justify the use of animals in their research.

At present, the Three R's Principle represents the main ethical framework for conducting research with animals. However, this principle was postulate over 60 years ago and it is addressed without any major change. Even though it is currently used to regulate the scientific use of animals (Bayne, Ramachandra, Rivera, & Wang, 2015; Burden, Chapman, Sewell, & Robinson, 2015), it has been recently questioned due to its limitations in considering all the cases of animal use in research (Rubilar & Crespi-Abril, 2017; Crespi-Abril & Rubilar 2018; Hermann & Jayne, 2019). In this work, we provide new insights that support the ethical consideration of the majority (if not all) of cases in which animals are used in research. Based on our arguments, we propose a broader principle that can be used as an ethical framework in research with animals.

Invertebrates in experiments

Invertebrates represents a heterogeneous group of animals that consist in more than 30 phyla ranging from sponges (extremely simple multicellular organisms) to cephalopods (organisms that display complex behaviors and can solve simple problems). Invertebrates accounts for more than 95 % of the total species on Earth (Kellert, 1993; Wilson, 1999) which represents more than 1 million different species.

Invertebrates are frequently used in experiments since they present a series of advantages over vertebrates such as: relatively

simple to maintain and culture, simpler and easier to manipulate organisms, shorter life cycle (Smith, Scimeca & Mainous, 2011). Additionally, invertebrates are considered as adequate biological models for other species of animals including humans (Horvarth et al., 2013). The most recognized invertebrates models are *Drosophila melanogaster*, *Caenorhabditis elegans*, *Aplysia californica*, sea urchins, squids and honeybees. Significant breakthroughs were conducted in biomedical research using only this few groups of invertebrates (Wilson-Sanders, 2011).

Usually invertebrates (except for cephalopods and decapods) are not included in the legislation and welfare of these animals is mostly neglected. This leads to a gap of knowledge regarding the number of individuals used in experiments since there is no need to record it (Harvey-Clark, 2011). Although there are no exact estimations, the numbers of individuals of particular species (*Drosophila melanogaster*, *Caenorhabditis elegans*, *Aplysia californica*) may reach millions in a single laboratory (Harvey-Clark, 2011).

Three R's principle: current situation

Three R's Principle introduces the three basic concepts of Refinement, Reduction and Replacement which any researcher should consider when conducting research involving animals. Basically, Russell and Burch (1959) defined Replacement as "any scientific method employing non-sentient material [to] replace methods which use conscious living vertebrates"; Reduction as diminishing "the number of animals used to obtain information of a given amount and precision"; and Refinement as a series of practices conducted to "decrease in the incidence or severity of [...] procedures applied to those animals which have to be used". These three concepts are considered to incur the same level of significant but were originally postulated as a sequence to achieve the goal of total replacement as a maxim: "[r]efinement is never enough, and we should always seek further for reduction and if possible replacement" (Russell & Burch, 1959). Clearly, this principle is only applied when using vertebrates since they are the only group of animals that are considered as sentient animals due to their similarities with humans in terms of the structure of the nervous system. In this sense, the principle fails to consider inclusion of invertebrates, whose capacity to sentience is still disputed. A strong assumption underlies the Three R's: This is that non-human animals which are lower on the zoological scale lack sentience (Tomasik, 2014). This represents a significant constraint, especially when it is considered that the number (and diversity) of invertebrates used in science is overwhelming. Despite the worldwide acceptance of the Three R's as a policy tool to ameliorate the suffering of animals and to reduce the use of animals in research, the failure in achieving these goals has been clearly noted (Blattner, 2019). In fact, the number of animals used for experimental purposes in the European Union (EU) is currently similar to the number registered in 1980s (Taylor & Rego, 2016). The main cause of this failure is that the language used in legislation regarding the replacement concept is frequently laxer that the language used in refinement. As an example, the EU Directive 2010/63/EU states "Member States shall ensure that, wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live non-human animals, shall be used instead of a procedure" (European Parliament, 2010, Article 4). This implies that replacement is only necessary if alternatives exist and are recognized by the normative. However, this comprises a limitation as, in practice, replacement alternatives are barely recognized in legislation and when they are, they do not refer to replacement in an absolute sense, but to fewer sentient animals. These conditions fail to exhort researchers to stop using animals in research. This, in turn, leads to an implicit hierarchical understanding of the Three R's in which refinement and reduction take priority over replacement (Gerritsen, 2015).

Relation between Ethics and Epistemology: alternative frameworks

In this section, we introduce the main relation between ethics and epistemology, which represents a metaethical analysis. However, it is not the aim of this paper to address exhaustively the field of metaethics.

Significant advances have been made on ethical considerations in animal experiments. Proof of this is seen in the fact that the Three R's Principle govern research practices. However, the principle is not applied to any species, but only to those considered sentient. In this sense, it is crucial to gather evidence to ensure that a particular species is sentient by establishing objective criteria involving behavioral, evolutionary and physiological considerations. Sentience is very complex to demonstrate, and researchers have been focused on demonstrating the existence of pain, as it is assumed to comprise a particularly important form of suffering (EFSA Panel on Animal Health and Welfare, 2005). In 2012, it was published the Cambridge Declaration on Consciousness on which scientific community concludes that animals are conscious living beings able of experiencing negative emotional sensations (pain).

The experience of pain is based on the presence of two components: nociception (physiological detection of nocive stimuli), and the experience of pain (negative emotional sensation produced after a nocive stimuli). If either of these two components is absent, it is assumed that there is no possibility of experiencing pain. If nociception is absent, stimuli are not detected, and if emotional sensations are absent, pain does not occur (nociceptive reflex responses). Seven criteria are applied to determine if individuals of a particular species are capable of experiencing pain (Smith, 1991; EFSA Panel on Animal Health and Welfare, 2005), and these can be summarized as follows (Andrews et al., 2013):

1. Possession of receptors sensitive to noxious stimuli, located in functionally useful positions on or in the body and connected by nervous pathways to the lower parts of the nervous system
2. Possession of higher brain centres (in the sense of integration of brain processing), especially a structure analogous to the human cerebral cortex
3. Possession of nervous pathways connecting the nociceptive system to the higher brain centres
4. Receptors for opioid substances found in the central nervous system, especially the brain
5. Analgesics modify the animal's response to stimuli that would be painful for a human
6. An animal's response to stimuli that would be painful for a human is functionally similar to the human response (that is, the animal responds so as to avoid or minimize damage to its body)
7. An animal's behavioral response persists and it shows an unwillingness to resubmit to a painful procedure; the animal can learn to associate apparently non-painful with apparently painful events

Most vertebrates fulfill these criteria and there is no doubt as to their capacity of experiencing pain. But in the case of invertebrates this is not that clear, and it is an area of longstanding concern and controversy (Fiorito, 1986; Mather, 2016) as the majority of the established criteria for pain are not met. This is a fallacious argument, as 'absence of evidence is not evidence of absence'. If one considers the large biodiversity of invertebrates (Kellert, 1993; Wilson, 1999) and the large variability in 'bauplan' (which includes the nervous system), it is likely that the capacity of experiencing pain can be achieved by other mechanisms and structures differing from those described for vertebrates (Riebli & Reichert, 2015). For instance, cephalopods are considered to be an exception as it has been already assumed that they can suffer and they were included in the ethical normative (Andrews et al., 2013; Smith et al., 2013; Della Rocca, Di Salvo, Giannettoni, & Goldberg, 2015) This applies in spite of the fact they have a different nervous system as compared to vertebrates. However, after much

research effort, controversy still remains due to the inherent difficulty of demonstrating the capacity of experiencing pain by this group of invertebrates (Harvey-Clark, 2011). With this framework, it is assumed that we first must evidence that animals are capable of suffering pain and, then, we should seek ethical considerations. This approach is denominated 'Epistemology-based Ethics' and subordinates ethical concern to scientific facts (Chandroo, Yue, & Moccia, 2004; Griffin & Speck, 2004; Elwood, 2011; Lewbart & Mosley, 2012; Mather, 2016).

A different approach has been proposed in which the relationship between epistemology and ethics is inverted where: ethics should guide epistemological practices. This implies that there is an initial need to establish a respectful relationship with living beings rather than a facts-based relationship. This, implies a limit (ethical one) on our actions, resulting in the need to rethink whether knowledge regarding the biology, physiology, ecology of animals should take precedence over the welfare of those living beings. In addition, it is important to note in this context that the utilitarian rationale purporting that human interest takes precedence over animal suffering is an insufficient ethical justification for the use of animals in experimentation as animals and humans equal rights as living beings. This approach is denominated 'Ethics-based Epistemology' (Cheney & Weston, 1999; Weston, 2009). This insight has been implemented in research with invertebrates, particularly regarding echinoderms (Rubilar & Crespi-Abril, 2017; Crespi-Abril & Rubilar, 2018). To biologists, this different approach may sound counterproductive as the focus of our own research may be, in fact invertebrates. However, we believe that science can be exercised within a new bio-ethical framework that includes these new concepts and ideas.

Precautionary Principle

The precautionary principle aims to take actions to avoid harm even though evidence is insufficient to provide certainties on the magnitude or probability of happening (Raffensperger, 1999; Kriebel et al., 2001). A simple way to phrase this is: until the risk of harm is controlled, be cautious, as it is better to be 'safe than sorry'. This rationale guides the vast majority of human, ordinary activities. This is not the same in current scientific practices involving animal experiments. As we stated above, the current approach is that until we do have the certainty that animals are capable of experiencing suffering (harm), we will not, ethically, give them consideration (action). Why do we act this way in science? The answer to this point does not rely on the fact that the principle leads in the wrong direction, but in the thought that it leads in no direction at all and threatens to be paralyzing (Sunstein, 2003). This thought generates resistance in the researcher community to start considering an ethical approach in scientific practices. With vertebrates there is a clear consensus on this point, but most invertebrates are still not treated relying on bio-ethical basis.

The precautionary principle is stated by incorporating different levels of restrictions that can be grouped into weak or strong versions. The weak version is less restrictive and allows preventive actions under the existence of the risk of harm, but does not require such preventive actions. Often, cost-benefits analyses are considered in order to postpone preventive actions and these analyses include factors other than scientific certainty, such as economic factors. In this case, the burden of proof (the requirements to justify) lies with those advocating precautionary actions. On the other hand, the strong version requires the taking of precautionary actions in the presence of the risk of harm, in spite of the costs incurred in doing so. Here, the burden of proof is inverted and lies with those who argue that certain activities will not cause significant harm. This means that society is not willing to accept any environmental risk, no matter the benefits (social or economic) that may be achieved (Di Salvo & Raymond, 2010). Four types of the precautionary principle were distinguished capturing both weak and strong types by Stewart (2002):

- *Non-preclusion Precautionary Principle*: Scientific uncertainty should not automatically preclude that regulation of activities posing a potential risk of significant harm.
- *Margin of Safety Precautionary Principle*: Regulatory controls should incorporate a margin of safety; activities should be limited below the level at which no adverse effect has been observed or predicted.
- *Best Available Technology Precautionary Principle*: Activities that present an uncertain potential for significant harm should be subject to best technology available requirements to minimize the risk of harm unless the proponent of the activity shows that they present no appreciable risk of harm.
- *Prohibitory Precautionary Principle*: Activities that present an uncertain potential for significant harm should be prohibited unless the proponent of the activity shows that it presents no appreciable risk of harm.

Currently scientific practices involving animal experiments are guided under a weak precautionary principle as it is necessary to demonstrate (to show scientific certainty) the existence of pain (harm) in order to take actions to diminish such pain and always only after a cost-benefit analysis has taken place to determine the benefit of the scientific practice. In an opposing position, one finds the ideals postulated by activists whose claims argue for a strong version of precautionary principle requiring the demonstration that pain is not present (absence of harm). Both of these are extreme positions and there are several flaws in the justification of each posture. Here, we propose to reach a position in the middle ground.

Five R's Principle: beyond Russell´s proposal

Problems emerging from an ethical approach regarding animal experimentation are more widely appreciated than the epistemological ones. Most researchers agree that subordinating ethics into epistemology (Epistemology-based Ethics), provides rigorous scientific ground whilst an empathic approach with animals (Ethic-based Epistemology) may bias experiments and conclusions due to the extreme reduction in the number of animals used or to the use of inappropriate replacements. In this way, it is assumed that keeping ethics out of the picture will provide the objectivity that is crucial for science. However, researcher desensitization may bias the results of experiments as animals may be kept in inappropriate environmental conditions (e.g. small spaces, intense noise, lack of environmental stimulation, poor quality of seawater, under fed, etc.) which produce significant levels of stress in the animals. This causes epistemological problems as stress impacts on the physiology of individuals, hence, modifying their response to treatments and, thereby, affecting the reliability of scientific data obtained from the animals (Baldwin, Primeau & Johnson, 2006; Burwell & Baldwin, 2006). None of these approaches are completely satisfactory since they present flaws, either at the epistemological level or ethical level. Consequently, there is no difference in our choice of levels to guide our scientific practices; in either case, we will not be able to fulfill epistemological and ethical grounds. One important point that is supposed to be a barrier in this context is that both approaches are seen to comprise opposite and irreconcilable frameworks, instead of complementary. We maintain that unifying both approaches under a broader conceptual framework may lead to the possibility of overcoming previous flaws.

The Three Rs' Principle was thought to provide ethical grounds (although limited only to certain species) for animal experimentation, but were not to address the epistemological problems arising from animal manipulation (Johnson & Degeling, 2012). We maintain that this principle needs to be extended in order to unify both approaches into a broader conceptual framework that considers the ethical and epistemological grounds at the same level here proposed to expand the principle including

two concepts involving personal commitment: Respect and Responsibility (Fig. 1).

Respect

Respect points to the establishment a respectful relationship with any living beings, regardless of their complexity or the knowledge we have of them. This implies that we are to be respectful with life, itself, rather than being in a facts-based relationship. The use of animals in experimentation implies a restriction on the ethical consideration to them. This not only implies leaving out from ethical consideration several individuals (such as any invertebrate), but also implies the restriction of our ethical actions to those animals which are considered ethically (e.g. individual suffering is justified if it is in favor of human benefits). Researchers often carry out procedures that have been defined and required by others (such as institutions or colleagues), which means that their ethical autonomy is not considered when conducting experiments with animals. They are trained to desensitize, by reframing their ethical relationship with animals, in order to accomplish the job when it involves animal experiments, as these experiments always imply animal suffering (Capaldo, 2004). At this point, researchers are no longer empathetically related with animals but, instead, take a neutral observer perspective, intentionally avoiding any commitment with them. This perspective distorts the ability to relate to animals by promoting reification of them (Honneth, 2006). Reification is very risky for those who are reified, since their interests or suffering are permanently denied (Johnson & Smajdor, 2019).

An empathic relationship with animals does not imply a barrier to achieving knowledge about them or to the use of the animals according to our needs. Although an empathic relationship in science is an innovative insight, it is worth noting that it is not fanciful or implausible, and is therefore a strategy that is worth considering. There are solid evidences among empathic relation and emotions communication between mammals and this could provide a novel field for studying empathic relations in other animals groups (Preston & de Wall, 2002; de Waal & Preston, 2017). There are two different approaches in which the key point is empathy. These are the Traditional Ecological Knowledge (TEK) and Animal-as-Patients approaches. The first one involves three aspects of nature: the cosmos (beliefs, emotions and symbolic representations), the corpus (general environmental knowledge), and the praxis (the behaviors carried out in relation to the use of Nature). Advances coming from ethnoecology revealed that several cultures relate with environment in a respectful manner (Toledo & Barrera-Bassols, 2008). They consider that environment is populated by living beings that have social relationships between themselves and with whom they establish social relationships. This provides a conceptual framework allowing a responsible management of natural resources, transcending mere utilitarian and economic aspects (Gadgil, Berkes, & Folke, 1993; Berkes, Colding, & Folke, 2000). This implies a type of knowledge that can be fully understood only if symbolisms and meanings that are attributed to natural entities are considered (Reyes-García & Martí, 2007). The second one, is to approach animals during experiments as patients (Pemberton, 2004; Haraway, 2008). This means to shift our current actions undertaken during experiments to treating animals as individual patients rather than as mass-produced and expendable objects. This means that animals should be included in experiments in a similar way as humans are enrolled into clinical trials (Johnson & Degeling, 2012). This approach is already implemented in animal experimentation in veterinary practice. When an animal is put through an experimental treatment, the care and welfare of the individual is not only evaluated by the neutral (professional) position of the veterinarian, but also by the empathy and feelings of the owner.

Responsibility

Responsibility when conducting animal experiments is the fifth concept that completes our proposal. The essence of scientific work requires a strong professional ethics since it is based on the confidence of the scientific community in the honesty of researchers. Although there is a rigorous peer review process, honesty in performing experiments, conducting data analysis and reporting the results is crucial. We argue in favor of expanding this responsibility and of including ethical commitment with animals as a part of our practices. Responsibility has already invoked by the Max Planck Society (MPG, 2020), mainly focusing on promoting animal welfare. In our proposal, responsibility calls for the personal commitment of researchers to conscientiously apply the ethical concepts. This goes beyond just promoting welfare, but also promotes the rethinking as to whether knowledge regarding the biology, physiology, ecology or medicine should be prioritized over the welfare of the animals involved in experiments. We need to act responsibly when using animals in experiments considering the real value of the life of each living being, and we need to be honest when asking if the life of any animal worth the knowledge obtained. The most controversial point is, of course, the use of animal experiments in medicine (Knight, 2019). There is a long tradition in using animals as physiological models in medicine, and currently this is an unavoidable step in medical trials in spite of the fact that there is overwhelming evidence that animals are not appropriate models (Ram, 2019). The unquestioned practice of using animals in medicine not only has ethical concerns, but also implies a problem in terms of human health (Greek & Kramer, 2019). This is a clear example of a scientific practice that can not stand a critical and responsible review and would need to be abandoned in most cases (Archibald, Coleman, & Drake, 2019).

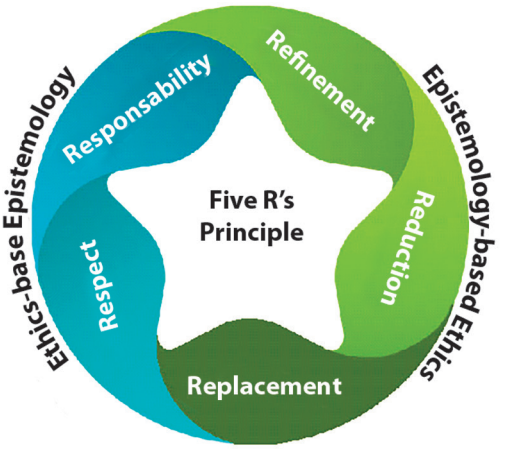

Fig. 1. Five R´s Principle. Equal level of importance of the ethical and epistemological approaches.

Concluding Remarks

The current framework allows researchers to relieve their ethical commitment through the implementation of the Three R's Principle. However, this is not enough for ethically responsible science. The Three R's Principle comprised a starting point in establishing an ethical approach in science but has remained almost unmodified for more than 60 years. As a community, we owe a critical review of our approaches to animals in experiments. It is necessary to keep permanently revising our approaches in order to develop new insights. We propose to extended the Three R Principle to a position in which the ethical and epistemological approaches are at the same level. This change may seem difficult to implement, but this should not be a barrier to developing new ways of thinking. All significant changes were seen as impossible when first postulated and are now commonplace. Scientific practice is no exception to the rule, it is worth to keep promoting ethical concern and real commitment in terms of how researchers relate to animals in the context of their own work and how this can be improved. We must stop accepting practices that in the long-term perpetuate the use of animals in experiments as an acceptable scientific standard. We are aware that this may not be seen as a straightforward way

of implementing changes, but communication between stakeholders (e.g. researchers, veterinarians, animal welfare activists, competent authorities) is crucial to promote change and keep moving forward on ethical commitment in animal experimentation. It is very important to include whole society in the debate since public perception and opinion is a strong influence in defining scientific practices and it is a challenge in invertebrates research (Drinkwater, Robinson, & Hart, 2019).

We propose to use the Precautionary Principle along with the Five R´s Principle, including Refinement, Reduction, Replacement but also Respect (to establish a respectful relationship with any living being regardless its complexity or the knowledge we have of that living being) and Responsibility (personal commitment of researchers to conscientiously apply ethics concepts). Under this new proposal, invertebrates are at the same level of ethical concern as vertebrates. This means that institutional animal care and use committee (IACUC) should evaluate all research project that requires the use of any invertebrate, and not only those projects that involves vertebrates. The high diversity of invertebrates requires that IACUC address with particular attention every case and this challenging for committee members (Harvey-Clark, 2001). However, in order for our proposal to succeed, it must be embraced in all of the different roles we as researchers assume and execute. First, in our own practices in the laboratory, then in our teaching (e.g. universities, schools, courses, etc) but, most crucially, in our role as reviewers. We can change the balance within and shift our own practices, but we can do even more in influencing others to follow in our tracks and, in the manner, initiate a real change.

Ethical statement: authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgements section. A signed document has been filed in the journal archives.

ACKNOWLEDGMENTS

We would like to thanks to Kathleen Anderson for the revision of the English and criticism of the manuscript. In addition, we thank the anonymous reviewers for their comments, which improved significantly the manuscript.

RESUMEN

Avanzando sobre la consideración ética de los invertebrados en experimentación: más allá del principio de las tres Rs

Introducción: El Principio de las Tres R (Refinamiento, Reducción y Reemplazo), postulado hace más de 60 años, es el principal marco ético que se aplica actualmente para realizar investigaciones en animales. Este principio no ha sido revisado desde una reflexión filosófica a pesar de tantos años y de lavariedad de estudios en ética animal que han presentado nuevas ideas. Este principio solo se aplica cuando se utilizan vertebrados sintientes, y no tiene en cuenta a los invertebrados, ya que su capacidad de sentiencia aún está cuestinada. De esta manera, los invertebrados son cosificados, lo que ha sido en su detrimento ya que su sufrimiento ha sido constantemente negado. El Principio de las Tres R fue diseñado para ser utilizado como una herramienta de política para mejorar el sufrimiento de los animales y reducir el uso de animales en la investigación, pero no ha logrado alcanzar estos objetivos. Como consecuencia, se necesitan nuevas ideas para mejorar las prácticas científicas. La epistemología y la ética siempre se han visto como enfoques opuestos. La concepción ética subordinada de a los hechos científicos se llama "Ética basada en la epistemología" y, en cambio, la "Epistemología basada en la ética" pretende que la práctica ética guie las prácticas epistemológicas. **Objetivo**: En este trabajo, proponemos que unificar ambos enfoques bajo un marco conceptual más amplio y eliminar la percepción de que son enfoques opuestos. Proponemos avanzar más allá del Principio de las Tres R , extenderlo y colocar un mismo nivel a los enfoques éticos y epistemológicos. También proponemos utilizar el Principio de precaución, ya que siempre es mejor prevenir que curar e incluir dos R más. **Métodos**: El trabajo está basado en un análisis de diferentes corrientes éticas utilizadas en biología y ecología que pueden ser implementadas en la experimentación con invertebrados. **Resultados**: El análisis de los trabajos reveló que hay diferentes posturas éticas que se usan frecuentemente en biología, pero no todas ellas son implementadas

en la experimentación con invertebrados. Bajos nuestra argumentación sostenemos que las consideraciones éticas usadas en cualquier campo de investigación puede ser implementada en la experimentación con invertebrados. **Conclusión**: De esta manera, proponemos un Principio de las Cinco R: Refinamiento, Reducción, Reemplazo para ser usados junto con el Respeto y la Responsabilidad (una relación respetuosa con cada ser vivo independientemente de su complejidad y un compromiso personal de aplicar conscientemente los conceptos éticos).

Palabras clave: principio de las Cinco R; responsabilidad; respeto; principio de precaución; ética; epistemología.

REFERENCES

Andrews, P.L., Darmaillacq, A.S., Dennison, N., Gleadall, I.G., Hawkins, P., Messenger, J. B., … Smith, J.A. (2013). The identification and management of pain, suffering and distress in cephalopods, including anaesthesia, analgesia and humane killing. *Journal of Experimental Marine Biology and Ecology*, *447*, 46-64.

Archibald, K., Coleman, R., & Drake, T. (2019). Replacing animal tests to improve safety for humans. In K. Hermann & K. Jayne (Eds.), *Animal Experimentation: Working Towards a Paradigm Change* (pp. 417-442). The Netherlands: Brill.

Baldwin, A.L., Primeau, R.L., & Johnson, W.E. (2006). Effect of Noise on the Morphology of the Intestinal Mucosa in Laboratory Rats. *Journal of the American Association for Laboratory Animal Science*, *45*(1), 74-82

Bayne, K., Ramachandra, G.S., Rivera, E.A., & Wang, J. (2015). The evolution of animal welfare and the 3Rs in Brazil, China, and India. *Journal of the American Association for Laboratory Animal Science*, *54*(2), 181-191.

Bentham, J. (1823). *An Introduction to the Principles of Morals and Legislation*. London, UK: W. Pickering.

Berkes, F., Colding, J., & Folke, C. (2000). Rediscovery of traditional ecological knowledge as adaptive Management. *Ecological Application*, *10*, 1251-1262.

Blattner, C.E. (2019). Rethinking the 3Rs: From whitewashing to rights. In K. Hermann & K. Jayne (Eds.), *Animal Experimentation: Working towards a paradigm change* (pp. 168-193). The Netherlands: Brill.

Burden, N., Chapman, K., Sewell, F., & Robinson, V. (2015). Pioneering Better Science through the 3Rs: An Introduction to the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs). *Journal of the American Association for Laboratory Animal Science*, *54*(2), 198-208.

Burwell, A.K., & Baldwin, A.L. (2006). Do Audible and Ultrasonic Sounds of Intensities Common in Animal Facilities Affect the Autonomic Nervous System of Rodents?. *Journal of Applied Animal Welfare Science*, *9*(3), 179-200.

Capaldo, T. (2004) The psychological effects on students of using animals in ways that they see as ethically, morally, or religiously wrong. *Alternatives to Laboratory Animals*, *1*(32), 525-532.

Chandroo, K.P., Duncan, I.J., & Moccia, R.D. (2004). Can fish suffer? Perspectives on sentience, pain, fear and stress. *Applied Animal Behaviour Science*, *86*(3), 225-250.

Cheney, J., & Weston, A. (1999). Environmental ethics as environmental etiquette: Toward an ethics-based epistemology. *Environmental Ethics*, *21*(2), 115-134.

Crespi-Abril, A.C., & Rubilar, T. (2018). Ética e invertebrados: análisis de los casos de los cefalópodos y equinodermos. *Revista Latinoamericana de Estudios Críticos Animales*, *8*, 210-232.

de Waal, F. B., & Preston, S. D. (2017). Mammalian empathy: behavioural manifestations and neural basis. *Nature Reviews Neuroscience*, 18(8), 498-509.

Della Rocca, G., Di Salvo, A., Giannettoni, G., & Goldberg, M.E. (2015). Pain and suffering in invertebrates: an insight on cephalopods. *American Journal of Animal and Veterinary Sciences*, *10*(2), 77.

Di Salvo, C.P., & Raymond, L. (2010). Defining the precautionary principle: an empirical analysis of elite discourse. *Environmental Politics*, *19*(1), 86-106.

Drinkwater, E., Robinson, E.J., & Hart, A.G. (2019). Keeping invertebrate research ethical in a landscape of shifting public opinion. *Methods in Ecology and Evolution*, *10*(8), 1265-1273.

EFSA Panel on Animal Health and Welfare (2005). *Opinion of the Scientific Panel on Animal Health and Welfare (AHAW) on a request from the Commission related to the "Aspects of the biology and welfare of animals used for experimental and other scientific purposes"*. EFSA J. 292, 1-136.

Elwood, R.W. (2011). Pain and suffering in invertebrates?. *Ilar Journal*, *52*(2), 175-184.

European Parliament. (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of the European Communities*, L276, pp. 33–79. [online] Available at: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32010L0063 [Accessed 4 September 2020].

Fiorito, G. (1986). Is there "pain" in Invertebrates?. *Behavioral Proceedi*ng, *12*, 383-388.

Gadgil, M., Berkes, F., & Folke, C. (1993). Indigenous knowledge for biodiversity conservation. *Ambio 22*, 151-6.

Gerritsen, V. (2015). Evaluation Process for Animal Experiment Applications in Switzerland. *Alternatives to Animal Experimentation: Proceedings*, *4*(1), 37-40.

Greek, R., & Kramer, L.A. (2019). The scientific problems with using non-human animals to predict human response to drugs and disease. In K. Hermann & K. Jayne (Eds.), *Animal Experimentation: Working towards a paradigm change* (pp. 391-416). The Netherlands: Brill.

Griffin, D.R., & Speck, G.B. (2004). New evidence of animal consciousness. *Animal cognition*, *7*(1), 5-18.

Haraway, D.J. (2008). *When Species Meet*. Minneapolis: University of Minnesota Press.

Harvey-Clark, C. (2011). IACUC challenges in invertebrate research. *Ilar Journal*, *52*(2), 213-220.

Hermann, K., & Jayne, K. (2019). *Animal Experimentation: Working Towards a Paradigm Change*. The Netherlands: Brill.

Honneth, A. (2006). *Reification: A Recognition Theoretical View. The Tanner Lectures on Human Values*. Berkeley: University of California.

Horta, O. (2011). La argumentación de Singer en Liberación animal: concepciones normativas, interés en vivir y agregacionismo. *Diánoia*, 56(67), 65-85.

Horvath, K., Angeletti, D., Nascetti, G., & Carere, C. (2013). Invertebrate welfare: an overlooked issue. *Annali dell'Istituto superiore di sanità*, 49, 9-17.

Johnson, J., & Degeling, C. (2012). Animals-as-Patients: Improving the Practice of Animal Experimentation. *Between the Species*, *15*(1), 43-58.

Johnson, J., & Smajdor, A. (2019). Human Wrongs in Animal Research: A Focus on Moral Injury and Reification. Human-Animal Studies. In K. Hermann & K. Jayne (Eds.), *Animal Experimentation: Working towards a paradigm chang*e (pp. 305-317). The Netherlands: Brill.

Kellert, S.R. (1993). Values and perceptions of invertebrates. *Conservation biology*, *7*(4), 845-855.

Knight, A. (2019). Critically evaluating animal research. In K. Hermann & K. Jayne (Eds.), *Animal Experimentation: Working towards a paradigm change* (pp. 321-340). The Netherlands: Brill.

Kriebel, D., Tickner, J., Epstein, P., Lemons, J., Levins, R., Loechler, E.L., & Stoto, M. (2001). The precautionary principle in environmental science. *Environmental health perspectives*, *109*(9), 871-876.

Lewbart, G.A., & Mosley, C. (2012). Clinical anesthesia and analgesia in invertebrates. *Journal of exotic pet medicine*, *21*(1), 59-70.

Mather, J.A. (2016). An invertebrate perspective on pain. *Animal Sentience: An Interdisciplinary Journal on Animal Feeling*, *1*(3), 12.

MPG. (2020). Reduce, refine, replace – responsibility. *Max Planck Society*. [online] Available at: https://www.mpg.de/10973438/4rs [Accessed 4 September 2020].

Pemberton, S. (2004). Canine technologies, model patients: The historical production of hemophiliac dogs. In S. Shrepfer & P. Scranton (Eds.), *American Biomedicine. In Industrializing Organisms* (pp. 191- 213). New York: Routledge.

Preston, S. D. & de Waal, F. B. M. (2002). The communication of emotions and the possibility of empathy in animals. In: S. Post, L. G. Underwood, J. P. Schloss, & W. B. Hurlburt (Eds.), *Altruistic love: Science, philosophy, and religion in dialogue*. (pp. 284–308). Oxford, UK: Oxford Univ. Press

Raffensperger, C., & Tickner J. (1999). *Protecting public health and the environment: Implementing the precautionary principle*. Washington, D.C.: Island Press.

Ram, R. (2019). Extrapolation of animal research data to humans: an analysis of the evidence. In K. Hermann & K. Jayne (Eds*.), Animal Experimentation: Working towards a paradigm change* (pp. 341-375). The Netherlands: Brill.

Regan, T. (2003). *Animal rights, human wrongs: an introduction to moral philosophy*. Rowman & Littlefield.

Regan, T. (2004). *The case for animal rights*. Univ of California Press.

Reyes-García V., & Martí, S.N. (2007) Etnoecología: punto de encuentro entre naturaleza y cultura. *Ecosistemas*, *16*(3), 46-55.

Riebli, N., & Reichert, H. (2015). Perspective - The first brain. In A. Schmidt-Rhaesa, S. Harzsch & G. Purschke (Eds.), *Structure and Evolution of Invertebrate Nervous System* (p. 67-74). Oxford: Oxford University Press.

Rubilar, T., & Crespi-Abril, A. (2017). Does Echinoderm research deserve an ethical consideration?. *Revista de Biología Tropical*, *65*, 11-22.

Russell, W.M.S., & Burch, R.L. (1959). *The principles of humane experimental technique*. London, UK: Methuen & Co. Ltd.

Singer, P (1975). *Animal Liberation: A New Ethics for our Treatment of Animals*. New York Review/Random House, New York.

Smith, J. (1991). The ethics of using animals in biomedical research: the findings of a working party of the Institute of Medical Ethics. *Bulletin of medical ethics*, 13-17.

Smith, J.A., Andrews, P.L.R., Hawkins, P., Louhimies, S., Ponte, G., & Dickel, L. (2013). Cephalopod research and EU Directive 2010/63/EU: Requirements, impacts and ethical review. *Journal of Experimental Marine Biology and Ecology*, *447*, 31-45.

Smith, S.A., Scimeca, J.M., & Mainous, M.E. (2011). Culture and maintenance of selected invertebrates in the laboratory and classroom. *ILAR journal*, 52(2), 153-164.

Smyth, D.H. (1978). *Alternatives to Animal Experiments*. London, UK: Scholar Press [for] the Research Defense Society.

Stewart, R.B. (2002). Environmental regulatory decision making under uncertainty. *Research in Law and Economics*, *20*, 71-126.

Sunstein, C.R. (2003). Beyond the precautionary principle. *University of Pennsylvania Law Review*, *151*(3), 1003-1058.

Taylor, K., & Rego, L. (2016). EU statistics on animal experiments for 2014. *ALTEX*, *33*(4), 465-468.

Toledo, V.M., & Barrera-Bassols, N. (2008). *La memoria biocultural. La importancia ecológica de las sabidurías tradicionales*. Barcelona: Icaria.

Tomasik, B. (2014). Suffering in Animals vs. humans, essays on reducing suffering. Retrieved from http://reducing-suffering.org/suffering-in-animals-vs-humans/

Von Staden, H. (1989). *Herophilus: The Art of Medicine in Early Alexandria*. Cambridge, UK: Cambridge University Press.

Weston, A. (2009). *The incomplete eco-philosopher: Essays from the edges of environmental ethics*. Nueva York: SUNY Press.

Wilson, E.O. (1999). *The diversity of life*. Harvard: Harvard University Press.

Wilson-Sanders, S.E. (2011). Invertebrate models for biomedical research, testing, and education. *ILAR journal*, 52(2), 126-152.