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OPINION

Embracing prospects for reducing the numbers of animals used in aquaculture research

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Abstract

The principles of three Rs—REPLACEMENT, REDUCTION, and REFINEMENT—govern the protection and use of animals, including fish, for research purposes in the European Union and Norway. In this paper, we discuss some straightforward steps to simplify the delivery of these principles at the idea stage and adapt some of these examples for conducting fish trials related to health and welfare. Although some of the approaches are well established in other animal science arenas, we believe there can be a timely recap of their key facets. We discuss a number of simple strategies to emphasize how a reduction in fish numbers can be achieved from initial project conception to implementation, highlighting not only their advantages but also their limitations. We also highlight the role that funding agencies can play in the implementation of the 3R principles in aquaculture research. These simple points can be used in frameworks to initiate a broader and dynamic intersectoral dialogue among stakeholders of aquaculture research on how to promote ethics and embrace opportunities for this within the tenets of the 3Rs.

KEYWORDS
animal welfare, aquaculture, fish welfare, research ethics, 3R principles

1 | INTRODUCTION

lovations in aquaculture production have been driven and supported by innovative research and development across the value chain. These innovations have been pivotal in aquaculture establishing itself as a major producer of proteins for the growing global population. Animal health and welfare remain two of the key drivers of sustainable aquaculture and are important to realizing the ambitions of the one health framework in aquacultural settings (Stentiford et al., 2020). Many aspects of aquaculture research have aims that can be linked to fish health and welfare during production, including but not limited to understanding the effects of production operations and rearing systems on animal biology, susceptibility and resistance to diseases, identification of optimal rearing conditions, and development of prophylactic and therapeutic measures. The protection and use of animals, including fish, for scientific purposes in the European Union (EU) are addressed and regulated by the Directive 2010/63/EU (European Commission, 2010), which is based on the principles of the three Rs—REPLACEMENT, REDUCTION, and REFINEMENT, first described by Russell and Burch in 1959 (Russell & Burch, 1959). An aim of the EU directive is, where possible, to replace the use of animals for scientific purposes with alternative methods. If this is not feasible, attempts should be made to reduce and refine their use in line with the 3R framework. Indeed, this is succinctly stated in the directive via the following text: *"While it is desirable to replace the use of live animals in procedures by other methods not entailing the use of live animals, the use of live animals continues to be necessary to protect human and animal health and the environment. However, this Directive*

represents an important step toward achieving the final goal of full replacement of procedures on live animals for scientific and educational purposes as soon as it is scientifically possible to do so. To that end, it seeks to facilitate and promote the advancement of alternative approaches."

In Norway in 2021, 2,008,597 animals were used in scientific procedures according to data reported to national authorities (https://www.mattilsynet.no/dyr/forsoksdyr/bruk-av-dyr-i-forsok, accessed 20.02.2024). Around 1.9 million of these experimental animals were fish, and the majority are species relevant for aquaculture and fisheries, with Atlantic salmon (*Salmo salar*) being the dominant species 2023. Aquaculture and fisheries are two of Norway's biggest industries, and modern innovations in these industries were made possible through years of research. So, it is logical to assume that at least a portion of these numbers can be attributed to the research-driven nature of these two industries in addition to other needs and questions within the wider fish biology community.

Directive 2010/63/EU classifies all scientific procedures based on their severity, and guidelines on assessing the severity of the procedures were drawn up in 2009 (https://ec.europa.eu/environment/chemicals/lab_animals/pdf/report_ewg.pdf), adapted for fish (Hawkins et al., 2011), and have also been updated since (Smith, Anderson, et al., 2018). The directive defines four categories for severity of procedures: SV1: non-recovery, SV2, (up to and including) mild; SV3, moderate; and SV4, severe (Table 1).

It is timely to consider how to reduce the number of fish for research through innovative and rational solutions, *where it is scientifically feasibly to do so*. Substantial multifaceted efforts will be required to achieve these overarching objectives. The reduction of animals used in research requires a diverse range of audited and secure alternative methods that can generate knowledge that is of equal or better utility for the end user. This development is ongoing and requires substantial investment. In addition to reducing the number of animals used, or the severity of the procedures they are subjected to, there are a range of further stakeholder benefits. It has been well documented that, where possible and appropriate, reducing the number of animals used for research purposes does not compromise scientific integrity (Kovalcsik et al., 2006) and is addressed in regulatory guidelines for carrying out scientific procedures. For example, under the requirements of the European 2010/63/EU directive, scientific procedures that are carried out by the member states must be authorized by a competent authority. During the authorization process, applications to conduct an experiment must demonstrate 3R compliance, with regard to the number of animals used, and also include harm–benefit analysis and a severity assessment of the procedure, to name a few. Unlike other animal models for research (e.g., rodents), you need to account for a variety of factors in handling aquatic animals due to the complexity of the aquatic environment. In addition, there are at least 600 farmed species in the world that require different biological and physical requirements (FAO, 2022). Aquaculture has changed dramatically in recent years (i.e., types of production systems, diversification of farmed species), which will greatly influence how research is conducted. However, excellent science and superior animal welfare can go hand in hand (Prescott & Lidster, 2017). Therefore, ensuring that experimental animals are handled in the most humane and ethical way possible will have a significant impact on the scientific robustness of the data generated from a trial.

TABLE 1 Criteria for the severity classes based on directive 2010/63/EU. Based on 2023 and 2011.

Category symbol and class	Description	Examples based on Hawkins et al. (2011)[a]
SV1: non-recovery	Procedures that are performed entirely under general anesthesia from which the animal shall not recover consciousness shall be classified as "non-recovery."	
SV2: (up to and including) mild	Procedures on animals as a result of which the animals are likely to experience short-term mild pain, suffering, or distress, as well as procedures with no significant impairment of the well-being or general condition of the animals shall be classified as "mild."	*Blood sampling under anesthesia where volumes and techniques are limited to those recommended by published guidelines and/or national legislation; research into some diseases, where humane endpoints are applied at the first clinical sign of disease or earlier.*
SV3: moderate	Procedures on animals as a result of which the animals are likely to experience short-term moderate pain, suffering, or distress, or long-lasting mild pain, suffering, or distress as well as procedures that are likely to cause moderate impairment of the well-being or general condition of the animals shall be classified as "moderate."	*Prolonged removal of fish from water for the purpose of inducing stress; disease studies where the disease in question is known to cause death but where the study can be controlled so that mortality does not occur.*
SV4: severe	Procedures on animals as a result of which the animals are likely to experience severe pain, suffering, or distress, or long-lasting moderate pain, suffering, or distress as well as procedures that are likely to cause severe impairment of the well-being or general condition of the animals shall be classified as "severe."	*Severe restriction of movement that interferes with normal activities over a prolonged period; infections with a prolonged disease course, in which substantial loss of condition or other overt clinical signs, which cause a significant and prolonged departure from normal health, are required for the purposes of the study.*

[a]For complete list of examples, refer to Hawkins et al. (2011).

A number of opportunities for ensuring that the needs of the 3Rs are being met in scientific studies can occur before the regulatory application phase of conducting research. For example, within a university, research institute, or experimental facility, a number of steps can be taken to ensure the maximal utility of individual animal experiments. When scientific procedures have themes of interest across different research fields, and where requirements for, for example, confidentiality allow it, researchers could take the time to openly discuss the aims and objectives of trials with other researchers within their department groups and beyond. This is a well-established practice in different research fields and is also used in fish-related aquaculture research (Czubala et al., 2022; Hubrecht & Carter, 2019; Rowan et al., 1993; Workman et al., 2010), and we should continue to retain and promote it wherever possible to ensure the potential for transparent interaction and collaboration on individual experiments that can ultimately maximize the utility of the studies undertaken.

In this perspective paper, we collate and highlight some of the existing and often well-established approaches that can often be overlooked but offer true utility when aiming to reduce the number of fish for research. This type of approach is established and well received in many other facets of laboratory animal science (ASRU et al., 2014). We highlight some examples from our experience, including challenges encountered employing the approach. We hope these simple steps and guidelines will be a platform for stimulating further discussion, highlighting the opportunities for maximizing the outputs of scientific studies involving animals in aquaculture research and potentially reducing the number of animals that need to be used by considering potentially synergistic research questions or challenges.

2 | TRANSPARENCY AND OPEN COMMUNICATION AS IMPORTANT DRIVERS

Internal communication, exemplified by transparency and openness, can be a key driver for reducing the number of experimental fish used in potentially synergistic trials. This is a well-established principle whose simplicity and utility should not be overlooked in the quest for maximizing the delivery of the 3Rs in aquaculture research. As most scientific trials are collaborative and multidisciplinary, that is, the research team often comprises researchers from different fields and facilities, formal and informal discussions during experimental preparations have become pivotal in sharing details of ongoing and incoming trials. These discussions have been used to identify shared or similar hypotheses, pinpoint potential areas for a common experimental approach, and undergo rigorous deliberations on combining different projects and hypotheses into one fish trial. Where opportunities and conditions allow, research questions from different associated projects can be realized in one trial. It must be reiterated however that this is not a single-solution approach and that it is a highly complex process requiring extensive discussions and deliberations. (Grunow & Strauch, 2023).

When we adapt this approach to our own research, some of the simple steps and questions we often ask at the start of a joint discussion between projects, during both the application and in progress, are as follows: (i) What is the main objective of each project, and are there similarities between the two? (ii) If there are similarities, what are the possibilities for combining the hypotheses of the two projects? (iii) Will synergy between the projects increase or compromise their scientific integrity? (iv) If there is a need to compromise, is it manageable given the resources? (v) How flexible are the available logistics and infrastructure for a joint trial? (vi) If a project is underway, what needs to be done to gain permission to deliver on these objectives and ensure the effective transition of information to, for example, external funding bodies, partners, or relevant stakeholders regarding the updated plans?

When we consider this approach in our own research context, we have a recent example where two projects funded by the Norwegian Seafood Research Fund (FHF) identified potential synergies in approach when one project was already underway and the other project was still in the proposal writing stage. These projects were Peragill (FHF 901472), a project that aimed to develop a treatment for amoebic gill disease, a parasitic infection in Atlantic salmon, that started in 2018; and CrowdMonitor (FHF 901595), a project that was started in 2020 that aimed to update our state of the art on the crowding of Atlantic salmon using a suite of existing and emerging health and welfare indicators. Both these projects involved fish crowding—one in relation *to parasitic treatment* and *the other in relation to how to improve our documentation of it*. Therefore, the two project teams suggested potential synergies between projects during the CrowdMonitor project application stage, stated this openly in the funding application and, when funding was awarded, deliberated and developed a joint trial where the hypothesis of each project could be addressed. The trial reduced the expected fish use by at least 50%, without compromising the experimental approach and objective of each project. In fact, the data generated by one project provided a different insight into the data of the other.

This simple example highlights the potential, at the institutional level, for optimizing the use of fish for research and the utility of the knowledge gained from each study. We know this approach is well established in other research groups and institutes but highlight our own experience with it in order to emphasise its often-forgotten value. The approach can markedly reduce the numbers of fish used by utilizing well-established, but sometimes overlooked, opportunities to have a thorough discussion and deliberation to find common ground and approach, especially in developing a joint experimental trial that can address and incorporate the objectives of at least two projects. We are also aware that this approach is both challenging and time demanding for planning and coordination, and can also have logistical constraints (e.g., clearance from external project collaborators as well as from the funding agencies). The openness of different parties to find similarities for a joint project is a decisive prerequisite. In our example, this has not been an issue, although we do acknowledge that each case requires different ways of actions, and there is a certain

level of collegial trust involved. In addition, combining animal trials has economic advantages through reduced cost expenditures, increasing the possibilities for a budget that may cover a broader analytical spectrum, thus maximizing the utility of each study and the impacts of the results it generates (Eggel & Würbel, 2021; Diederich et al., 2022). Also, addressing similar but different hypothesis in a single trial may increase the number of analytical approaches applied. Utilizing experimental animals across projects may thus strengthen the scientific outcome of each trial (Lazado, Iversen, et al., 2023; Ytteborg, Lazado, et al., 2023).

3 | DEVELOPMENT OF METHODS USING MATERIALS FROM PREVIOUS STUDIES

Method development is central to delivering research that is timely and relevant. To develop and verify the suitability of these methodologies, one often requires specimens and source materials from various experimental or production origins. A strategy to streamline the delivery of these source materials is not to run several new trials but rather utilize ongoing trials and specimens from previous studies as biological materials for the development of different methodologies. This is a well-established procedure and a criteria of the 2010/63/EU directive; see Article 18 "Member States shall facilitate, where appropriate, the establishment of programmes for the sharing of organs and tissues of animals killed." For example, the development of an immunocompetence test (i.e., ImCom assay) for Atlantic salmon smolts employed this approach (Krasnov et al., 2020), and the ImCom assay was developed and verified without running a single fish trial.

Similar work has also been carried out to develop artificial intelligence histopathological tools. Histological evaluation is an integral tool in fish health research. It allows us to study structural changes (e.g., the extent of damage of tissues/organs) after exposure of fish to various physical/biological/chemical stimuli. A machine learning–based method for histological evaluation of Atlantic salmon skin (Sveen et al., 2021) has been developed, and several algorithms developed for other tissues/organs and species are currently in the pipeline. These algorithms have been developed, trained, and verified using a combination of samples from finished trials and ongoing trials. This way, histological sections from several different biological cases, production regimes, animals with varying disease status, fish species, and life stages can be included in the machine-learning steps (Sveen et al., 2021), thus improving the robustness of the analysis with a variety of samples, as well as reducing the need for running separate trials.

4 | IN VITRO MODELS

Establishing alternatives to running fish trials can significantly reduce the number of experimental animals. *In vitro* models offer systems to study fish cells, tissues, or organs in the laboratory, with a limited number of fish. *In vitro* studies are often carried out using 3–15 fish, depending on the type of experimental setup. In aquaculture research, it is possible to design lab-based trials where we can test how fish (i.e., cells, tissues, organs) respond to different stimuli relevant to production, such as new dietary ingredients, pathogens, toxins, and chemotherapeutics, among a few others (Chang et al., 2021; Goswami et al., 2022; Grunow et al., 2021; Løkka et al., 2023; Pasquariello et al., 2023). However, these strategies are sometimes met with skepticism because of the argument that the multiwell plates cannot reflect the complex aquatic environment, which to some extent is true. However, we would like to point out that these *in vitro* model systems are *targeted* replacements to fish trials and can be utilized as a way of refining future large-scale trials (Rehberger et al., 2018). These *in vitro* models are excellent systems for understanding the underlying biological mechanisms of a specific stimulus, which can be challenging in an *in vivo* trial where several factors are interacting, thus posing compound effects that are difficult to isolate (Cabillon & Lazado, 2022; Torrissen et al., 2023). *In vivo* exposure trials involving aquaculture-relevant stimuli often require a significant number of fish and are costly. Cell and explant models are valuable to fine-tune the *in vivo* trials because tests for treatment combinations (e.g., concentration, duration) are not limited with the number of fish. For example, this strategy can narrow down the concentration range to which the host is responsive to the stimuli.

Skin and scale explants from salmon, cod, and lumpfish have been used to study skin cells (keratocytes) in culture, where their barrier functions are in focus (Ytteborg et al., 2020; Ytteborg, Falconer, et al., 2023; Ytteborg, Lazado, et al., 2023). Culturing keratocytes in the laboratory allows us to study their migration capacity, proliferation rate, wound healing potential, and response to different stimuli, such as toxic components and environmental factors. Often these studies can add functional insight into histological sections of skin, helping us, for example, better understand the biological significance of skin damage caused by different lice treatments (Karlsen et al., 2018; Ytteborg et al., 2020). We have explant models for the gills that were employed to investigate how the branchial barrier functions are affected by environmental toxicants, including oxidants (Lazado & Voldvik, 2020) and the toxic gas hydrogen sulfide (Alipio et al., 2022). These models are helpful when one is interested in understanding the mechanism of action. For instance, we have used the model to investigate the involvement of mucus in the gill responses to H_2S through pharmacological inhibition/stimulation of mucus production in the explant models (Alipio et al., 2022). We have also developed models to study how the nasal microenvironment of Atlantic salmon responds to different aquaculture-relevant stressors (Alipio et al., 2022; Cabillon & Lazado, 2022; Lazado et al., 2020). For instance, the cell and explant models that we have for the olfactory organ have been used to elucidate the molecular processes underlying the adaptation of Atlantic salmon to a sulfide-rich environment.

Finally, another *in vitro* approach that should not be overlooked is using the same fish for different cell isolation purposes. (Alipio et al., 2022; Cabillon & Lazado, 2022; Lazado, Voldvik, et al., 2023).

Open and transparent communication before and during the procurement of fish means the same individual fish can be used for multiple purposes instead of securing different fish stocks for two separate occasions. This thereby reduces the overall number of fish needed to secure the samples and has been long proposed by Johansen et al. (2006), and recent discussion was highlighted by Grunow and Strauch (2023) in line with the aims of Directive 2010/63/EU.

5 | EMERGING TOOLS

Innovations are rapidly evolving in aquaculture technologies, which also propel advancements in performing tank-based *in vivo* trials. Research related to recirculating aquaculture systems (RAS) can often require large systems to mimic commercial scenarios (Terjesen et al., 2013), but this also means that a significant number of fish is needed to keep the system performance at optimal. Small experimental modular RAS units are alternatives to traditional experimental setup (Mota et al., 2022; Pedersen et al., 2012). Despite the size and technical limits of these types of systems, they are fitted to answer important questions in aquaculture, including water quality, disinfection, biosecurity breach, among others. However, one must always consider the effects of tanks size (Espmark et al., 2017).

Development of minimally invasive tools to assess health and welfare has been the focus of an increasing number of research initiatives in recent years, especially targeting nonterminal samplings for reducing the number of fish for research. Most fish research conducted in Norway has euthanized fish for tissue collection, and the recent report on the minimal reuse of experimental animals reflects this (Champetier & Smith, 2023). Nonterminal sampling, whenever applicable, should be adapted. We acknowledge that key experimental questions, severity of distress of the trial, and required response variables will dictate the applicability of nonterminal sampling. Nonetheless, tools must be developed and advanced to assess fish health, thus avoiding euthanasia as this can allow the reuse of experimental animals. For instance, morphological injuries are well-established operational welfare indicators for farmed fish but can be challenging to manually audit in high numbers. The emergence of a number of digital tools can streamline this process and audit injuries in realtime without handling the fish (see e.g. Gupta et al., 2022). Camera-based assessment of body shape has also been explored as a means of assessing welfare of fish, and attempted to correlee them to physiological indicators of growth and health (Barreto, et al., 2022; Føre, et al., 2018; Timmerhaus, et al., 2021). If the fish still need to be handled, an additional tool for welfare scoring can be hyperspectral imaging, which uses a spectroscopic technique that captures reflection of light at narrow-wavelength bands with a level of detail that exceeds the visual perception of humans and traditional cameras. This has been used as a tool for digital scoring of welfare traits in Atlantic salmon (Lindberg et al., 2023). Using mucus instead of plasma/serum as a matrix for biochemical analysis to assess health and welfare has also been explored to investigate responses to stress (Carletto et al., 2022; Lazado et al., 2021; Raposo de Magalhães et al., 2023; Sanahuja et al., 2023).

5.1 | Starting from the beginning

Better experimental design and an improved consideration of the statistical requirements that are needed to undertake the scientific procedure are still one of the best approaches to reducing the number of fish in research (Grunow & Strauch, 2023; Sneddon et al., 2017). Starting such consideration from day 1 provides researchers with a breadth overview of factors that should be rigorously considered and opportunities to explore in the design of the trial. Questions such as "how many animals are needed?" dictate crucial information that must be identified in running trials, including trial duration, frequency of sampling, and allocation of treatment groups. According to Directive 2010/63/EU, all fish experiments conducted on independently feeding larval forms of fish that involve scientific procedures which may have a mild, moderate or severe impact upon the animal need to secure approval from the competent national authority, which in Norway is the Norwegian Food Safety Authority (Mattilsynet), and the number of fish to be used in a trial must always be justified in the application. Power calculations are an integral component in ensuring that the required number of animals are used for the trial, which provide statistically robust results (de Blas, et al., 2020; Hawkins, et al., 2013; Ling, Cotter, 2003; Mota, et al., 2022) and several web-based applications are available that are easily accessible tools for researchers to calculate the number of animals for research (e.g., Laboratory Animal Services Centre of the Chinese University of Hong Kong, ClinCalc). One of the most extensive collection of guidelines in preparing to conduct animal studies has been developed by Norecopa, the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines (Smith, Clutton, et al., 2018). It covers the three broad areas that determine the quality of the preparation for animal studies, including formulation of the study (e.g., literature review, legal issues), dialogue between scientists and the animal facility (e.g., objectives and time scale, funding and division of labor), and quality control of the components in the study (e.g., experimental animals). Besides well-established aspects, the PREPARE guidelines include topics that are frequently not mentioned in reporting guidelines but are regarded critical to the validity and reproducibility of research. These include facility evaluation, education and training; health risks; waste disposal and decontamination; quarantine and health monitoring; and humane killing, release, reuse, or rehoming. As of this writing, the PREPARE guidelines have already been translated into 34 different languages and are readily available for download (see https://norecopa.no/prepare).

5.2 | Maximizing 3R opportunities from funding agencies

Should 3Rs be strictly implemented in securing funding? Indeed. The way to reduce the use of experimental animals in research should be the responsibility of not only the researchers but also the funding agencies. This has previously been highlighted by Grimm et al. (2023).

Though the role of funding agencies may be indirect because, in most cases, they do not involve in the design of the trial, they do have the opportunity to significantly influence how the 3R principles are to be implemented in the project. Funding agencies and grant reviewers should evaluate the ways the research proposal will implement the 3R principles, and this should be clearly taken into consideration in the evaluation marks of a proposal. In some research funding bodies, this is not explicitly asked in the required components of the proposal. The Research Council of Norway (RCN) is the major funding agency in Norway that provides grants for both fundamental and applied research. Applicants, especially those submitting proposals to programmes on biological sciences, are required to provide information on how the 3Rs will be implemented in the project. Though a minor section, it allows researchers to highlight the approaches and methodologies that may facilitate the reduction of fish for research, application of alternative systems, and identification of humane endpoints, among others. However, in the current portfolio plan for the Oceans programmes (where aquaculture is included), there is no mention of the priorities of animal experimentation, especially in the context of the 3Rs. We urge the board of the Oceans portfolio to consider this aspect as the programme undergoes changes in the next years. The FHF is another funding agency in Norway, mainly financing aquaculture and fisheries research. It is a limited company owned by the Ministry of Trade, Industry and Fisheries and financed by the industry through a levy on exports of Norwegian seafood at 0.3%. Specific requirements to disclose how the 3Rs will be implemented in a research project have recently been introduced in most of its research calls, which was earlier not the case. The description of 3R implementation in the proposed project is acknowledged and evaluated positively in proposal evaluations. In addition, the link among different projects is given positive marks during assessments, as it highlights synergies and provides opportunities for maximal utility of experimental animals. In addition to FHF and RCN, the Norwegian Animal Protection Alliance, a national foundation for animal welfare in Norway, is funding research that will improve animal welfare, including how to apply the 3R principle in aquaculture research. This fund was first active between 2008-2012 and has again been active since 2019. In the current funding framework of Horizon Europe (as of November 2023), the research and innovation funding programme launched by the European Commission, there are two main calls related to the implementation of 3Rs, which are also relevant in aquaculture research: Tools and Technologies for a Healthy Society (two stage-2024) (HORIZON-HLTH-2024-TOOL-05-two-stage) and A Competitive Health-Related Industry (single stage-2024) (HORIZON-HLTH-2024-IND-06). Different countries in and around Europe also have national funding agencies related to the advancement and implementation of the 3R principles in animal experimentation, such as the National Centre for the 3Rs (NC3Rs) and FRAME.org in the United Kingdom, Bundesministerium für Bildung und Forschung (BMBF) in Germany, Belgian Council Laboratory Animal Science (BCLAS) 3R's Fund in Belgium, and the Swiss 3R Competence Centre in Switzerland. In the United States, several funding agencies are also available, such as the Alan and Helene Goldberg In Vitro Toxicology Grant at Johns Hopkins Center for Alternatives to Animal Testing (CAAT), the Alternatives Research & Development Foundation (ARDF), and the International Foundation for Ethical Research (IFER).

When funding agencies explicitly state that applicants must contemplate and disclose 3R considerations in their proposals, it puts welcome pressure on researchers to comprehensively consider this significant aspect of their research. This will be a step closer to more intersectional discussion and implementation of the 3Rs, particularly in reducing the number of fish in research and looking for alternatives to some of the procedures that we use. Disclosure on how the 3R principles are to be implemented should not stop at the grant application process but could be followed up by the funding agencies during the implementation of the project, particularly in the project's progress report. This may take some time and effort to have a more streamlined process, but it can help ensure the delivery of high ethical standards in aquaculture research.

6 | SUMMARY AND FUTURE PERSPECTIVES

We hope that some of the overlooked options we have highlighted, some of which are simple and some of which are complex, provide further evidence that we are increasingly in a scientific position and technological epoch where we can reduce the number of experimental fish for research, where is it scientifically feasible and without compromising scientific integrity. There is currently no universal solution, and one cannot exclude occasions where compromises must be made, which can be dictated by research questions and logistics. This highlights that this is not a straightforward approach but rather a dynamic process. However, there are a range of options and better alternatives to incorporate the 3Rs in research and strategies to reduce the number of fish for fish health research—it should start from research conception and should not end when a fish trial terminates. We also believe that the obligation to reduce the number of fish should not be the responsibility of the researchers alone, though they are the central drivers of the whole process. This discussion requires intersectoral representation where different actors in the value chain of aquaculture research participate and demand accountability on how the reduction in experimental animals is taken into consideration. This is a complex topic, and several roadblocks are expected but not unattainable. A 3R center in each country would be ideal to further advance the application of the 3R principles and at the same time provide a platform for stakeholders to discuss challenges and opportunities of its implementation. Many European countries, but not all, have one of these already.

AUTHOR CONTRIBUTIONS

Carlo C. Lazado, Elisabeth Ytteborg, and Chris Noble were involved in conceptualization. Carlo C. Lazado wrote the first draft of the paper. Carlo C. Lazado, Elisabeth Ytteborg, and Chris Noble revised and reviewed the paper. All authors agreed with the final submitted version of the paper.

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