Workshop
Etica, Benessere animale e 3R

Comunicazioni

The legal protection of animals in scientific experimentation

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Animal experimentation represents one of the most delicate and controversial areas in balancing scientific progress, biomedical research needs, and the protection of non-human living beings. This presentation offers an in-depth analysis of the current legal framework governing the use of animals for scientific purposes, highlighting the main European and national regulations as well as the underlying ethical and bioethical principles. At the European level, Directive 2010/63/EU is the key normative reference and promotes the principle of the so-called "3Rs": Replacement (substitution of animals with alternative methods), Reduction (reduction in the number of animals used), and Refinement (improvement of techniques to minimize suffering), guiding research toward more sustainable practices that respect animal welfare.

In Italy, Legislative Decree no. 26/2014 transposed the directive with some stricter provisions, introducing additional restrictions such as the ban on breeding dogs, cats, and non-human primates, as well as limitations on their use. These measures have sparked intense debate among the scientific community, institutions, and animal rights organizations. The presentation also delves into the legal concept of animals throughout history and the progressive recognition of animal welfare as a legally relevant value, particularly in light of the recent amendment to Article 9 of the Italian Constitution, which extends environmental protection to include animals explicitly as entities worthy of legal protection.

The role of the bodies responsible for overseeing and authorizing experimental protocols—such as the competent authorities—is examined, along with the responsibilities of those involved, including researchers, veterinarians, and institutions, with regard to transparency, training, and reporting obligations. Particular attention is given to the comparison between scientific needs, the development of alternative methods, and the growing ethical awareness of public opinion.

The overarching goal is to stimulate critical reflection on the effectiveness of the legal protection of animals in laboratories, assessing the future evolution of legislation, also in light of recent jurisprudence and the position of some European institutions toward the gradual phasing out of animal experimentation, in favor of a more ethical, sustainable, and responsible science.

Welfare and care of the laboratory animals: new milestones achieved in the facilities

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Since the enactment of the European Directive 2010/63/EU on the protection of animals used for scientific purposes, the activities carried out in the animal facilities are conducted in full compliance with the 3R principle (Russell & Burch, 1959) with the aim to reduce the number of animals involved to a minimum, compatible with the scientific goals; to refine the housing methods and experimental techniques in compliance with the biological needs of the species; to replace the animal model, where possible.
To ensure the proper regulation and welfare of research animals, the standards of laboratory animal welfare (how animals should be correctly cared for) are continuously updated as the Commission delegated directive (EU) 2024/1262 of 13 March 2024 regarding the requirements for establishments and for the care and accommodation of animals.
In Italy the legislative context has been implemented by the Decree 26/2014 and new training obligations have recently been identified by the Ministry of Health according to the Decree of 5 August 2021 and the Decree of 18 March 2022 for all personnel who work with animal models.
Such personnel must be adequately trained and must know the basic and specific biology of the single species in relation to anatomy, physiological characteristics, reproduction, genetics and genetic alteration; animal behaviour (ethology), breeding and enrichment; management methods and procedures specific to the species bred.
The competence of personnel is assessed by the Animal Welfare Body composed of the designated Veterinarian (DV), the Scientific Member (SM) and the Animal Welfare Officer (RBA). The RBA, a completely new role recently introduced, must have in-depth knowledge of Animal Biology to ensure the "well-being" of the animals and the right accommodation of the animals as well as the activity and skills of the technical and research personnel who work with the animals.
The specie-specific skills of the staff become even more important since the use of emerging animal models, i.e. species that are currently recognised as having less neurological complexity than those protected by legislation, is constantly increasing and represents a new and promising alternative in the field of animal testing (partial replacement).

Invertebrates and Humans: perspectives from Science, Ethics, and Policy

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Recent advances in cognitive ethology, neurobiology, and evolutionary biology increasingly challenge anthropocentric paradigms that have historically denied sentience to invertebrates. Accumulating evidence reveals complex cognitive and behavioral capacities across several invertebrate taxa, particularly cephalopods, hymenopteran insects, and decapod crustaceans. These organisms show adaptive problem-solving, long-term memory, behavioral flexibility, nociceptive responses consistent with pain perception, and signs of metacognition. Such findings support a paradigm shift toward viewing consciousness as a graded, biologically distributed phenomenon rooted in diverse neurophysiological substrates.

Within this framework, the ethical implications are profound. Recognizing sentience in invertebrates requires extending moral consideration beyond vertebrates. Applying the precautionary principle amid scientific uncertainty compels us to treat invertebrates as potential subjects of suffering, requiring refinement of experimental protocols and husbandry practices. This aligns with the 3Rs—Replacement, Reduction, and Refinement—promoting alternatives to invasive methods, minimizing animal use, and mitigating distress.
From a policy perspective, this evolving scientific and ethical landscape is shaping legislation unevenly. Cephalopods have been protected under Directive 2010/63/EU since 2013, while other taxa, such as decapods, have gained growing recognition in countries like the UK and Switzerland. In 2025, Italy amended its Penal Code by introducing Title IX-bis, explicitly recognizing animals as sentient beings and subjects of rights not limited to vertebrates, opening the door to protecting invertebrates where scientific evidence supports it.
Practical implications include that boiling crustaceans alive may be classified as mistreatment, insect farming may require minimum welfare standards, and cephalopods—already protected in research—could receive broader safeguards. This affirms that where there is sentience, there must be protection, regardless of taxonomy.
This interdisciplinary analysis highlights the scientific, ethical, and political challenges in reassessing invertebrate welfare. It advocates combining rigorous research, ethical deliberation, and policy reform to advance humane treatment of invertebrates, contributing to a redefinition of human-animal relationships and biodiversity conservation.

Rethinking Invertebrate Research: where do we draw the line between use and protection?

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For decades, even complex vertebrate species such as birds were regarded as mere "reflex machines," deemed incapable of flexible or sophisticated responses due to their brain being organized into nuclei rather than a layered cortex. Some of these species, fundamental to human nutrition worldwide, are unfortunately still exploited in intensive farming, with ethical, environmental, and health-related consequences.

However, as early as 2002, the international forum on avian brain nomenclature (Reiner et al., 2004) highlighted how mistaken this view was: with a different neural architecture, birds turned out to be capable of complex cognitive functions comparable to those of supposedly more evolved organisms, to the point of being described as "feathered apes" (Emery, 2004). This emblematic case prompts reflection on two crucial situations today.

The first concerns the widespread belief that complex cognition necessarily requires a centralized nervous system. Jellyfish often fall victim to this bias, commonly labeled "brainless" due to the radial and decentralized organization of their nerve net. This prejudice is reinforced by the scarcity of targeted behavioral studies. Yet, in juvenile Aurelia spp., we have observed behavioral markers of cognitive processes, such as short-term memory and novelty preference, challenging the notion of a complete absence of cognitive abilities (Agrillo et al., accepted).

The second concerns Tenebrio molitor, an insect promoted as a sustainable protein source (EFSA NDA, 2021) and a potential protagonist of future intensive farming. Currently lacking specific regulatory protections, T. molitor has demonstrated the ability to recognize and prefer more advantageous alternatives when motivational values of stimuli change, suggesting forms of intentional (or at least goal-directed) behavior that deserve immediate attention (Dissegna et al., 2024). This is crucial to avoid repeating, with insects, the same ethical issues - and associated impacts on animal welfare and human health - already observed in poultry farming.

These findings urge a profound reconsideration of the criteria by which we define cognitive complexity and, consequently, the boundaries between the use and protection of invertebrates. Ignoring their cognitive potential risks not only perpetuating scientific prejudices but also jeopardizing the recognition of their welfare rights, precisely at a time when new species are entering global production chains.

Wellbeing of fish used in scientific research

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The professional and technical skills required to work as figure a), b), c) and d), according to the article 23, of the Italian Legislative Decree 26/2014 must be certified as per the Italian Ministerial Decree of the 5th of August 2021, article 6 and the Italian Directorial Decree of the 18th of March 2022, article 3. The certification is obtained after the completion of specific mandatory courses. An example is the course "The use of different species of fish in scientific research" (i.e., sea bass, Salmonidae, sea bream, sturgeons and others). These courses must include the following modules and topics:
- Module 3.1: Basic and appropriate biology – species-specific
- Module 4: Animal care, health and management – species-specific
- Module 5: Recognition of pain, suffering and distress – species-specific
- Module 7: Minimally invasive procedures without anaesthesia – species-specific

All the personnel must be fully trained to accomplish their specific duties.
The Italian Ministerial Decree of the 5th of August 2021 outlines all the steps required to obtain the appropriate certifications, maintain the certifications over time and demonstrate the acquisition of the appropriate skills. Being in possess of the proper certifications is mandatory for all the personnel working as:
a) carrying out procedures on animals;
b) designing procedures and projects;
c) taking care of animals;
d) sacrificing animals.

Moreover, a Designated Doctor in Veterinary Medicine and an Appointed Officer in charge of Animal Care and Wellbeing must always be present.
When not under experimental procedures, all animals must be maintained under appropriate conditions to guarantee their wellbeing.

Swimming our way through science: the zebrafish as a research model

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The use of the zebrafish (*Danio rerio*) as a research model is gradually and continuously growing. Nowadays, the zebrafish is considered one of the most complete and flexible models in a variety of research fields: biomedical, cardiovascular, neurodegeneration, behavioural and even drug screening. This is all thanks to several specific features: embryos are transparent, which allows for better visualisation of organs and tissue development, develop all major organs within the first 5 days of life and adults have high reproduction and fertilisation rate. However, it is important to keep in mind that the zebrafish can't completely replace mammals just yet, but is considered a good alternative method that helps to drastically reduce the number of higher animals used in the scientific research. In this context, the zebrafish allows to fulfil the concept of *Reduction*, since the use of embryos helps to reduce the number of adults used, and *Replacement*, since the embryos are not regulated by the Directive 2010/63/EU and the Italian D.lgs 26/2014 due to the low neural development. It is also important to remember that the 3Rs concept also applies to the zebrafish: considering that the adults can be used for research purposes, their number should be kept to the minimum required to obtain a statistically significant result. It is also necessary to use refined techniques that can limit the amount of distress and pain caused to the animal as much as possible. Finally, even in this case, the use of embryos within 120 hours post fertilisation instead of adults is considered a replacement method. In conclusion, the zebrafish is leading the way to new possibilities and will continue to do so in the future, allowing for a gradual decrease in the number of mammals or other animals used in the scientific research.

Adaptive evolution and individual ontogenetic trajectory: the biological dimensions of welfare

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Since Ruth Harrison's 1964 book "Animal Machines" and its profound impact on public opinion and quality of life of farmed animals, scientific research on non-human animal welfare expanded to other captive settings such as research laboratories, zoos and shelters. Multiple conceptual frameworks have been produced and shared in attempts to define the complex concept of animal welfare, as well as different philosophies and scientific approaches to assess it and improve it. No matter what the emphasis has been put on (e.g., the basic health and biological functioning, a natural living, or the affective states of the animal), the more scientific research has been carried on, the more progressively the concept of welfare as an emergent property of the "animal system" has established itself. As such, welfare may be differently perceived by each individual, as the expression of the combination between its species-specific morpho-anatomical, physiological, and behavioral adaptations, together with its ontogenetic trajectory and experiential life, combining genetics with epigenetics, and species-specific with individual needs. The more recent "Five Domains Model" has prompted scientists to focus on what is now almost universally recognized as the core of an animal welfare state: its mental state. In this talk I will discuss for example the role in animal welfare and key value of: experience and emotions, with emotions teaming up with individual experience and cognition during the process of assessing situational contingencies - preparing the organism for an optimal response - as parts of the evolutionary mechanisms underlying animal decision making; the ability to cope with environmental challenges; having opportunities to exert choice and control over the environment, enhance feelings of autonomy, i.e., 'animal agency'. In the context of animals under human care, animal-centered welfare management should prioritize providing opportunities for comfort-related activities, as they are determinants of animal quality of life - 'a life worth living' – aimed at helping animals not merely to survive, but thrive. Animals under human care too often face a mismatch between their built-in "equipment" (adaptations) and the challenges of their current circumstances, whether wild or domestic species. Emphasis will also be given to the lexicon used in welfare science, of its evocative and often misleading power, since definitions and terms used affect the way animals are treated.