Effects of the Directive 2010/63/EU on Research on Wild-Sourced Animals: A Review of Publication Trends in Studies on Captive Wild Mice (*Apodemus* sp.)

Maílis Carrilho[1], Sophie von Merten[1,2], Maria da Luz Mathias[1], Joaquim T. Tapisso[1]

[1] CESAM – Centre for Environmental and Marine Studies and Department of Animal Biology, Faculty of Sciences of the University of Lisbon, 1749-016 Lisbon, Portugal

[2] Department of Environment and Biodiversity, University of Salzburg, Hellbrunner Straße 34, 5020, Salzburg, Austria

Corresponding author: Maílis Carrilho (mdcarrilho@fc.ul.pt)

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ABSTRACT

Most animals used in experimentation are small mammals. In the European Union, the Directive 2010/63/EU regulates the use of laboratory animals for experimental purposes. However, there are only few guidelines for the use of wild-sourced animals, which cover permits, experimentation, transport, maintenance, and setting free after experiments. To evaluate the

effect of the Directive on the study of wild-sourced animals, we conducted a systematised literature review focusing on three widespread rodents of the genus *Apodemus*: *Apodemus agrarius*, *A. flavicollis* and *A. sylvaticus*. We selected studies performed across the EU, published before (2000-August 2010), during (September 2010-2012) and after the implementation of the Directive (2013-2022). From those, we collected data on three main topics: i) authorization; ii) care and accommodation and iii) methods of killing. We found that after the implementation of the Directive a higher proportion of studies published provided information about authorization. In contrast, there was no significant difference over time in the information given about care and accommodation of animals and the methods of killing. As such, our analysis suggests that there is still room for improvement to achieve consistency across journals publishing research involving wild-sourced small mammals. Specifically, editors should require the provision of detailed information by authors regarding proper animal care (e.g. more detailed care and accommodation protocols). To harmonize the information requested by different editorial boards, we recommend the addition of specific guidelines in the Directive regarding wild animals, particularly on proper accommodation, manipulation, enrichment and veterinary control.

1. INTRODUCTION

In 2019, the total number of animals involved in research and testing for the first time in the European Union and Norway was around 10.4 million.[1] About 62% of these animals were mice and rats, which remain the preferred models, especially for biomedical studies.[1,2] The use of laboratory animals for experimental and/or other scientific purposes is currently well-regulated across the European Union by the Directive 2010/63/EU (referred to as “Directive” from now on),[3] a community directive that each member state transposed to national law. This Directive classifies

45 the severity of experimental procedures (i.e. non-recovery, mild, moderate or severe) and
46 standardizes the laboratorial conditions in which they should be performed,[4,5] in order to ensure
47 that the tested animals receive adequate care, and any suffering, distress and pain inflicted during
48 the studies is minimized. The importance of these guidelines has already been shown,[6,7]
49 especially regarding the 3Rs principles (reduction, replacement and refinement; introduced by
50 Russell & Burch, 1959[8]). While the Directive was elaborated mainly considering laboratory
51 animals, these principles are also applied when wild-sourced models are used in experimental
52 procedures. Additionally, some recommendations are given for the acclimatization and release of
53 these animals after the experimental captivity period.

54 Studies with wild small mammals in laboratory conditions can be highly relevant as a
55 complement to field studies.[9] However, the maintenance of wild animals in a captive environment
56 can induce behavioural and physiological adjustments that may greatly differ from those of
57 laboratory animals, as the latter are reared and maintained in captivity throughout their live.[10]
58 These differences justify the relevance of studying the specificities related to the maintenance of
59 wild small animals in captivity.[11]

60 The Directive does not give any specific details on necessary permits to perform experiments,
61 transport, housing, and procedures of setting free when referring to the use of wild-sourced
62 animals in experimental research.[12] In addition, no information is provided on the most adequate
63 duration of captivity or on the methods of killing, when necessary. Given the lack of specific
64 recommendations, it is important to understand the current interpretation of the Directive by
65 researchers studying wild animals. While the impact of the Directive on studies involving
66 laboratory animals has been analysed,[13,14] we are at this moment unaware of similar analyses
67 focussing on wild-sourced animals, especially small mammals. Such studies are crucial to
68 understand whether the Directive's requirements and recommendations have influenced

researchers' approaches to the care and experimentation of wild models in captivity and the way they document their findings in scientific publications.

The objective of our study was to assess the impact of the Directive on studies involving the use of wild-sourced animals in the European Union. Specifically, we aimed to determine the impact of the Directive on parameters linked to i) the reporting of permits, ii) the description of care and accommodation, and iii) the methods of killing. As model species for this study, we choose three species of rodents of the genus *Apodemus*: the striped field mouse (*Apodemus agrarius*), the Yellow-necked mouse (*A. flavicollis*) and the wood mouse (*A. sylvaticus*). These species are widely distributed and relatively abundant across Europe and are not protected by any law or convention (IUCN status: Least concern).[15–17] In addition, they are easily captured and commonly maintained in captivity for research purposes. Our expectations were to gather insights into the interpretation of the Directive recommendations by zooming into the use of wild-sourced *Apodemus* sp. in experimental procedures conducted in captivity.

2. METHODS

2.1. Literature Search and Study Selection

To analyse the impact of the Directive on the study of wild-sourced animals, we conducted a systematised literature review focusing on three widespread rodent species: the striped field mouse (*Apodemus agrarius*), the yellow-necked mouse (*A. flavicollis*) and the wood mouse (*A. sylvaticus*).

We performed a systematic search for scientific articles that involved at least one of the three target species and were published between 2000 and 2022. The main search was performed in June 2024 using three databases: Web of Science, Scopus and PubMed. An initial

search had already been conducted in December 2020 on Web of Science. However, all the publications retrieved in 2020 were also found in 2024. The search strings used contain different ways of writing the scientific identification of the target species and were adjusted according to the specifications of each database (**Table 1**). The results were retrieved from each database and managed using a spreadsheet (Microsoft Office Excel). We excluded all double entries and removed any studies not published in English. The studies were then downloaded from Web of Science in pdf format if available, or directly from the journal website. We screened all the retrieved studies for eligibility according to the following two criteria: i) studies conducted in the European Union countries compliant with the Directive **(Table S1** – Supplementary Material**)**, and ii) studies involving one of the three mouse species maintained in captivity for experimentation or retention purposes. The screening process was performed manually by one author (MC) by reading the abstract and methods section, resulting in a new spreadsheet containing relevant information for the identification of each selected study (i.e. title, authors, abstract; see Supplementary Material - "**Data_Directive_Apodemus.xlsx**"). In order to detail how this systematised literature review was conducted, a filled PRISMA 2020 Checklist [18] is provided as Supplementary Material ("**PRISMA_2020_Checklist.docx**").

Table 1. Search strings obtained from each database search.

Database (2024)	Search String	Specific Definitions
Web of Science	TS=("apodemus agrarius" OR "apodemus flavicollis" OR "apodemus sylvaticus" OR "a. agrarius" OR "a. flavicollis" OR "a. sylvaticus" OR "a.agrarius" OR "a.flavicollis" OR "a.sylvaticus") AND PY=(2000-2022)	Not applicable
PubMed	(Apodemus agrarius) OR (Apodemus flavicollis) OR (Apodemus sylvaticus) OR (a. agrarius)) OR (a. flavicollis) OR (a. sylvaticus) OR (a.agrarius) OR (a.flavicollis) OR (a.sylvaticus)	Time interval: 2000-2022
Scopus	TITLE-ABS-KEY("apodemus agrarius" OR "apodemus flavicollis" OR "apodemus sylvaticus" OR "a. agrarius" OR "a. flavicollis" OR "a. sylvaticus" OR "a.agrarius" OR "a.flavicollis" OR "a.sylvaticus") AND PUBYEAR > 1999 AND PUBYEAR < 2023	Not applicable

2.2. Data Extraction

From all studies included in the review, relevant data (see below) was extracted and registered in the spreadsheet containing the list of the selected scientific publications. The choice of data to extract was made considering the specific topics addressed by the Directive. For each variable, a specific column was created in the spreadsheet. All selected scientific publications were read twice by the same author (MC). The relevant data was simultaneously extracted by the same author. A number of randomly selected studies in the resulting table was checked and entries confirmed by reading the respective study by two other authors (SvM, JTT). All the chosen variables, as well as the extracted data, are presented as Supplementary Material - **"Data_Directive_Apodemus.xlsx".**

The data extracted from the selected articles can be grouped in three main topics: (1) authorization, (2) care and accommodation and (3) methods of killing animals in lethal studies. For each topic, we registered the following parameters:

(1) Authorization:

i) Capture permit – binary variable indicating if an article refers to permits issued by a competent authority allowing the capture of wild animals (e.g. capture licence number such as: "*ASAB/ABS, 2012*"[19], "*49/2008/CAPT*"[20]);

ii) Project/Ethical permit – binary variable indicating if an article refers to a project and/or an ethical permit issued by a competent authority (e.g. ethical license number such as: "*protocol # 066/2010*"[21]);

(2) Care and accommodation of animals:

i) Husbandry/care of animals – binary variable indicating if an article refers to the type of provision of food or water (e.g. "*ad libitum*"[22] or controlled[23]) and/or type of food (e.g. "*standard chow diet*"[24]; "*seeds*"[25]).

ii) Acclimatization/quarantine – binary variable indicating if an article refers to an acclimatization or quarantine period in terms of duration and macro-environmental conditions such as room temperature, humidity or photoperiod;

iii) Housing – binary variable if an article refers to any aspect of housing conditions such as cage type, substrate or environmental enrichment like shelter (e.g. "*cardboard tube-shelter*"[26], "*plastic tubes*"[27]) or nesting material (e.g. "*hay*"[28], "*pressed cotton*"[23]);

iv) Transport – binary variable indicating if an article refers to transport conditions such as containers, means of transport or duration (e.g. transportation in the trap[29], transportation in a standard rodent cage[30]);

v) Setting free of animals, if applicable – binary variable indicating if an article refers to any kinds of measures taken prior to setting free of animals, such as verification of health status, rehabilitation programs, habitat adequacy or provision of food and water during transportation (e.g. transportation in a waterproof shelter with provision of hay and food[31], previous feeding before setting free[32]) .

(3) Methods of killing animals in lethal studies:

Euthanasia procedures – binary variable indicating if an article refers to the method of killing, and, if such reference exist, categorical variable indicating the specific method used (anaesthetic overdose, carbon dioxide, cervical dislocation, concussion, decapitation, other methods).

From all the information retrieved, proportions of articles referring to the respective parameter could be calculated for statistical analysis.

Following the data extraction, we grouped the resulting papers into three different time periods, according to the year of publication, for further analysis: i) 2000-August 2010, ii) September 2010-2012 and ii) 2013-2022, representing, respectively, the periods before, during and after the implementation of the Directive.

Additionally, we compared the proportion of articles referring to a capture or project/ethical permit between different types of studies and between invasive and non-invasive studies. Types of studies were behavioural (e.g. cafeteria tests to assess food preference[33], test spatial memory

retention in the Morris water maze[34]), physiological (e.g. blood sampling to evaluate metal levels[35], measurements of body temperature[36]), post-mortem (e.g. collection of bone marrow[37], epididymal sperm extraction[30]) and none (only for retention purposes – e.g. equipping animals with collars for identification[38] or avoidance of recaptures within a certain amount of time[39]). Invasiveness of each study was defined according to the Chapter I - Article 3 (Definitions – Point 1) of the Directive (examples of invasive procedures: toxicity testing, tumour inducing, severe movement restriction; examples of non-invasive procedures: open-field testing, non-invasive imaging of animals, assessing body composition by non-invasive measures).

2.3. Data Analysis

All the data was analysed using the statistical software R.[40] To test the differences between the proportions of scientific publications referring to specific topics during the different time periods (before, during and after the implementation of the Directive), we performed Chi-square tests using the function "CrossTable" of the package "gmodels".[41] Yates correction was used in cases with one degree of freedom and/or small frequencies, as in the case of post-hoc Chi-square tests performed to test for differences between the specific pairs of time periods. We tested for differences between time periods in the parameters related to authorization (i.e. reference to capture and project/ethical permit), care and accommodation (i.e. reference husbandry/care, acclimatization/quarantine, housing, transport and setting free) and methods of killing (i.e. reference to and type of euthanasia procedures). Additionally, we tested for differences between time periods in reference to capture and project/ethical permits by invasiveness of study (i.e. invasive and non-invasive) and type of study (i.e. behavioural, physiological, post-mortem and none).

2. RESULTS

Our literature search resulted in 2598 studies from Web of Science, 1132 from PubMed and 1789 from Scopus, which included at least one of the three target *Apodemus* species and were published between 2000 and 2022 **(Figure 1)**. One hundred and forty-two of those papers involved studies with individuals maintained in captivity for various scientific purposes **(Figure 1)**. All the selected studies found on PubMed and Scopus were also found on Web of Science. Sixty-four of them involved studies published before the implementation of the Directive (2000- August 2010), 20 during (September 2010-2012) and 58 after (2013-2022) **(Figure 1)**. This set of scientific articles considered for analysis is presented as Supplementary Material **("Data_Directive_Apodemus.xlsx")**.

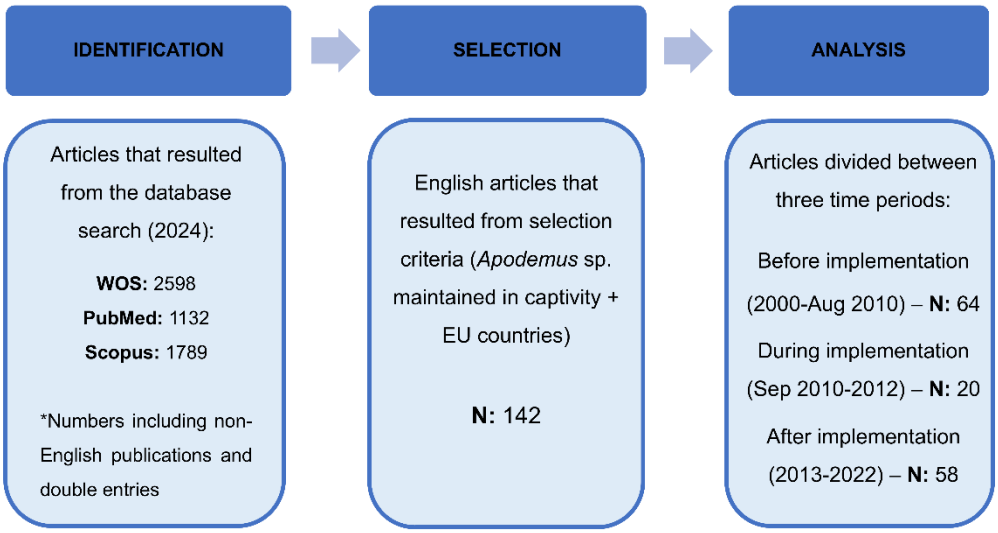


Figure 1. Protocol design. This flow diagram gives the numbers of articles resulting from the three stages of the literature search, namely identification, selection and analysis. "Implementation" refers to the implementation of the Directive 2010/63/EU. WOS: Web of Science.

3.1. Data Analysis Results

Regarding authorization, we found a significant association between the proportion of studies with a reference to a capture permit and/or a project/ethical permit and the time of publication **(Table 2**). After the implementation of the Directive, more studies referred to permits than before its implementation (**Figure 2; Table 3**).

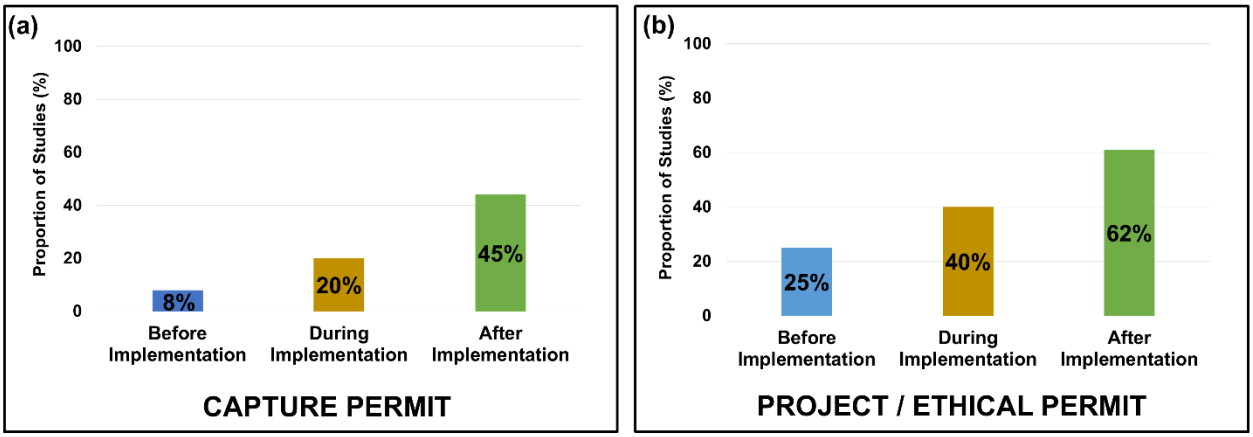


Figure 2. Authorization: Proportion of studies with reference to (a) Capture permit and (b) Project/Ethical permit.

Table 2. Results of Chi-square tests analysing the impact of time period (before, during and after the implementation of the Directive) on the proportion of studies that reported parameters related to authorization, care and accommodation and methods of killing. Significant results are highlighted in bold (p≤ 0.05).

Variable	*Chi-square (X^2)*	*df*	*p*
Capture permit	**22.716**	**2**	**< 0.001**
Ethical permit	**17.183**	**2**	**< 0.001**
Husbandry/care	1.878	2	0.391
Acclimatization/quarantine	0.709	2	0.702
Housing	2.463	2	0.292
Transport	2.543	2	0.280
Setting free	1.030	2	0.598
Methods of killing	1.523	2	0.467
Specific methods of killing	12.803	10	0.182

Table 3. Results of post-hoc Chi-square tests (with Yates correction) analysing the impact of time period (before, during and after the implementation of the Directive) on the proportion of studies that reported information regarding capture or project/ethical permits. Significant results are highlighted in bold (p ≤ 0.05).

Variable	Pairwise	*Chi-square (X^2)*	*df*	*p*
Capture Permit	Before Directive – During Implementation	1.264	1	0.261
	During Implementation – After Directive	2.895	1	0.089
	Before Directive – After Directive	**20.085**	**1**	**< 0.001**
Ethical Permit	Before Directive – During Implementation	1.025	1	0.311
	During Implementation – After Directive	2.117	1	0.146
	Before Directive – After Directive	**15.614**	**1**	**< 0.001**

The increase of references to capture permits was significant for behavioural, physiological and post-mortem studies. The increase of references to project/ethical permits was significant for physiological and post-mortem studies (**Table 4; Figure 3**). The proportion of studies with a reference to capture or project/ethical permits did not differ between invasive studies (capture permit vs invasiveness: p=0.821; project/ethical permit vs invasiveness: p=0.460) (**Figure 4**).

Table 4. Results of Chi-square tests analysing the impact of time period (before, during and after the implementation of the Directive) on the proportion of studies that referred to capture or ethical permits, separated by type of study (behavioural, physiological, post-mortem and none). Significant results are highlighted in bold ($p \leq 0.05$).

Type of Study	Variable	*Chi-square (X^2)*	*df*	*p*
Behavioural	Capture Permit	**15.975**	**2**	**< 0.001**
	Ethical Permit	5.571	2	0.062
Physiological	Capture Permit	**6.120**	**2**	**0.047**
	Ethical Permit	**11.659**	**2**	**0.003**
Post- Mortem	Capture Permit	**8.885**	**2**	**0.012**
	Ethical Permit	**9.170**	**2**	**0.010**
None	Capture Permit	4.444	2	0.108
	Ethical Permit	3.429	2	0.180

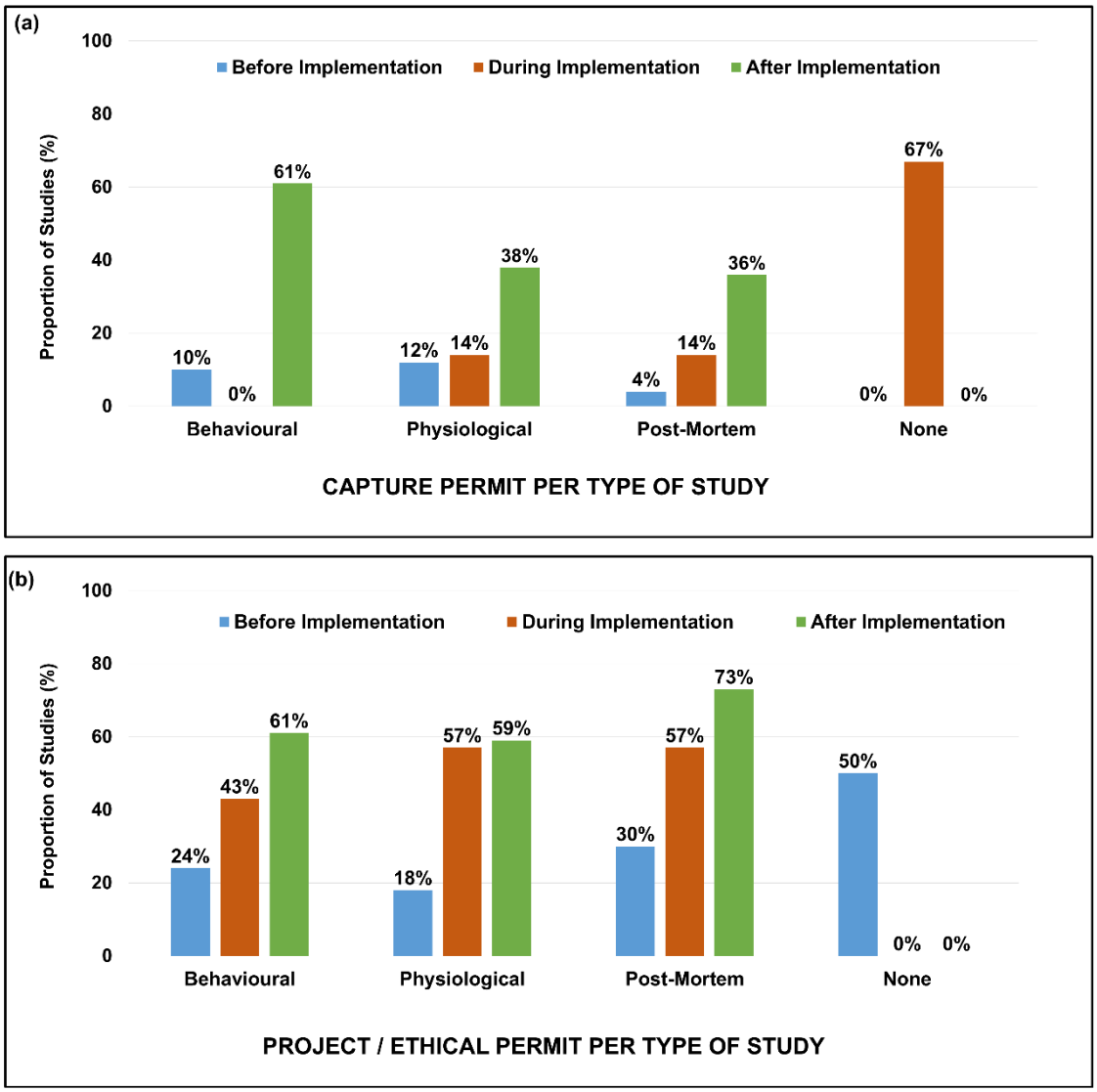


Figure 3. Permits by type of study: Proportion of studies with reference to (a) Capture permit and (b) Ethical permit, by type of study. Types of study are behavioural, physiological, post-mortem and "None" (i.e. only for retention purposes).

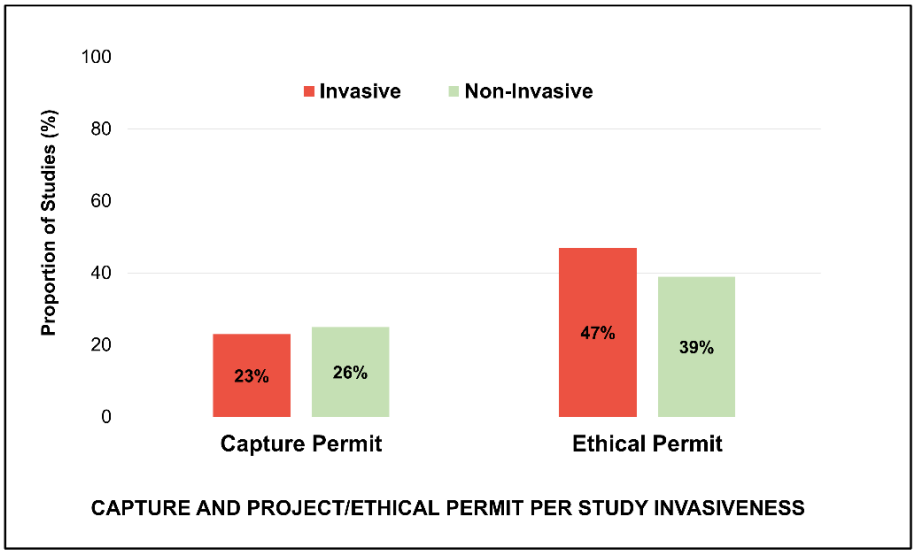
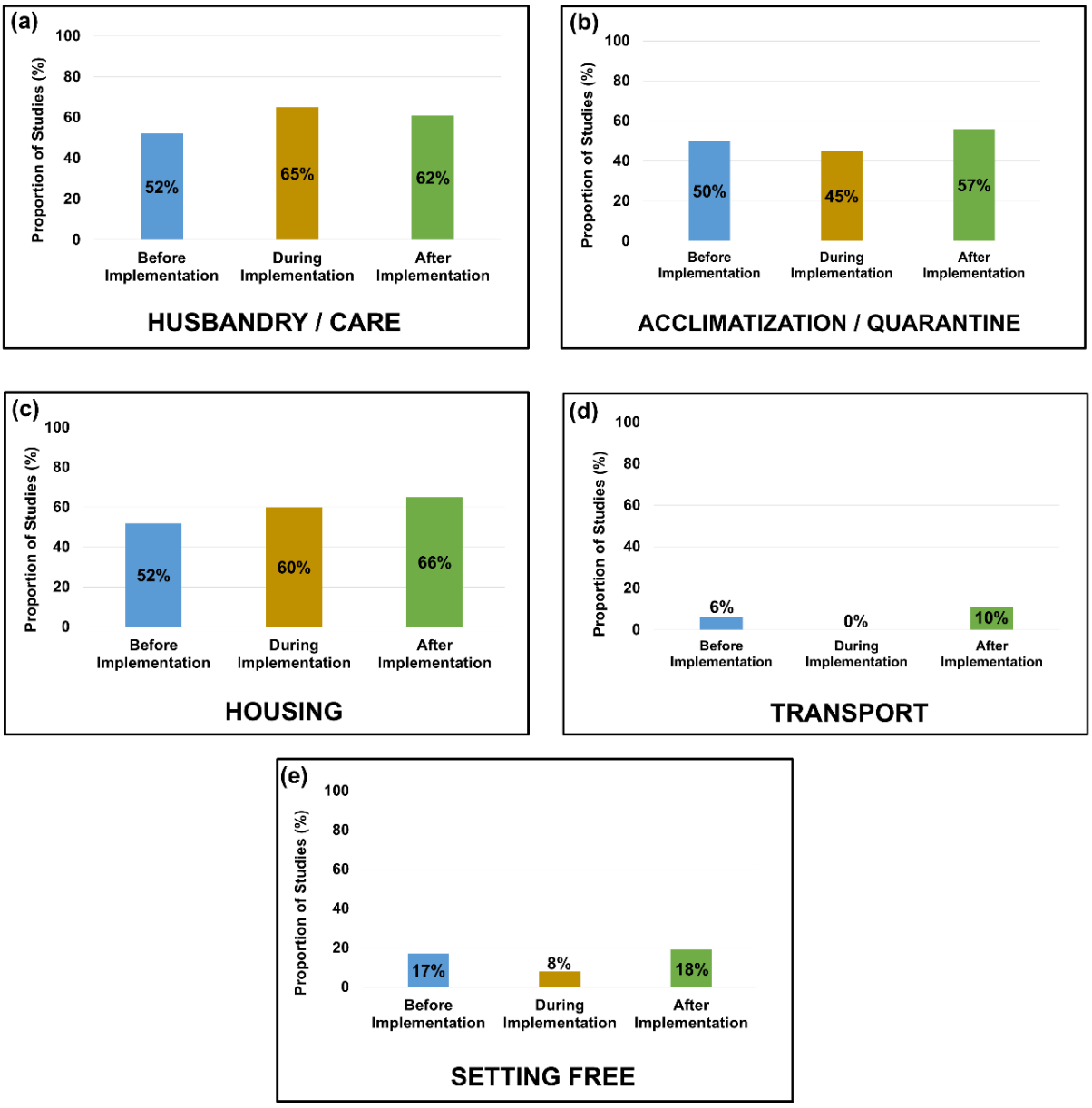


Figure 4. Permits by study invasiveness: Proportion of studies with reference to Capture permit and Ethical permit by study invasiveness (i.e. invasive and non-invasive studies).

Regarding care and accommodation, we did not find a significant association between the proportion of studies with a reference to the analysed parameters and time of publication. While references to husbandry/care, acclimatization/quarantine and housing increased slightly after the implementation of the Directive, none of the differences were significant (**Figure 5; Table 1**). Regarding transport conditions and setting free of animals, we noticed a lack of information in most scientific publications analysed, which may mask any differences (**Figure 5**).



287 **Figure 5.** Care and accommodation: Proportion of studies with reference to (a)

288 Husbandry/care, (b) Acclimatization/quarantine, (c) Housing, (d) Transport and (e) Setting free.

289

Forty four percent of the selected studies (n=62) involved lethal procedures, that is, animals were killed during the respective study. Among those, 29 studies were conducted before, eight during and 25 after the implementation of the Directive. We found no significant association between the proportion of studies with reference both to the method of killing and to the specific method of killing and the time period. **(Figure 6; Table 1)**

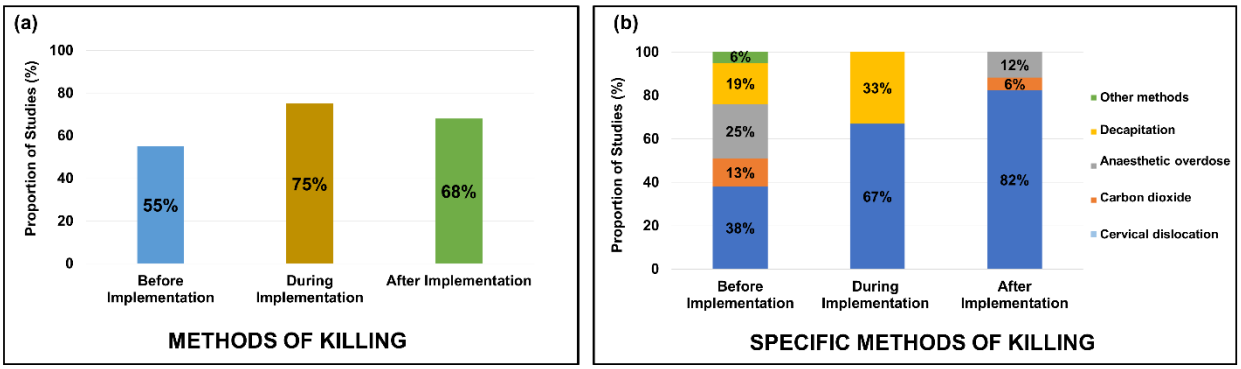


Figure 6. Methods of killing: (a) Proportion of lethal studies with reference to the method of killing and (b) Proportion of lethal studies with reference to a specific method of killing.

3.2. Information Lacking in Analysed Studies

During the extraction of data, we noticed a lack of information regarding several parameters among those previously chosen according to the topics of the Directive. The parameters with more than 80% of data absence (i.e. stated as "NOINFO" in Supplementary Material - **"Data_Directive_Apodemus.xlsx"**) and those that we considered to be redundant for this review were disregarded for the analysis. We found a lack of information about the duration of trapping sessions and trap mortality. The same was observed for the conditions of transport

311 (duration and cage type) used to bring the captured animals to the keeping facility. There was
312 also a lack of information regarding several macroenvironmental variables (e.g. sound insulation,
313 ventilation and humidity conditions of the keeping facilities and several microenvironmental
314 variables (e.g. specific enrichment type, nesting and shelter material). Information regarding
315 regular monitoring of animals during the captivity period was also lacking. Finally, we observed a
316 lack of information regarding methods of setting free, including transport and control of stress
317 indicators prior to release.
318

319 ## 4. DISCUSSION

320
321 In this review, we found that subsequent to the implementation of the Directive
322 2010/63/EU, the proportion of publications reporting information on permits requested in studies
323 involving wild rodent species (e.g. *Apodemus'* sp.) was significantly higher. This was consistent
324 across different types of studies. In contrast, there was only a slight and not significant increase
325 in information provided on care and accommodation and methods of killing. Additionally, we noted
326 a lack of information regarding transport and setting free of animals, both before and after the
327 implementation of the Directive.
328

329 ### 4.1. Impact of the Directive on Information on Permits

330
331 The increase in references to capture or project/ethical permits might be explained by the
332 increasing requirements of scientific journals regarding welfare policies for the publication of
333 fundamental research. As highlighted in a review focused on field studies, until late 2017,

334 approximately one third of journals in the fields of “Biodiversity Conservation” and “Zoology”
335 lacked explicit animal care policies.[42] Even among those journals that already had such policies,
336 only 34% enforced compliance from authors, and only 14% required documentation of relevant
337 permits (permit/licence numbers). However, the cited review focused on field studies, and we are
338 not aware of a similar analysis on studies involving the maintenance of wild animals in captivity.
339 The lack of specific recommendations for keeping wild animals in captivity might affect the
340 editorial boards of journals to not require specific proofs of compliance, such as permit numbers
341 issued by competent authorities. For example, a review on field research on fishes revealed that
342 about half of the journals analysed did not mention any requirements or recommendations
343 regarding ethics and animal welfare in their publication guidelines, and only 18% had specific
344 ethic guidelines.[43] Nonetheless, despite the Directive primarily targeting laboratory animal
345 research, its implementation may have encouraged several journals to incorporate additional
346 requirements in their editorial rules regarding animal care policies for studies involving wild
347 animals. The Directive clearly states the need for authorization for studies or projects involving
348 animals brought from the wild, specifying that a justification must be provided as to why the
349 scientific aim of the study cannot be achieved with animals raised in a laboratory.[3] In many cases,
350 research with wild animals is conducted in the field. However, when there is a need to capture
351 wild animals and bring them in captivity for research purposes, they may experience the stressful
352 effects of transport and the exposure to a new and more restrictive environment.[44] Therefore, the
353 requirement of project/ethical and capture permits issued by competent authorities is justified as
354 a certification that researchers have the competence to follow the best practices concerning
355 capture, manipulation and maintenance of wild animals. Also, the requirements set by funding
356 agencies regarding ethics can play a crucial role in ensuring compliance with animal care policies.
357 For example, Horizon 2020, one of EU's largest research and innovation funding programmes,

require ethics self-assessment to be added to the research proposal and the provision of the necessary permits for animal acquisition and experiments.[45]

4.2. Impact of the Directive on Information on Care and Accommodation and Methods of Killing

The reporting of care and accommodation practices did not increase significantly during or after implementation. This might be due to a comparatively high proportion of studies already providing such information before the implementation of the Directive, which was not the case for the permits. One explanation is that well before the implementation, the 3Rs principles[8] had already been addressed, contributing for further development of techniques and guidelines regarding the maintenance of laboratory animals, which researchers could follow and report in a way that allows the reproducibility of studies. Nonetheless, while researchers might feel compelled to adapt guidelines targeted on laboratory animals to wild animals, the inherent differences between them must be acknowledged. Generally, information on the transport was rarely provided. We believe this issue is related to the lack of specific information on these aspects in the Directive.[3] However, this topic should not be disregarded as studies have found an influence of transportation on stress levels both for laboratory mice (e.g.[46]) and rats (e.g.[47]). Such an influence may be even more pronounced in wild rodents, as high mortality rates after the transport of wild small mammals have been reported in some cases[48]. Also, a lack of information on setting free of animals was observed. This might be related to a lack of specific guidelines in the Directive and the fact that researchers possible consider such information irrelevant, as the release of animals does not influence the outcome of their own study.

Finally, there was also no increase in the information provided on killing methods after the implementation of the Directive. Instead, the proportion of lethal studies, independent of the method used, before, during and after the implementation of the Directive stayed approximate the same (45% before; 40% during; 44% after). This result can likely be explained by the fact that lethality is more related to the type of study being conducted than ethical concerns. A review on the use of lethal, invasive or non-invasive DNA sampling techniques in several animal groups showed that in rodents, lethal sampling accounted for 56% of the studies.[49] While the mere purpose of DNA sampling does not necessarily require killing of animals, in certain types of studies killing is an implicit requirement (e.g. [50] – internal parasites collection; [51] – bone marrow collection). In this regard, it is crucial to consider minimizing the suffering of the animals to the greatest extent possible[7,52] during invasive techniques or killing, in which the "refinement" of the techniques is primordial. While, next to refinement and reduction, also the replacement of animals is included in the 3Rs principles,[7,52] many studies have yet to adopt the use of in-vitro or in-silico techniques as viable alternatives. One reason might be the costs, which can compel researchers to not choose this option due to financial constraints.[53]

On a final note, it is important to recognize that researchers face several constraints while studying wild animals, since their traits are more closely linked to natural conditions.[11] One major obstacle for the compliance with good animal practices is the weakness of ethics training in biological sciences across Europe. A study from 2017 revealed that only 9% of the undergraduate programs in biology, ecology and life sciences include ethics as a mandatory course.[54] This can result in researchers lacking knowledge concerning ethical questions and requirements, as well as best practices for the maintenance and experimentation involving wild animals. The need for training is also addressed in Annex V of the Directive, with a list of topics that should be included in experimentation science training.[3] The solution for the recognition of competences has been

set by FELASA (Federation of European Laboratory Animal Science Associations) through the development of courses that provide accreditation for carrying out functions related to research involving animals (A - carrying out procedures on animals, B - designing procedures and projects, C - taking care of animals, D - killing animals).[55]

4.3. Potential Limitations

While we included several databases and used a predefined protocol regarding the selection of publications and the extraction of data from the selected publications, some limitations should be considered. One of the limitations is the selection of scientific publications and extraction of data by one observer. However, two additional authors cross-checked the data to guarantee that all the necessary information was correctly extracted. Further, as our study does not present a meta-analysis on data that had been collected in the course of the analysed publications, but rather a de-novo analysis of information provided inside those publications, we do not present effect measures or statistical synthesis methods. A potential source of a risk of bias in the studies included in this review might stem from the editorial policies of journals, which might have different strategies regarding the documentation of ethical concerns and animal care practices. This is indeed an important point and was discussed above.

5. CONCLUSIONS

We found a significant increase in references to authorization parameters, but only a slight and not significant increase in reference to care and accommodation and methods of killing in publications following the implementation of the Directive 2010/63/EU. However, even with the increase on the reporting of some information in the last years, there is still room to improve the

transparency on scientific studies using wild-sourced small mammals. An effective approach to enhance accurate reporting in animal studies is through policies implemented by funding agencies, ethical committees and editorial boards. Funding agencies should require strict ethical assessments by ethical committees and, thus, guarantee the adequacy of the methodologies regarding best animal care practices. Finally, editorial boards of journals publishing studies involving experimentation of wild animals should require authors to include all necessary information regarding proper animal care, including permit numbers. In order to adjust the Directive for wild animal researchers, we suggest the inclusion of more specific guidelines for wild-sourced animals, such as those related to accommodation (e.g. more space), manipulation (e.g. avoid direct manipulation for biosafety reasons), enrichment (e.g. increase the complexity of environmental enrichment and adapt it to the species needs), and a tighter veterinary control (e.g. control the health conditions of animals more closely in order to guarantee the animals welfare and avoid biosafety issues). Such additions to the Directive could then be used by editorial offices and would thus help to harmonize the information provided in the publications of different journals. Additionally, we suggest to perform the same type of analysis presented in this review for other type of wild-sourced animals that are also frequently brought to captivity such as fresh water fish[56]. Societal expectations and concerns regarding the welfare of animals has been increasing in the last years. It is thus pivotal to clarify the need of using animals in research, especially wild animals, and the responsibility of scientists in ensuring their proper maintenance and testing. The need of such transparency might have also an important role in influencing the animal care policies in research in addition to the Directive, in order to guarantee the reproducibility and ethical correctness of the studies.

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DATA AVAILABILITY

All relevant data (i.e. selected scientific publications and extracted data) is presented as supplementary material.

DECLARATION OF CONFLICTING INTERESTS

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621 # SUPPLEMENTARY MATERIAL

622 **Table S5.** List of countries compliant with the Directive 2010/63/EU.

List of Countries - Directive 2010/63/EU	
Actual Members	Austria; Belgium; Bulgaria; Croatia; Cyprus; Czech Republic; Denmark; Estonia; Finland; France; Greece; Germany; Hungary; Ireland; Italy; Latvia; Lithuania; Luxembourg; Malta; Netherlands; Poland; Portugal; Romania; Spain; Slovenia; Slovakia; Sweden.
Past Members	United Kingdom*

623 * Until 2020

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