ABSTRACT: The European Union agreed on a directive (DIR) for the protection of animals used for scientific purposes in 2010 which was implemented by member states at the onset of 2013. The DIR applies to animals used for science or education that are subjected to pain, suffering, distress or lasting harm equivalent to, or higher than that caused by a needle. The DIR changes the legal framework for wild animal research and requires educational and training standards of staff involved in capturing, planning, or performing research. Both wild animals studied in or taken from the wild into captivity are covered by the DIR. An animal welfare body must be established that includes a scientific member and at least one person responsible for animal welfare, and they must receive input from a designated veterinarian. The DIR will aid and improve wild animal research because standards of animal welfare and research ethics must be met. Although similar standards for moose research were employed previously in Scandinavia, future moose research and conservation will likewise benefit.

INTRODUCTION

The European Union (EU) developed a new directive (DIR) for the protection of animals used for scientific purposes in 2010 (Directive 2010/63/EU), that was implemented by member states (MS) at the onset of 2013. The principle reasons for the DIR were to standardize legislation between MS and to improve the welfare of animals used in scientific research and procedures. This action was actualized by the increasing scientific knowledge about factors that influence animal welfare (AW), as well as the capacity of animals to sense and express pain and suffering. Ethical concerns of the general public were also influential, and the desire to replace the use of animals for scientific purposes with non-animal alternatives. It was recognized that animals have an intrinsic value which must be respected, they should always be treated as sentient creatures, and their use should be restricted to benefitting human or animal health, or the environment. Thus, MS must ensure that live animals are not used when a scientifically satisfactory method or testing strategy not entailing the use of live animals is available. Wildlife research with wild ruminants had been regulated previously by national legislation in Scandinavian countries; however, no comprehensive European regulations existed.

Moose (Alces alces) in the wild have been used for scientific purposes in northern Europe for decades and been used in theoretical and applied ecological research covering a wide range of topics: predator-prey interactions, herbivore-plant interactions, ecology, behavior, migration, veterinary medicine, disease, and moose-human
interactions using a variety of scientific methods and procedures, and technical and analytical approaches. Moose provide an excellent case study for educating and explaining the DIR to current wildlife researchers. In this paper we aim to inform researchers about the content and intent of the DIR, and identify the consequences of its implementation for future moose research.

**DESCRIPTION**

The DIR states that animals taken from the wild (e.g., ruminants) shall not be used for scientific studies unless a competent authority has granted an exemption. The exemption shall only be granted if the purpose of the procedure cannot be achieved by the use of an animal which has been bred for such use. Captures must be performed with care by competent persons such that an animal is not caused any avoidable pain, suffering, distress, or lasting harm. If a wild animal is found to be injured or in poor health at or after capture, it must be examined by a veterinarian or other competent person; action shall be taken to minimize the suffering of the animal. Competent authorities may grant exemptions from the requirement to minimize suffering of the animal if there is scientific justification.

Scientists using research animals need to understand the DIR legislation to identify which parts apply to and affect their research. This will facilitate experimental design and planning, minimize the number of animals used, and ensure that their research is within the legal framework. The DIR applies to all animals that are used for scientific or educational purposes that are subjected to pain, suffering, distress, or lasting harm equivalent to, or higher than that caused by the insertion of a needle, in accordance with good veterinary practice. This includes moose studied in or taken from the wild and kept in captivity if subjected to procedures that cause “pain” or the equivalent to pain; e.g., an immobilized moose fitted with ear tags or radio-collar. All users of moose for scientific purposes must be authorized and registered by a competent authority.

Research projects must pass an ethical evaluation to receive authorization for use of animals in research or teaching; the use is evaluated and justified relative to the societal, scientific, and/or educational purpose of the project. The project should be designed such that procedures are in compliance with the requirement of the 3R’s (i.e., Replacement of the use of animals for scientific studies, Reduction of the number of animals used, Refinement of procedures; Russell and Burch 1959). The evaluation of the project shall be performed in an impartial manner, the evaluation process must be transparent, and it shall weigh the predicted societal and scientific benefits, or educational value of the project against the harm and suffering of the research animals. Furthermore, the evaluation shall verify that the project is designed such that procedures are performed in the most humane and environmentally sensitive manner possible. The ethical evaluation shall also determine whether the project should be evaluated retrospectively, concerning the AW and outcome of the study. For all projects, a non-technical summary written by the project leader/researcher shall be published by the MS to facilitate communication with the general public. Before initiation of research, any staff involved in capturing, handling, planning, or performing research must be formally educated and trained until proven competent to perform their tasks.

All users and breeders of animals for research must form an AW body which shall include the person responsible for AW and care, and in the case of a user, a scientific member; the body shall also receive input from the designated veterinarian. The
primary task of this body is providing advice on AW issues and the outcome of AW in projects. The body should also foster a climate of care and provide tools for the practical application and timely implementation of recent technical and scientific developments in relation to the principles of the 3 R’s to enhance the life-time experience of research animals. The advice of the AW body should be properly documented and open to scrutiny during inspections.

The MS shall ensure that an animal may only be reused in a new procedure provided that the actual severity of any previous procedure was ‘mild’ or ‘moderate’ and that the general state of health and well-being has been fully restored; veterinary advice shall be taken into account regarding the lifetime experience of the animal. The MS may allow animals used in procedures to be returned to a suitable habitat appropriate to the species, provided that the state of health of the animal allows such and there is no danger to public health, animal health, or the environment. Appropriate measures shall be taken to safeguard the wellbeing of the animal.

APPLICATION TO MOOSE

A typical radio-collaring project with moose that is managed by a university or research institute in Europe needs to comply with the DIR by adopting the following protocol chronologically: 1) the department has to be granted a general permit to use animals in research for a limited period (years); 2) a local group (e.g., AW organization) should be formally appointed at the department level to help oversee AW; 3) the project leader should write an application describing the purpose (what, why, when, how) of the study relative to AW and how the project complies with the 3 R’s; 4) the application is signed by both the project leader and head of the local AW group; and 5) the application passes a review by a national ethics board. A permit to radio-collar a defined number of moose would then be approved for a maximum of 5 years.

Research studies involving capture and restraint can be stressful and cause measurable harm to moose, and can also influence experimental assumptions and data. The DIR permits competent authorities to exempt the requirement to minimize the suffering of wild-caught animals found to be in poor health or injured, given scientific justification. The DIR states that the assessment of health and welfare of the animals must be performed by a competent person. Assessment of competence is based upon an appropriate level of understanding animal behavior, biology, and ecology of the species, and the ability to recognize poor health, injury, discomfort, pain, and distress. It is important to minimize the disturbance of a study population and understand the potential pathologies related to the capture activity, and how to prevent sickness and take appropriate actions in the case of poor health or welfare of a captured animal. Proven competence is required for capture, handling, and restraint techniques including the operation and maintenance of any trapping devices. The idea of wild animals suffering is of concern for the legislating bodies, as well as scientists and the general public.

Concern regarding AW is related not only to marking methods, but also capture and handling procedures prior to, during, and after marking and release. Combined long-term effects associated with these procedures and activity can occur at both the individual and population level.

To our knowledge, the first chemical immobilization of a wild moose for research purposes in Europe took place in 1975 at Grimsö Wildlife Research Area in south-central Sweden (Sandegren et al. 1987); > 2800 moose have been immobilized throughout Scandinavia since (Arnemo et al. 2006). Moose are usually darted from a helicopter
and marking is performed under general anesthesia with typical surveillance including pulse- and respiratory rate, body temperature, and sometimes arterial oxyhemoglobin saturation (SpO₂). Chemical capture and anesthesia of free-ranging mammals involves some risk of mortality even in healthy animals. A Scandinavian study on immobilization of moose estimated such mortality as 0.7% (n = 2,816) with 0.2% related directly to the immobilization procedure (Arnemo et al. 2006). Even if mortality is the most apparent negative side-effect of marking wild animals, other subtle, stress-induced biases are critical to identify because of their potential influence. For example, it is recommended to omit data from the initial 5 days post-capture when measuring moose movement and distribution (Neumann et al. 2011).

To help achieve zero mortality in Scandinavian moose research, 3 factors have been identified: 1) use an experienced, trained professional capture team, 2) develop and follow a species-specific capture protocol, and 3) require a mortality assessment after any capture-related death to provide a knowledge-based evaluation of the capture protocol. This approach complies with the intent of the DIR and should be followed by all moose researchers.

When recapturing and re-marking the same animal, the three R’s should be employed to reduce the risk of impaired AW in moose research, conservation, and management. Specific considerations are: 1) replacement strategies by which the required information may be obtained by other means than marking live wild animals, 2) educational strategies to use the fewest animals possible for providing valid information and statistical significance, and 3) refining strategies to use the most humane, least invasive marking techniques with the goal of minimizing pain and distress. These requirements are already in the spirit of most, if not all contemporary moose researchers.

In addition to ecological studies with radio-collared moose, both physiological and pathological studies have been performed on captive moose housed indoors. For example, several successive Swedish studies of infectious diseases in moose including brainworm (Elaphostrongylus alces) and moose wasting syndrome involved calves obtained from the wild, and subsequently penned and raised in stalls (Stéen et al. 1997, Broman et al. 2002). The DIR classifies facilities where wild animals are housed for experimental studies as ‘establishments’ and defines them as any installation, building, group of buildings, or other premises, and may include a place that is not wholly enclosed or covered, or a mobile facility. The MS must ensure that an establishment has installations and equipment suited to the species of animal housed and related research procedures.

The DIR states that animals removed from the wild shall not be used for scientific studies unless a competent authority has granted an exemption; of concern is the future of research requiring the capture, mark, and indoor housing of wild animals. To grant exemption for future studies of this type, competent authorities will require adequate knowledge of the behavior, biology, and veterinary studies of multiple wild species. If granted, the requirements of the DIR are beneficial for all study participants including researchers, veterinarians, and field assistants since they will participate in project planning. This planning would include educational requirements of the various participants and their responsibilities, experimental design, ethical concerns, and the AW of the study animal. The practical implications of the DIR should be advantageous for research with wild animals overall.
and specifically benefit moose research and conservation globally.

REFERENCES


