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MAKING ROOM FOR THE 3RS PRINCIPLES OF RESPONSIBLE ANIMAL USE IN ECOLOGY: POTENTIAL ISSUES IDENTIFIED THROUGH A PILOT SURVEY

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Abstract.
Research on animals is one of the most controversial ethical issues in our society. The concern for animal welfare has been increasing in recent years and it is therefore imperative that any potential harm and distress to animals used in research is minimized. This could be achieved through the implementation of the so-called 3Rs principles for animal research (Replace, Reduce, Refine), which are now anchored in many legislations worldwide. In this scientific forum article, I comment on how the 3Rs might be useful specifically for ecological research on wildlife. While the main benefit of the 3Rs principles is improved animal welfare, their implementation also provides opportunities for better science, saved costs, public support, and innovation. However, the awareness and implementation of the 3Rs, and attitudes about animal use in ecological research and education, have never been examined before. In order to close this gap, I conducted a pilot survey among ecologists working with wildlife. Even though the responses from 107 respondents from 23 countries are unlikely to represent the whole community of ecologists and should be, therefore, interpreted with caution, they provided several important insights. The responses from ecologists across different age classes and career stages revealed that lethal and invasive research methods might be prevalent when working with both invertebrate and vertebrate species, and that more than half of the respondents have never heard of the 3Rs principles for animal research. The reported lack of calculation of the minimum sample size and the widespread dissection classes as a part of education may also be of concern. Based on these findings, it is highly recommended to implement rigorous ethical and methodological standards for ecological practice and education and enforce the implementation of the 3Rs principles in wildlife research.

Introduction

There is a consensus that ecological research and conservation efforts are necessary for the preservation of biodiversity (Cooke et al., 2017; Hone et al., 2018). In order to provide sound information for species management, ecologists need to assess, for example, how species interact (Zemanova et al., 2017a), the population densities of endangered animals (Molina et al., 2017), or gene flow among populations in a fragmented habitat due to land-use change (Zemanova et al., 2017b). While the impact of human activities such as forestry or agriculture on the welfare of wildlife has been acknowledged, the potential of ecological research itself to negatively affect the welfare of individual animals has been recognized less frequently (but see Fraser & MacRae, 2011; Beausoleil, 2014; Costello et al., 2016; Zemanova, 2020).

This is despite the fact that ecological research can involve many practices that affect animal welfare – for instance, by causing stress through trapping (Harcourt et al., 2010), invasive marking (Powell & Proulx, 2003; MacRae et al., 2018), or invasive or lethal genetic sampling (Zemanova, 2019). Invasive methods, i.e., methods affecting the physical integrity of the animal (Lefort et al., 2019), are very likely to cause discomfort and even pain in animals with a discernible nervous system (Smith & Lewin, 2009). Therefore, it is important to promote responsible animal use in all fields of life sciences (Jewell, 2013; Zemanova, 2020).

Probably the most often implemented guidance on responsible animal use in research and safeguarding animal welfare are the so-called 3Rs principles. These principles were proposed by Russell and Burch over sixty years ago (Russell & Burch, 1959) and serve as a basis for research without the use of animals whenever possible (Replacement), with as few animals as possible (Reduction), and in which the animal's welfare is as good as possible (Refinement). The 3Rs principles have been developed and traditionally applied in laboratory research, where the research focus is often on human health (Russell, 1995; Wurbel, 2017). This focus has been also

reflected in the implementation strategies. For instance, one of the common approaches to implement the Replacement principle is to use the so-called organs-on-a-chip instead of a laboratory animal (Zhang et al., 2018). An organ-on-a-chip is a microfluidic structure containing human cell cultures that is capable of simulating the mechanics and physiology of an entire organ. This Replacement strategy would not be applicable to ecological research. For example, it is not possible to use cell cultures to determine the abundance or the population structure of deer. However, one obvious strategy to implement Replacement or Refinement in research on wildlife is the use of non-invasive research methods (Zemanova, 2019). Other approaches might include avoiding redundant measurements (de Jong, 2019), or improved study design (Zemanova, 2020).

There are multiple advantages associated with the implementation of the 3Rs principles in ecological research on wildlife (Fig. 1). The first obvious benefit is improved animal welfare. For instance, instead of using blood samples for genetic studies, we might use what animals leave behind – faeces, saliva, or urine (Zemanova, 2019). Capture can be extremely stressful for free-living animals, thus using a study design in which we do not even need to touch the animal avoids any potential complications such as capture myopathy or injury incurred from traps (Montané et al., 2002; Ponjoan et al., 2008). Moreover, scientific results might be invalid if they are affected by the stress or injury caused to the animal through invasive research methods (Hurst & West, 2010). Implementing the 3Rs principles in wildlife research is therefore not only important for the well-being of animals under study, but it is also crucial to ensure robust science (Poole, 1997; Prescott & Lidster, 2017).

The 3Rs principles can also serve as a practical ethical tool. Ecological studies and research on wildlife usually enjoy public support, as both scientists and the general public consider conservation of species – and the associated ecosystem services they provide – crucial for our long-term survival (IPBES, 2019). However, in a study by Bruskotter et al. (2019), the majority of respondents, identifying themselves as conservationists, support the idea that wildlife possess intrinsic value, and that people have an obligation to treat wildlife in an ethical way, not compromising their welfare. Consequently, research that has a high potential of harming the animal might be encountered with public outrage (McMahon et al., 2007).

Nowadays, the 3Rs principles are an integral part of many legislations worldwide (Blattner, 2014). For instance, within the European Union, the 3Rs are implemented in the EU Directive 63/2010. Its Article 13 prescribes the choice of research methods in the following way: "In choosing between procedures, those which to the greatest extent meet the following requirements should be selected: (a) use the minimum number of animals; (b) involve animals with the lowest capacity to experience pain, suffering, distress or lasting harm; (c) cause the least pain, suffering, distress or lasting harm; and are most likely to provide satisfactory results." Furthermore, anyone working with animals is required to undertake specific training (EU Directive 63/2010). Implementation of the 3Rs principles is therefore in many countries a legal requirement. Other benefits include the cost-effectiveness of some of the non-invasive research methods (Ford et al., 2017; Alldredge et al., 2019) and the development of innovative approaches to wildlife research (Kersey & Dehnhard, 2014; Hodgson et al., 2018).

So far, only a few studies have documented the experience, knowledge, and adoption of the 3Rs principles among researchers (e.g., Pollo et al., 2004; Leenaars et al., 2009; Franco & Olsson, 2014; Franco et al., 2018), and none of them focused on ecologists. Considering the potentially substantial impact of ecological research on animal welfare (Zemanova, 2020) and the benefits associated with the imple-

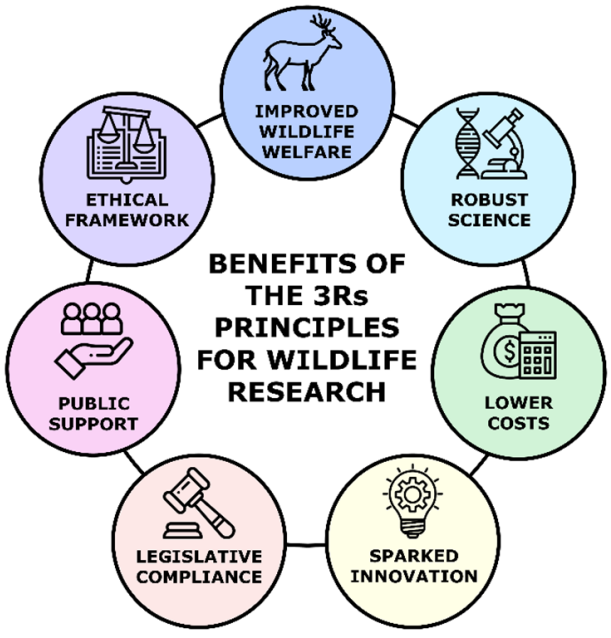

Figure 1: Benefits provided by the implementation of the 3Rs principles into ecological studies on wildlife.

mentation of the 3Rs principles (Fig. 1), the present study aimed to explore the experience, attitudes, and knowledge regarding animal use and the 3Rs principles among ecologists working with animals through a pilot online survey, and to provide strategies to contribute to responsible animal use in ecological research and education.

Materials and Methods

A confidential and anonymous questionnaire was designed by following several guidelines and previous studies (Boynton & Greenhalgh, 2004; Dillman et al., 2014; Franco et al., 2018). Five researchers, who were not included in the target sample, were asked to read the questionnaire and provide feedback regarding its clarity, readability, and length. Small adjustments were made to the questionnaire following the information provided by the pilot study participants before the launch through the online platform SurveyMonkey (http://www.surveymonkey.com).

The final version of the questionnaire (Appendix 1) consisted of seven thematic sections: a) animal use in ecological research, b) ethical concerns about animal use, c) awareness of the 3Rs principles, d) animal welfare, e) animal use in education, e) training in ethics and animal welfare, and f) socio-demographic characteristics. The questionnaire started with a brief background and purpose of the survey. Respondents were informed that the completion of the questionnaire was anonymous and that responses would be used for scientific and educational purposes. The completion time was estimated to be 10 minutes. Respondents had to answer all questions within a section before being able to move on to the next section, but the option "I don't know" was available for most questions. Survey logic was used to filter questions based on previous responses to ensure question relevance.

To recruit respondents, I contacted 38 ecological and zoological societies and groups across Europe (Appendix 2, Table S1) and requested assistance with enrolling ecologists into the study through their mailing lists. As some participants notified me of doing snowball sampling, i.e. recruiting other respondents from among their acquaintances, it was not possible to determine the exact response rate. The geographical constraint was implemented due to the unified animal welfare legislation within the European Union. No incentives were provided for participation. The survey was open from the 8th of October 2018 until the 25th of January 2019. Since some of the respondents did not answer all of the questions in the questionnaire, responses to questions are reported as percentages with the actual number of respondents for each question indicated in brackets.

In order to assess any potential effect of socio-demographic factors (age, gender) on the attitudes, I used the Chi-square test for nominal variables and the Wilcoxon test for ordinal variables (Franco et al., 2018). Only fully answered questionnaires were included in the analyses (N = 96). Significance for all tests was set at $p < 0.05$. All statistical analyses were conducted in R 3.3.3 (R Core Team, 2017).

Results

Socio-demographic characteristics

In total, 107 respondents from 23 countries fully or partially completed the questionnaire. Forty-seven percent of the respondents were females, 52% were males, and one percent chose not to reveal their gender. Ninety-six respondents chose to provide an-

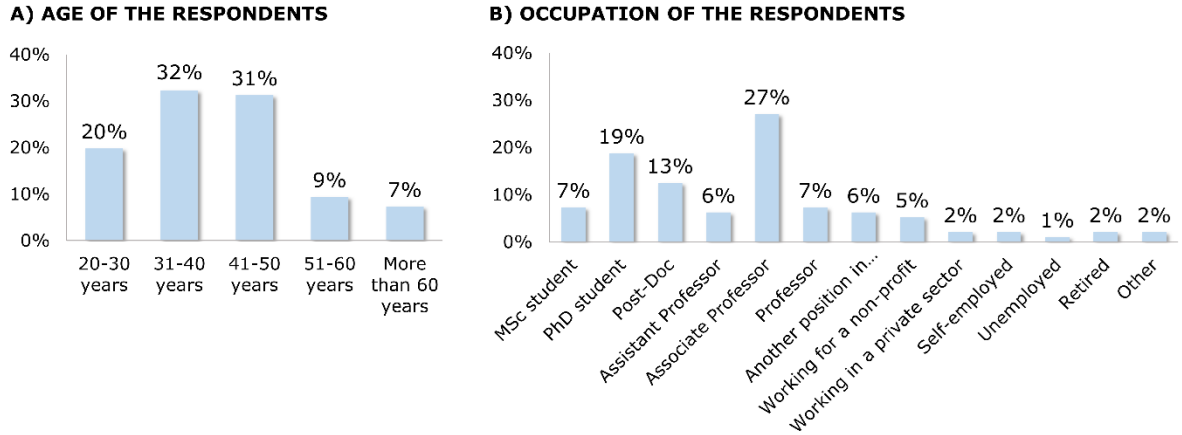

Figure 2: Age (A) and occupation (B) of the respondents (N = 96).

swers about their socio-demographic characteristics. The majority of respondents were either from France (22%), Germany (20%), or the Czech Republic (9%). Although this survey targeted researchers residing in Europe, six non-EU countries were also represented (Appendix, Table S2). The majority of respondents belonged to the age groups 31-40 years old (32%) and 41-50 years old (31%; Fig. 2A). PhD students, Post-Docs, and Associate Professors constituted half of the respondents (Fig. 2B).

Animal use in ecological research and awareness of the 3Rs principles

Majority of the respondents (64 %) used in their latest research more than 100 animals (Fig. 3A; N = 107) and worked with vertebrates (Fig. 3). More than a quarter of the respondents reported that the animals in their latest research had to be killed and 18% used an invasive research technique, defined as a technique that is likely to affect animal welfare (for example, blood taking, toe-clipping, marking; Fig. 3B; N = 107). Only 44% of the respondents calculated the minimum sample size before they started their last study (Fig. 3C; N = 107).

Respondents were asked if they heard of the 3Rs principles for animal research, and if they answered "yes" they were then asked to list the principles. The majority of the respondents (55%) admitted being completely unaware of the 3Rs principles for animal research (Fig. 3D; N = 104). Only 39% of the re-

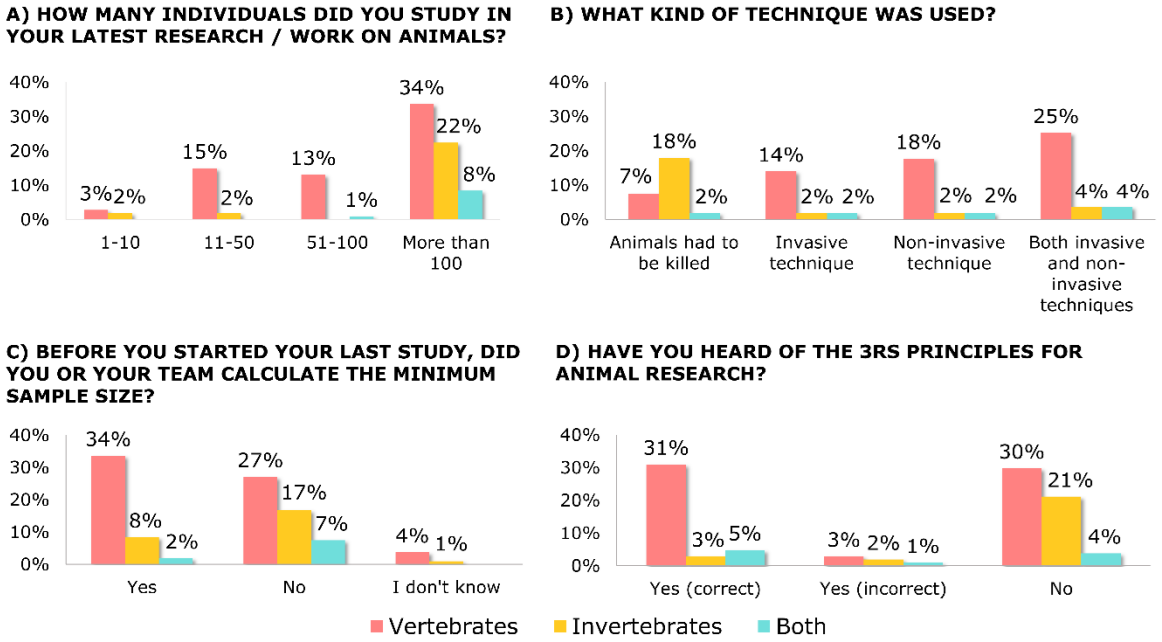

Figure 3: Number and type of animals used in the latest work of the respondents (N = 107). B) The technique used in the latest work of the respondents (N = 107): the invasive technique was defined as a technique likely affecting animal's welfare (for example, blood-taking, toe-clipping, or marking), non-invasive technique was defined as a technique without any impact on animal's welfare (for example, collection of feces or using camera traps). C) Calculation of the minimum sample in the latest work of the respondents (N = 107). D) Awareness of the 3Rs principles for animal research among the respondents (N = 104).

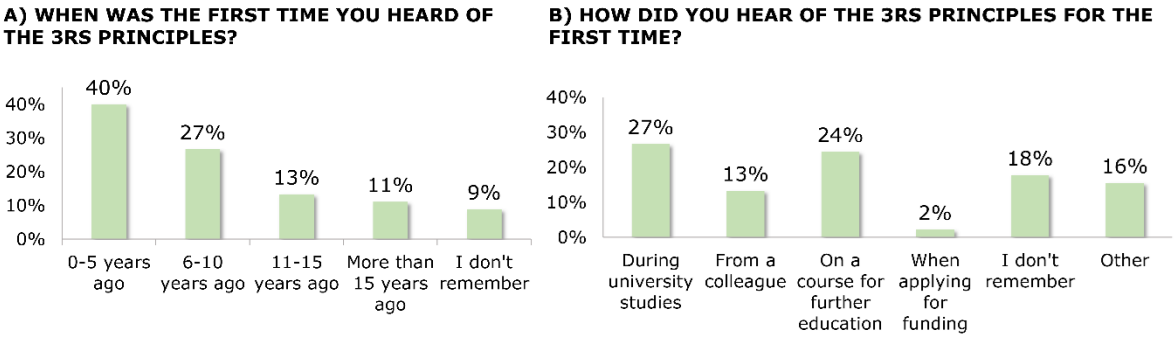

Figure 4: The first time (A) and the way (B) the respondents heard of the 3Rs principles (subsample of Fig. 3D; N = 45).

spondents did hear of them and were able to list them correctly. The rest of the respondents (6%) claimed that they knew the 3Rs principles, but failed to name them, or confused them with other 3Rs (e.g., the "reduce, reuse, recycle" principles for waste management). The majority of those who knew the 3Rs principles said that they first heard about them in the last 5 years (40%; Fig. 4A; N = 45), and either during university studies (27%) or in a course for further education (24%) (Fig. 4B).

Access to information about non-invasive research methods

In terms of access to information on non-invasive research methods, 9% considered it difficult and 24% were not sure (Fig. 5A; N = 98). To the question of what they perceive as barriers to implementing more non-invasive research methods into ecological research, respondents could choose multiple answers. Financial constraints, lack of awareness, and lack of established laboratory protocols were chosen most frequently (Fig. 5B; N = 98). Other reasons mentioned by the respondents were, e.g., that "standard methods are difficult to replace", or that "researchers are conservative-minded and do not want to change previous protocols" (Appendix, Table S3).

Ethical concerns about animal use

The majority of the respondents (66%) experience to varying extent ethical doubts or concerns regarding animal use in their research or work (Fig. 6A; N = 105) and discuss the ethical aspects of their work with colleagues (Fig. 6B; N = 105). There were however statistically significant differences among the age groups (p = 0.006), with a higher proportion of the younger respondents (20-50 years old) expressing ethical concerns than older respondents (>51 years old). Furthermore, among respondents stating that they do experience ethical concerns (sometimes, usually, always) there was a higher proportion of women (p = 0.021).

Training in animal welfare and ethics

The majority of the respondents (70%) felt that their research included sufficient consideration for animal welfare (Fig. 7A; N = 98). However, the topic of animal welfare was covered in the education of only 38 % of the respondents (Fig. 7B; N = 97). Ethics classes were attended by only a third of the respondents (Fig. 7C, D; N = 97). Still, the majority

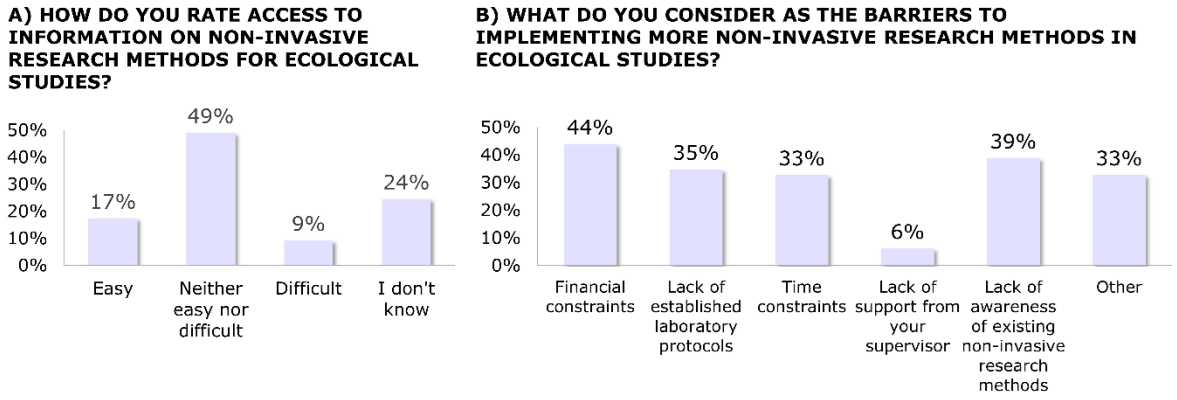

Figure 5: A) Perceived ease of access to information on non-invasive research methods among the respondents (N = 98). B) Perceived barriers to implementing more non-invasive research methods in ecological studies among the respondents (multiple choice; N = 98). Detailed responses from the category "Other" are reported in Appendix, Table S3.

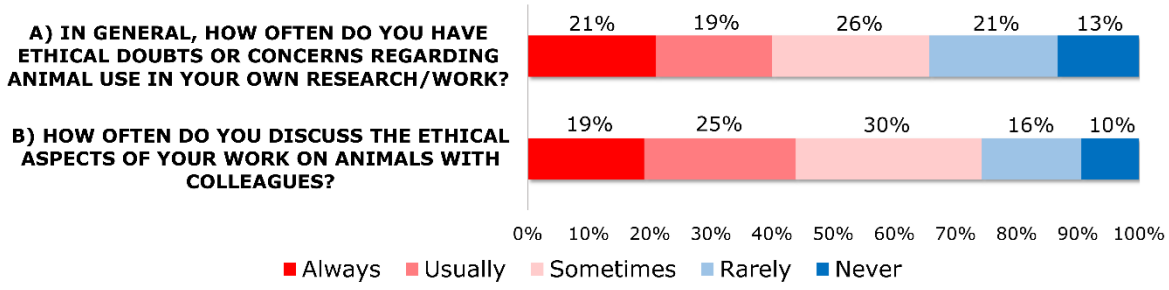

Figure 6: A) Frequency of ethical concerns regarding animal use in their work among the respondents (N = 105). B) Frequency of discussions about the ethical aspects with colleagues (N = 105).

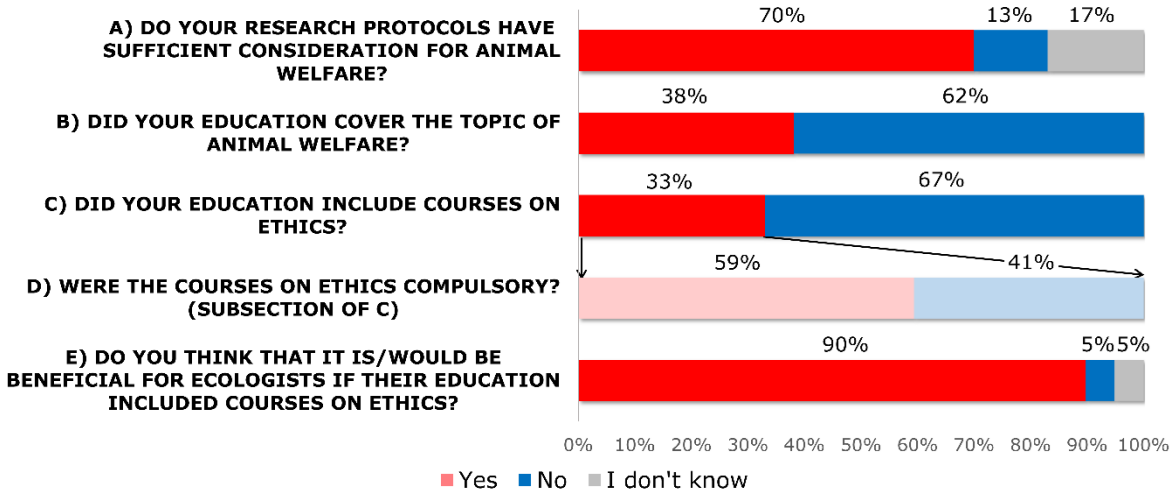

Figure 7: A) Perceived status of animal welfare consideration in research protocols of the respondents (N = 98). B-E) Experience and opinion on animal welfare and ethics classes among the respondents (N = 97).

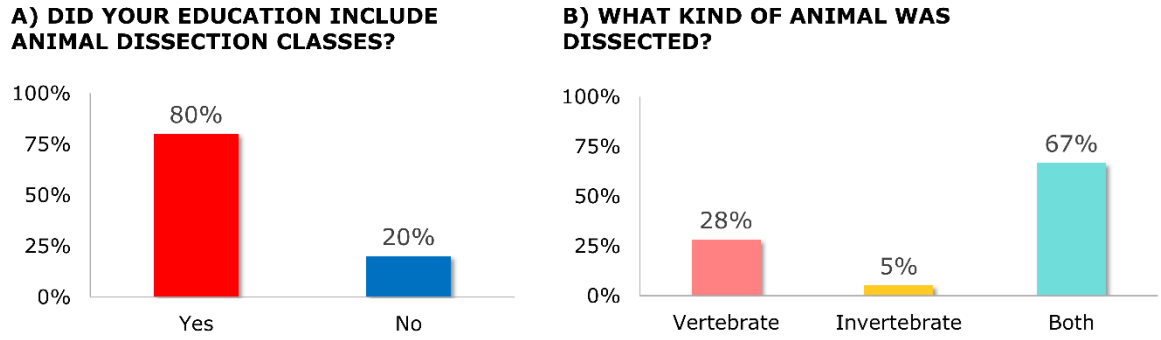

Figure 8: A) Experience with animal dissection as a part of education among the respondents (N = 98). B) Type of animal used in dissections (subsample of Fig. 8A; N = 78).

of the respondents (90%) thought that it would be beneficial for ecologists if their education included courses on ethics (Fig. 7E; N = 97).

Animal use in education

For the vast majority of the respondents (80%), animal dissection was a component of their education (Fig. 8A; N = 98). The respondents experienced dissection of both vertebrate and invertebrate species most frequently (67%), followed by dissection of vertebrates (28%; Fig. 8B; N = 78).

Discussion

Unfamiliarity with the 3Rs principles

The implementation of the 3Rs principles could help prevent research and management in which animal welfare is compromised (Zemanova, 2020). My findings however revealed that more than half of the respondents never heard of the 3Rs principles, or were not able to list them correctly (Fig. 3D). Respondents working with invertebrates were overall less aware of the 3Rs principles than respondents working with vertebrates, but half of the respondents working with vertebrates had not heard of the 3Rs either. These responses are in accordance with other studies looking into awareness of the 3Rs principles among researchers working or intending to work with animals (Franco & Olsson, 2014; Franco et al., 2018). Van Luijk et al. (2013) pointed out that the information about the 3Rs principles and their implementation may be difficult to access. The issue with effective searching for the 3Rs principles was highlighted also in the study by Leenaars et al. (2009), in which 67 scientists said that they have limited knowledge of the 3Rs databases and access to information is limited. Another important source of information about the 3Rs principles and animal welfare are colleagues (Fig. 4B) and strengthening communication among researchers might be one of the strategies to

distribute knowledge about the 3Rs (van Luijk et al., 2011; van Luijk et al., 2013).

Additionally, more than half of the respondents did not calculate the minimum sample size in their latest study (Fig. 3C), which is the most straightforward way to implement the principle of Reduction. While the calculation of the minimum sample size in ecological research has not been enforced (Field et al., 2019), several pioneering studies have been recently published, e.g., in seabirds (Thaxter et al., 2017) or moose (Girard et al., 2002).

Prevalence of invasive research methods

Most of the respondents worked in their latest study with vertebrates (Fig. 3) and only 22% used a non-invasive research method (Fig. 3B). This could be explained either by the non-availability of a suitable non-invasive technique or by failure to implement existing methods, as was reported in previous studies (Waugh & Monamy, 2016; Russo et al., 2017; Zemanova, 2019). In the current survey, two of the most commonly cited barriers to implementing non-invasive research methods were time constraints and lack of awareness of existing non-invasive techniques (Fig. 5B). The lack of awareness might be mitigated with the emergence of the 3Rs guidelines and databases designed specifically for researchers working with wildlife (https://3RsWildlife.info/; Zemanova, 2021).

Specifically for invertebrates, the most common research method implemented was lethal sampling (Fig. 3B). However, we are currently still lacking research investigating the appropriateness of different methods of invertebrate euthanasia (Drinkwater et al., 2019). While invertebrates do not fall – with some exceptions – under legislation on animal protection, debates on considerations for animal welfare have recently expanded to include invertebrates as well (Adamo, 2016; Keller, 2017). With the emergence of studies pointing out invertebrates' ability to feel pain (Crook & Walters, 2011; Elwood, 2011) and experience emotions (Bateson et al., 2011; Plowright, 2017), it might be important to exercise the precautionary principle and implement non-invasive methods even for invertebrates whenever possible.

Lack of training in animal welfare and ethics

More than two-thirds of the respondents stated that they had ethical doubts or concerns about their work at least occasionally (Fig. 6A) and discuss these concerns with their colleagues (Fig. 6B). Consistently with other studies, my results confirmed that gender and age are some of the most significant indicators of personal attitudes and opinions about animals. Women have been shown to express more concern for animal suffering and are more likely to object to animal use (Kellert & Berry, 1987; Herzog, 2007; Phillips et al., 2011; Magnani et al., 2017), and the use of animals in research is often less accepted among young people (Hagelin et al., 2003; Ostovic et al., 2017).

While the majority of the respondents think about the ethical implications of their work (Fig. 7A), they rarely receive training in animal welfare and ethics (Fig. 7B, C). This result is consistent with my previous work (Zemanova, 2017) in which I determined that majority of undergraduate biology programs at European universities do not provide stand-alone courses in animal ethics. Courses in ethics could help ecologists navigate the terrain of moral problems encountered during their work (Appendix, Table S3; Zemanova, 2017), and animal welfare classes can increase the confidence of the students when handling animals (Hazel et al., 2011; Johnstone et al., 2019).

Animal dissection is an intrinsic part of education in ecology

The responses from the survey also suggest that dissections might be a very much ingrained part of education in ecology (Fig. 8). Animal dissection for educational purposes is, however, a highly controversial topic, with several studies contesting this practice on environmental, economic, and ethical grounds (Hug, 2008; Oakley, 2013). Several studies have shown that the educational merit of dissection is often very low in comparison with humane teaching alternatives (Lalley et al., 2010; Lombardi et al., 2014; Moro et al., 2017; Zemanova & Knight, 2021). Following the 3Rs principles, the shift towards using non-harmful animal use and animal-free alternatives that we witness in medical (Pawlowski et al., 2018; Gala & Crandall, 2019) and veterinary education (Pereira et al., 2017; Grevemeyer & Knight, 2018) should be implemented in all life sciences – including ecology.

Limitations of the survey results

It is important to note that there are two major limitations of this survey. Firstly, the questions may not have captured the full range of animal use in ecological research and education. Secondly, even though the rather small sample size is comparable

or even larger than in some other studies on the 3Rs implementation and attitudes towards animal welfare (e.g. Leenaars et al., 2009; van Luijk et al., 2013; Dignon, 2016), the results of the survey cannot be interpreted as generally representative of the attitudes and experience of all ecologists working with animals. Therefore, caution should be exercised when interpreting the responses. Nevertheless, the respondents represented a wide range of nationalities, ages, genders, and positions, and it should be therefore possible to draw some general tendencies in the attitudes and experiences.

Conclusions

The question of how we can ensure responsible research using animals remains a controversial and sensitive issue that needs to be addressed also within the ecological research community. The results of this pilot study pointed out that careful consideration of how the 3Rs principles, animal welfare, and ethics in ecological research are taught, communicated, and enforced is necessary and overdue. In summary, it is important to 1) keep raising awareness of the 3Rs principles for animal research among ecologists, 2) support the implementation of non-invasive research methods through grants and institutional assistance in order to ensure no unnecessary harm to research animals, 3) promote courses in statistics for ecological study design, 4) establish compulsory education in animal welfare and animal ethics, 5) move away from dissections towards non-harmful animal use or animal-free alternatives, and 6) encourage peer discussions about responsible animal use. The findings reported here could serve as a baseline for assessing the success of such measures.

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Conflicts of Interest

None declared.

Author Contributions

MAZ declares being the sole author of this work.

Data Availability Statement

All results and wording of the questionnaire are reported in the main text and appendices. The raw data that support the findings of this study are available on request from the corresponding author.

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Appendix 1
Data A1: Questionnaire

Animal use in ecological research and education

Welcome

Welcome to the survey on animal use in ecological research and education!

This survey is being conducted by the University of Basel in Switzerland in order to understand how ecologists conduct and think about their studies on animals.

Please take a moment to complete this short questionnaire. Your responses will help us to assess the current situation and propose suggestions for improvement.

The questionnaire is anonymous and should take a maximum of 10 minutes to complete.

If you have any questions about the survey please feel free to contact Dr. Miriam Zemanova by email at miriam.zemanova@unibas.ch.

Thank you!

Animal use in ecological research and education

Animal use in ecological research

How many individuals did you study in your latest research/work on animals?

- ◯ 1-10
- ◯ 11-50
- ◯ 51-100
- ◯ More than 100

What kind of animal did you study?

- ◯ Vertebrate
- ◯ Invertebrate
- ◯ Both
- ◯ Other (please specify)

1

What type of technique was used?

- ◯ Animals had to be killed
- ◯ Invasive technique - likely affecting animal's welfare (for example: blood taking, toe-clipping, marking)
- ◯ Non-invasive technique - without any impact on animal's welfare (for example: collection of feces, camera traps)
- ◯ Both invasive and non-invasive techniques were used
- ◯ I'm not sure
- ◯ Other (please specify)

Before you started your last study, did you or your team calculate the minimum sample size?

- ◯ Yes
- ◯ No
- ◯ I don't know

University of Basel **Animal use in ecological research and education**

Ethical concerns

In general, how often do you have ethical doubts or concerns regarding animal use in your own research/work?

- ◯ Always
- ◯ Usually
- ◯ Sometimes
- ◯ Rarely
- ◯ Never

How often do you discuss the ethical aspects of your work on animals with colleagues?

- ◯ Always
- ◯ Usually
- ◯ Sometimes
- ◯ Rarely
- ◯ Never

University of Basel **Animal use in ecological research and education**

2

3Rs principles

Have you heard of the 3Rs principles for animal research?

- ◯ Yes
- ◯ No

University of Basel **Animal use in ecological research and education**

3Rs principles - continued

Could you please list the 3Rs principles?

When was the first time you heard of the 3Rs principles?

- ◯ 0-5 years ago
- ◯ 6-10 years ago
- ◯ 11-15 years ago
- ◯ More than 15 years ago
- ◯ I don't remember

How did you hear of the 3Rs principles for the first time?

- ◯ During university studies
- ◯ From a colleague
- ◯ On a course for further education
- ◯ When applying for funding
- ◯ I don't remember
- ◯ Other (please specify)

University of Basel **Animal use in ecological research and education**

Considering animal welfare in ecology

Do your research protocols have sufficient consideration for animal welfare?

- ◯ Yes
- ◯ No
- ◯ I don't know

How do you rate access to information on non-invasive research methods for ecological studies?

- ◯ Easy
- ◯ Neither easy nor difficult
- ◯ Difficult
- ◯ I don't know

What do you consider as the barriers to implementing more non-invasive research methods in ecological studies? (select all that apply)

- ☐ Financial constraints
- ☐ Lack of established laboratory protocols
- ☐ Time constraints
- ☐ Lack of support from your supervisor
- ☐ Lack of awareness of existing non-invasive research methods
- ☐ Other (please specify)

University of Basel **Animal use in ecological research and education**

Animal use in education

Did your education include animal dissection classes?

- ◯ Yes
- ◯ No

University of Basel **Animal use in ecological research and education**

Dissection classes

What kind of animal was dissected?

- ◯ Vertebrate
- ◯ Invertebrate
- ◯ Both

University of Basel **Animal use in ecological research and education**

Education

Did your education cover the topic of animal welfare?

- ◯ Yes
- ◯ No

Did your education include courses on ethics?

- ◯ Yes
- ◯ No

University of Basel **Animal use in ecological research and education**

Education - philosophy

Were the courses on ethics compulsory?

- ◯ Yes
- ◯ No

University of Basel **Animal use in ecological research and education**

Education - ethics

Do you think that it is/would be beneficial for ecologists if their education included courses on ethics?

- ◯ Yes
- ◯ No
- ◯ I don't know

University of Basel **Animal use in ecological research and education**

Socio-demographic characteristics

In this last section, we would like to know a little bit about you - your gender, age, country of residence and occupation.
These characteristics will help us put your responses into context.

What is your gender?

- ◯ Female
- ◯ Male
- ◯ Other

What is your age?

- ◯ Less than 20 years
- ◯ 20-30 years
- ◯ 31-40 years
- ◯ 41-50 years
- ◯ 51-60 years
- ◯ More than 60 years

What is your country of residence?

Which of the following best describes your current occupation?

- ◯ MSc student
- ◯ PhD student
- ◯ Post-Doc
- ◯ Assistant Professor / Junior Researcher
- ◯ Associate Professor / Senior Researcher
- ◯ Professor
- ◯ Working in another position in academia
- ◯ Working for a non-profit
- ◯ Working in a private sector
- ◯ Self-employed
- ◯ Unemployed
- ◯ Retired
- ◯ Other (please specify)

Appendix 2
Supplementary Tables

Table S1: Ecological societies and wildlife research groups that were contacted in order to recruit questionnaire participants.

GEOGRAPHICAL COVERAGE	SOCIETY / GROUP
ALBANIA	Institute for Nature Conservation in Albania
AUSTRIA	Austrian Entomological Society
AUSTRIA	Zoological-Botanical Society Austria
BELGIUM	Belgian Biodiversity Platform
BOSNIA AND HERZEGOVINA	Society for Biological Research and Protection of Nature
CROATIA	Croatian Ecological Society
CZECH REPUBLIC	Czech Society for Ecology
DENMARK	Danish Society Oikos
FINLAND	Finnish Association for Nature Protection
FRANCE	French Society for Ecology
FRANCE	French Society for the Study of Animal Behavior
GERMANY	German Society for Mammalian Biology
GERMANY	German Zoological Society
GERMANY	German Society of General and Applied Entomology
GERMANY, AUSTRIA, SWITZERLAND	Ecological Society of Germany, Austria and Switzerland (Facebook group)
GREECE	HELECOS Hellenic Ecological Society
HUNGARY	Hungarian Ecological Society
ICELAND	Ecological Society of Iceland
IRELAND	Irish Ecological Association
ITALY	Italian Ecological Society
MACEDONIA	Macedonian Ecological Society
MOLDOVA	Biotica Ecological Society Moldova
NETHERLANDS	Dutch-Flemish Association for Ecology
NORWAY	Norwegian Organic Society
POLAND	Naturalist's Club
PORTUGAL	Portuguese Ecological Society
ROMANIA	Romanian Ecological Society
SERBIA AND MONTENEGRO	Ecological Society Endemit Serbia and Montenegro
SPAIN	Spanish Association of Land Ecology (Facebook group)
SPAIN	Spanish Society for the Conservation and Study of Mammals (FACEBOOK group)
SPAIN	Spanish Ecological Society
SWEDEN	Swedish Oikos Society (Facebook group)
SWITZERLAND	Swiss Society of Wildlife Biology
UK	British Ecological Society (Facebook group)
EU	European Ecological Federation
EU	Society for Conservation Biology Europe (Facebook group)
WORLDWIDE	Ecology and Evolutionary Biology (Facebook group)
WORLDWIDE	Wildlife Biologists (Facebook group)

Table S2: Number of respondents and their country of residence. In total, 107 respondents from 23 countries participated in the survey.

COUNTRY	RESPONDENTS (%)
AUSTRIA	3.13%
BELGIUM	5.21%
BOSNIA AND HERZEGOVINA	1.04%
CZECH REPUBLIC	9.38%
FRANCE	21.88%
GABON	1.04%
GERMANY	19.79%
GREECE	2.08%
HUNGARY	2.08%
IRELAND	1.04%
KOREA, SOUTH	6.25%
MACEDONIA	2.08%
MALAYSIA	1.04%
POLAND	1.04%
PORTUGAL	4.17%
SERBIA	1.04%
SPAIN	3.13%
SWEDEN	5.21%
SWITZERLAND	4.17%
TAIWAN	1.04%
TURKEY	2.08%
UNITED KINGDOM	1.04%
UNITED STATES	1.04%

Table S3: Specific responses to the question: "What do you consider as the barriers to implementing more non-invasive research methods in ecological studies?"

ID	OTHER (PLEASE SPECIFY)
1	The research question
2	lack of time to check out alternative methods - it's often easier to use what has been use before...
3	We use standard methods that are difficult to replace
4	N.-i. methods for fish in running water e.g. eDNA not very developed
5	non-invasive methods not necessary, e.g. when only trapping animals for later release
6	Sometimes individuals need to be captured and measured, that is per se invasive
7	not applicable for experiments on insect development
8	the question does not apply: tiny insects must be killed for identification, birds must be caught for ringing
9	Jurisdiction, law, law enforcement
10	It sure depends on what kind of animals you are working on
11	Invertebrate determination not possible non-invasive
12	lack of appropriate methods
13	when working with insects 1-5 mm in size, these methods are simply not applicable
14	catching, identifying (as detailed as possible) and releasing invertebrates is a lot of effort and is very time limited - this reduces the number of sites and traps you can work with during a certain time period significantly. If time of day or day of the year vary too much, comparisons between sites become impossible. Animals caught alive can attack and eat each other, and they have only little energy reserves - so time in trap should be as short as possible.
15	I do not know about the barriers, we use non-invesive research methods.
16	I work on wild animals: stress is much less documented/investigated than for lab animals, what impairs easy and regular assessment of invasiveness (i.e. the level of stress induced, and its indirect consequences)
17	no real barriers
18	a lack of respect for animal life and nature in general
19	For our research we need to euthanize small mammals
20	Lazyness to find other ways to do it (the case of my current lab only)
21	I think that most non-invasive methods I have seen do not really offer viable alternatives to existing methods.
22	I worked as a field assistant so I did not design our experiments. Certain invasive methods are simply necessary (such as ear tagging individuals) while alternatives are possible for others.
23	Experimental standard protocols
24	no barriers - methods depend on questions
25	The constrains related to the study of physiological mechanisms that may need tissue sampling
26	Non-invasive is used whenever but genomics data usually requite high amounf of DNA so blood or tissue are the only way worth it (feathers could be used for microsatts). There is no point taking a feather if the data out of it is not worth sampling an individual
27	In some cases non-invasive research methods do not exist (yet)
28	Lack of robust substitute methods
29	many of those techniques are indirect indicators of what we want to measure and therefore less accurate. I prefer to use an invasive technique on a small number of animals, but have a clear answer to my scientific questions
30	We study fish (migration) behaviour in rivers. Visual observation is no option. We make use of mark (fin clip, floy tag, VIE tag, ...) and recapture methods and telemetry (radio-, PIT- and acoustic) methods.
31	I am not aware of any, I just do research that cannot be done without killing the studied invertebrates.
32	I generally think researcers are very conservative-minded and dont want to change previous protocols, even if less invasive ones are available