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Ethical considerations for field research on fishes

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Collection of data from animals for research purposes can negatively impact target or by-catch species if suitable animal ethics practices are not followed. This study aimed to assess the ethical requirements of peer-reviewed scientific journals that publish primary literature on fishes, and review the ethical considerations and animal care guidelines of national and international documents on the ethical treatment of animals for research, to provide an overview of the general ethical considerations for field research on fishes. A review of 250 peer-reviewed, ISI-rated journals publishing primary research on fishes revealed that nearly half (46%) had no mention of ethics, treatment of animals or ethical requirements for publication in their author guidelines or publication policies. However, 18% of the journals reviewed identify a specific set of ethical guidelines to be followed before publishing research involving animals. Ethical considerations for investigators undertaking field research on fishes, common to most animal care policies, legislation and guiding documents, include adhering to relevant legislation, minimising sample sizes, reducing or mitigating pain and distress, employing the most appropriate and least invasive techniques and accurately reporting methods and findings. This information will provide potential investigators with a useful starting point for designing and conducting ethical field research. Application of ethical best practices in field sampling studies will improve the welfare of study animals and the conservation of rare and endangered species.

Conservation implications: This article provides a list of ethical considerations for designing and conducting field research on fishes. By reviewing sampling techniques and processes that are frequently used in field research on fishes and by highlighting the potential negative impacts of these sampling techniques, this article is intended to assist researchers in planning field research to minimise impacts on fish populations. It is envisaged that this review will be a useful resource for journal editorial committees intending to introduce ethical requirements for publication and for researchers, managers, conservation practitioners and research organisations when designing field studies on fishes, applying for ethical clearance and developing institutional ethical guidelines.

Introduction

Studies on wild populations of animals are integral to scientific research (Basel Declaration Society 2010). Sampling for such research may be non-invasive (e.g. simple observation, visual census and video surveys), intrusive (e.g. external marking, fin-clipping, blood extraction, internal transmitter implantation) or lethal (e.g. voucher specimens, specimens sacrificed for biological and ecological data collection). While intrusive techniques may provide information that is not obtainable via non-invasive methods (Heupel & Simpfendorfer 2010), such sampling can negatively impact target or non-target (by-catch) species at individual or population levels or damage the surrounding habitat (*Animal Behaviour* Editorial 2012). Investigators, therefore, need to weigh up the costs and benefits in each case and select sampling methods and experimental procedures that minimise the potential disturbances to target and non-target organisms and their habitat (Canadian Council on Animal Care 2005; South African Bureau of Standards 2008; Use of Fishes in Research Committee 2014).

In much of the world, the humane care and use of animals for scientific purposes is guided by the ethical framework of the three *R*'s: *R*eplacement of animals by non-animal models where possible; *R*eduction in the number of animals used to the minimum number required for valid scientific results and *R*efinement of procedures and animal care standards to minimise pain, suffering, distress or lasting harm (Russell & Burch 1959). Studies employing sampling of wild populations should therefore eliminate, mitigate or minimise the potential for pain and distress and the duration of exposure to pain (CCAC 2005; Murray & Fuller 2000; SABS 2008).

Accordingly, numerous guiding documents have been produced, such as *The care and use of animals for scientific purposes* (SABS 2008) and *Guide for the care and use of laboratory animals* (National Research Council 2011), which identify important factors for consideration before and during field sampling studies. Furthermore, certain peer-reviewed scientific journals (e.g. *Fisheries Research*, *Fisheries Management and Ecology* and *Canadian Journal of Fisheries and Aquatic Sciences*) prescribe ethical considerations that need to be met for research involving live animals to be published. However, many journals do not require information on the ethical treatment of study animals, while those that do, lack consistency.

Pain can be defined as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage' and nociception as 'unconscious detection of potentially injurious stimuli by peripheral, spinal, and subcortical levels of the nervous system' (American Fisheries Society 2014:20–21). Recent research suggests that fishes have highly developed cognitive capabilities and the necessary physiological capacity for pain reception, nociception and suffering, resembling those found in other vertebrate groups (Bshary & Brown 2014; Chandroo, Duncan & Moccia 2004). Ethical considerations for research on sentient animals should therefore be applied to fish as they are to other vertebrates (Metcalfe & Craig 2011). However, many of the guiding documents are largely or entirely directed at the ethical use and treatment of mammalian animals, particularly captive animals (Cooke *et al*. 2016), with few addressing the ethical use and treatment of fishes, particularly in the wild. Furthermore, because of the diversity of animal taxa under research and the multitude of techniques available, a single set of guidelines is neither practical nor possible (UFRC 2014).

This article aimed to assess the ethical requirements of peer-reviewed scientific journals that publish primary literature on fishes and identify the guiding documents most commonly cited in instructions of these journals to submitting authors. The article also aimed to highlight the common ethical considerations for designing and undertaking field research on fishes, with particular relevance to the commonly used techniques in ichthyological and fisheries research in southern Africa. These considerations are based on international and national animal care and use policies and animal ethics guiding documents and published literature. It is envisaged that this review will be a useful resource for journal editorial committees intending to introduce ethical requirements for publication and for researchers, managers, conservation practitioners and research organisations when designing field studies on fishes, applying for ethical clearance and developing institutional ethical guidelines.

Journal ethical requirements for publishing primary fish research

Scientific journals that publish primary research on fishes were identified through a structured literature search, using the online citation indexing service Web of Science, maintained by Thomson Reuters. A text search for 'fish*', under the research areas of 'fish biology' and 'fish ecology' performed in February 2015 returned articles from more than 500 English-language, ISI-rated journals. The instructions to authors and publication policies of the 250 journals with the most articles relating to fish research were scrutinised to identify their editorial policies relating to the publication of primary research involving the use of animals.

Of the 250 journals reviewed (Appendix 1), 54.0% (135) have some mention of animal ethics requirements: 18.0% (45) stipulate a specific guiding document, to which submitted work must adhere for publication (Category 1), 21.6% (54) require formal approval by an institutional, national or international animal ethics committee (AEC) (Category 2) and 14.4% (36) require only a statement within the manuscript on the welfare of study animals (Category 3). The instructions to authors of the remaining 46.0% (115 journals), however, make no reference to animal ethics requirements (Category 4), suggesting that the treatment of animals in almost half of these articles had not been questioned. A similar review, of 288 randomly selected English-language journals publishing original research on animals, revealed that nearly half the journals assessed had no editorial policy relating to the use of animals in research (Osborne, Payne & Newman 2009). These two searches therefore highlight the large discrepancies in journal ethical requirements, with almost half not requiring adherence to ethical standards, while journals that do, lack consistency.

The 45 journals that require authors to adhere to a specific set of ethical guidelines (Category 1) together identify 15 guiding documents (Table 1). The scope of these documents varies considerably, from highly prescriptive, for example, *Guide for the care and use of laboratory animals* (NRC 2011), to providing a general tone for investigators to follow, for example, *Basel Declaration* (BDS 2010). These included guidelines relating specifically to reporting, such as the ARRIVE guidelines (Animal Research: Reporting of in Vivo Experiments; Kilkenny *et al*. 2010); national legislation, such as the *United Kingdom's Animals (Scientific Procedures) Act 1986*; animal use guidelines published specifically by the respective journal (i.e. *Animal Behaviour* and *Journal of Fish Biology*) and guiding documents prepared specifically for the ethical treatment of fishes, such as *Guidelines on the care and use of fish in research, teaching and testing* (CCAC 2005) and *Guidelines for the use of Fishes in Research* (AFS 2014) (Table 1).

These guiding and policy documents (for web links, see Appendix 2) provide a useful starting point for investigators wishing to explore each of the common aspects for ethical consideration in more detail. While the documents range broadly in their scope, background and applicability, many share several common aspects for ethical consideration (Table 2). These aspects appear repeatedly in most comprehensive animal care and use guiding documents and several countries' legislation relating to the ethical treatment of animals in research.

TABLE 1: Guiding documents for the ethical treatment of animals in research, stipulated by 45 ISI-rated, peer-reviewed journals that publish primary research on fishes, which must be followed by authors submitting research for publication in these journals.

Guiding document or policy or legislation	Citation	Number journals
National Centre for the Replacement, Refinement and Reduction of Animals in Research: ARRIVE (Animal Research: Reporting of in vivo Experiments) Guidelines	Kilkenny *et al.* (2010)	15
Animal Behaviour Journal: Guidelines for the treatment of animals in behavioural research and teaching	Editorial (2012)	8
World Medical Association: Declaration of Helsinki	WMA (2008)	6
Council for International Organization of Medical Sciences/International Council for Laboratory Animal Science: International Guiding Principles for Biomedical Research Involving Animals	CIOMS & ICLAS (2012)	3
Basel Declaration Society: Basel Declaration (2010)	BDS (2010)	2
European Commission: Directive 2010/63/EU on the protection of animals used for scientific purposes	EU (2010)	2
United Kingdom Government: *Animals (Scientific Procedures) Act 1986* (revised 2013)	UK Government (1986)	2
Canadian Council on Animal Care: Guidelines on the care and use of fish in research, teaching and testing	CCAC (2005)	2
Society of Environmental Toxicology and Chemistry: Code of Ethics	SETAC (n.d.)	1
National Cancer Institute, Frederick National Laboratory for Cancer Research: Animal Care and Use Committee Guidelines	NCI (n.d.)	1
Journal of Fish Biology: Ethical justification for the use and treatment of fishes in research	Editorial (2011)	1
American Fisheries Society: Guidelines for the use of fishes in research	AFS (2014)	1
The National Academies: The Brazilian legal framework on the scientific use of animals	Filipecki *et al.* (2011)	1
Council of Europe: European convention for the protection of vertebrate animals used for experimental and other scientific purposes	COE (1986)	1
National Research Council: Guide for the care and use of laboratory animals	NRC (2011)	1

TABLE 2: Common aspects for consideration for the ethical treatment of animals in research, mentioned in the guiding documents identified in the author guidelines of 45 ISI-rated journals that publish primary research on fishes, as well as in numerous animal care and use guiding documents and national policies.

Aspects for consideration	ARRIVE	*Animal Behaviour Journal*	Declaration of Helsinki	CIOMS & ICLAS	Basel Declaration	EU Directive 2010/63/EU	*UK Animals Act 1986*	Canadian Council on Animal Care	SETAC Code of Ethics	NCL-Frederick ACUC	*Journal of Fish Biology*	American Fisheries Society	Brazilian Legal Framework	European Convention	National Research Council
Legislation	1	1	-	1	-	1	1	1	1	-	1	1	1	1	1
Personnel	-	1	-	1	1	1	1	1	-	-	-	1	1	1	1
Animal welfare	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Principle of the 3 R's	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1
Cost–benefit analysis	1	1	-	1	-	-	-	-	-	1	-	1	1	-	1
Endangered species	1	1	-	-	-	1	-	1	-	-	1	1	-	-	1
Handling		1	-	-	-	1	-	1	-	-	-	1	-	-	1
Holding	1	1	-	1	-	1	1	1	-	1	-	1	1	1	1
Standardised procedures	1	1	-	1	-	1	1	1	-	1	1	1	1	1	1
Anaesthesia/ analgesia	1	1	-	1	-	1	-	1	-	1	1	1	1	1	1
Euthanasia	1	1		1		1	1	1		1	1	1	1	1	1
Capture methodology	-	1	-	-	-	1	-	1	-	-	-	1	-	-	-
Collecting fluid/tissue	-	-	-	-	-	-	-	1	-	1	-	1	-	-	-
Marking/ tagging	-	-	-	-	-	-	-	1	-	-	-	1	-	-	1
Reporting	1	1	-	-	1	-	-	-	-	-	-	-	1	-	-

The clear and unified theme across almost all guiding documents is the need to maintain the welfare of study animals (Table 2). To this end, many of these guidelines advocate adopting the principle of the three *R*'s – *R*eplace, *R*educe, *R*efine (Russell & Burch 1959). Most guiding documents also highlight the importance of adhering to regional, national or international legislation and gaining approval from at least a local or institutional AEC, and many identify the requirements and considerations for holding (i.e. keeping in captivity), breeding and husbandry of animals. Specific procedures, such as the sampling of blood or tagging of fishes, receive relatively less focus in these documents, although most documents identify the need for standardised, approved procedures and prescribe that investigators familiarise themselves with the adverse effects associated with different methods and choose the least invasive techniques to suit the aims of the study and the information required (e.g. behavioural observations, genetic studies or specimen collections) (CCAC 2005; UFRC 2014).

The following subsections of this review expand on these common ethical considerations, providing information (with citations to relevant literature) for researchers, managers and conservation practitioners, when undertaking sampling or field research on fishes and for journals intending to develop or specify ethical requirements for publication. Particular focus is placed on the considerations for capture, handling and processing of fishes, which are relatively under-represented in many guiding documents.

Ethical considerations for field research on fishes

Based on the guiding documents identified in the previous section and numerous institutional, provincial, national and international guidelines for the care and use of animals in research, we expand on the key ethical aspects for consideration before, during and after undertaking field research on fishes, which we group into five categories: regulations, experimental design, handling and holding of study animals, experimental procedures and reporting of findings.

Regulations

Prior to any study involving live animals, investigators should familiarise themselves with the regulations and legislation pertaining to the proposed species, techniques and study area and obtain all necessary permits (Murray & Fuller 2000). All research activities and field sampling should follow accepted policies, standard operating procedures and international guidelines for the care and use of animals in research (CCAC 2005). In South Africa, animal research is governed by the *Veterinary and Para-Veterinary Professions Act* (South African Government, Act No. 19 of 1982), with guidelines provided by the *South African National Standard (SANS) 10386: The care and use of animals for scientific purposes* (SABS 2008). Investigators should also ensure that all methods, sample sizes, target species and study locations are approved by at least an institutional or preferably national or international AEC. All persons involved in the capture, handling, holding, treatment and processing of experimental animals must be appropriately trained for their specific roles during an experimental procedure (Bradford, Korman & Higgins 2005; Murray & Fuller 2000).

Experimental design

Animal welfare, sample size and the principle of the three R's

The most repeated consideration, in most guiding documents, is to maintain the welfare of the study animals and minimise or mitigate pain, suffering and distress (AFS 2014; CCAC 2005). This may be achieved through less invasive techniques and refining procedures in ways that reduce the intensity and durations of exposure to pain, suffering and distress (Mohr 2013; NRC 2011).

The number of individuals captured, manipulated or killed for the purpose of research is a fundamental consideration of any scientific study involving live animals. The appropriate sample size is contingent on the nature and objectives of the proposed research. Most guiding documents therefore advocate the principle of the three R's (Russell & Burch 1959). For experimental research, particularly field research in which animal subjects cannot be replaced by computer or other surrogate models, the number of study animals should be minimised as far as practicably possible, but sufficient to provide representative information (UFRC 2014).

Cost–benefit analysis

A key principle of justifying animal experimentation is that the cost incurred to the individual or population (e.g. stress, deaths) is outweighed by the benefit of the research (e.g. advancement of knowledge) to society (Knight 2011). Thus, in proposed studies for collecting or manipulating fishes, a cost–benefit analysis should underlie decisions regarding sample size (*Animal Behaviour* Editorial 2012). For biodiversity studies, a sample should be sufficient to capture phenotypic or genotypic variation, although this may be difficult to determine without prior knowledge of a taxon's natural genetic variation (Miyamoto *et al*. 2008). In experimental biology, a minimum sample size may be determined to satisfy the required statistical power (Knight 2011). Voucher specimen collections should sacrifice the minimum number of animals necessary for effective species confirmation, taking into account sexual dimorphism, ontogenetic change in identifiable features and similarity to related species (Animal Research Review Panel n.d.).

Species and sampling localities

During field collection, vulnerable life-history stages or life-cycle events, such as aggregations of breeding fish, and sensitive habitats should be avoided (AFS 2014). Species regarded as imperilled or threatened should not be subjected to invasive sampling studies, unless deemed necessary for the specific purpose of gaining information for improved conservation (AFS 2014). By-catch of non-target species should be minimised, but if captured these should be released promptly and with minimal injury (AFS 2014). The number of sampling sites, depending on habitat variation, distribution range and the purpose of collection (e.g. confirming range shifts), should be minimised. Investigators should also select a sampling method that has the least impact on the fishes and the local ecosystem (CCAC 2005).

Recent debate around the conservation ethics of killing endangered species for scientific research highlights the ethical challenge of justifying the lethal sampling of fishes in field work (Heupel & Simpfendorfer 2010; Minteer *et al*. 2014). For lethal sampling, particularly of threatened species, the proportion of the population affected by sampling (with implications for the viability of the sampled population) and the purpose of collecting, as it applies to the conservation management of the species, should be considered.

Certain fisheries journals, such as *Journal of Fish Biology* and *Indian Journal of Fisheries*, have explicit policies on the sizes and justification of lethal collections reported on in submitted manuscripts and will not accept manuscripts that involve the unmotivated killing or damage of IUCN Red List threatened or endangered species.

Handling, holding and release of experimental fishes

Captured fish should be handled in a manner that minimises pain, distress, suffering and unnecessary loss of external mucus or scales (CCAC 2005). The duration of handling and the experimental procedure should be kept to a minimum, to avoid unnecessary stress and exposure time (AFS 2014).

Investigators should assess the suitability of holding study animals prior to, during or after experimentation. If holding tanks are to be used, they must allow fish to rest comfortably, minimise risk of escape or injury, be adequately aerated, maintain constant temperature and minimise the risk of disease transmission (Barker *et al*. 2002). Holding tanks should be disinfected between uses and holding areas must be safe, quiet and hygienic (Barker *et al*. 2002). While post-experimentation captivity may allow recovery monitoring, this may be undesirable for species vulnerable to stress from captivity, and holding of animals in field research, particularly large-bodied animals, may be impractical (Jepsen *et al*. 2002).

Where appropriate, fish should be released as soon as possible after completion of processing (e.g. measuring, tagging or collection of tissue). All fish having undergone an experimental procedure must be in good health when released and able to return to their natural environment with 'normal' physiological and behavioural functioning (Bradford *et al*. 2005; CCAC 2005). All necessary steps must be taken to prevent predation and injury upon release and the introduction of pathogens or harmful chemicals into the environment (SABS 2008).

Experimental procedures

Sampling methods for fish larvae

High levels of natural mortalities and rapid recoveries from population reductions are characteristic events in the early life histories of many fish species (UFRC 2014). It is therefore generally regarded by most ethical standards that the sampling of fish larvae that have not developed beyond exclusive reliance on their own yolk nutrients does not require ethical clearance (Canadian Council on Animal Care 2007). However, according to the SABS (2008), live, sentient non-human vertebrates, including eggs, foetuses and embryos of fish, are to be cared for and used in ways that are judged to be scientifically, technically and humanely appropriate. Furthermore, investigations involving the early life stages of fishes typically require the sacrifice of large numbers of study organisms. Therefore, a precautionary approach should be followed, in which sample sizes and by-catch are minimised, particularly when threatened species constitute the target or possible by-catch species (CCAC 2005).

For fish larvae, killing by immersion in ice or a fixative such as ethanol or formalin is generally acceptable; however, this should be preceded by an overdose of an appropriate anaesthetic (Craig 2006). Any proposed non-use of anaesthesia on the grounds of compromising the aim of the study should be fully justified *a priori* (Metcalfe & Craig 2011). When fish larvae become independently feeding forms, ethical authorisation must be obtained (CCAC 2007).

Non-invasive fish sampling techniques

Visual sampling, such as underwater visual census (by snorkelling or SCUBA) and underwater video surveys, is common in marine, estuarine and freshwater environments (Ellender *et al*. 2012; Willis, Millar & Babcock 2000) and provides a valuable tool for collecting qualitative and quantitative fishery-independent data on fish density, diversity, community structure and behaviour (Brock 1982). Underwater videography is increasingly used for assessing and monitoring fish communities, as digital video provides standardised methodology and permanent records (Langlois *et al*. 2010). The non-destructive nature of these methods makes them suitable for sensitive habitats and protected areas (Mallet & Pelletier 2014), and in most cases, ethical justification for their use is not necessary. In some instances, however, divers conducting visual or video observations or the deployment of remote equipment may cause habitat damage. Investigators should thus attempt to minimise any potential impact on the habitat.

Capture sampling techniques

Numerous sampling techniques involve the actual capture (such as trapping, netting or hooking) of target fishes and are thus inherently invasive. Investigators therefore need to understand the uses, limitations and threats of these methods, in order that the most appropriate method be employed, to provide the most satisfactory result, while minimising pain, suffering and distress (EU 2010).

Netting: There are a variety of netting methods for the capture of fishes, some passive and some active. Gill nets are passive nets, hung vertically in the water column. The gills or fins of fish swimming into the net become entangled in the mesh, preventing their escape. Mesh size is an important consideration, as larger mesh sizes usually result in the capture of larger fish, while providing some form of exclusion for smaller species or individuals. The use of gill nets for sampling is generally accepted, although gill nets can result in cuts on the body from the filament of the net or the drowning of obligate air-breathing fishes, amphibians, reptiles and mammals (Ellender *et al*. 2016).

Fyke nets are also passive nets, comprising a vertical wall of net that guides fishes into an enclosed cod-end or bag at either end. They are generally considered non-destructive, as fish are free to swim in the cod-end once captured, allowing them to be released relatively unharmed. However, fyke net placement is an important consideration, as nets set in areas of low oxygen concentration can result in elevated fish mortality. Furthermore, fyke nets are not species selective and mortality of by-catch, particularly drowning of obligate air-breathing animals, can occur (Larocque, Cooke & Blouin-Demers 2012). Mortality can also result from predatory fishes, frogs or crabs that are caught in the cod-end or from external sources, such as otters attacking fishes through the net (Ellender *et al.* 2016). Given the associated predation risk to captured fishes, fyke nets may be unsuitable in certain environments, particularly if they pose a high capture threat to endangered species. Simple gear modifications, such as creating air spaces using floats to allow captured animals to breathe (Larocque *et al.* 2012) or selective mesh placed over the entrance to exclude mammals or reptiles, can however reduce mortality and by-catch. Reducing soak time or checking nets at shorter intervals enables by-catch and target species to be processed and released more rapidly. Owing to the potential injuries, by-catch and mortalities associated with gill and fyke netting, motivation for their use is warranted and identification of potential by-catch species and an assessment of their conservation status should form an integral part of the planning phase of any project using these gears.

Active net gears include, *inter alia*, seine nets and various forms of trawl nets. Seine nets are vertical nets, with a floating top line and weighted bottom line, actively hauled to encircle a set area of water and pull the fish within that area to shore. They are a preferred gear for sampling fish communities in shallow littoral environments such as beaches, estuaries and artificial impoundments (Lapointe, Corkum & Mandrak 2006). A cod-end at the centre of the net acts as a corral point for fish as they are brought to shore. Seine netting is generally regarded as non-destructive, although mortality of small-bodied species and post-release mortality of discarded by-catch species have been recorded (Kennelly & Gray 2000). Fish mortality can be minimised by handling fish within the submerged cod-end before bringing the net onshore. In some cases, seine netting can damage submerged macrophyte habitat in haul regions, although the impact is usually minor (Bayley & Herendeen 2000).

Trawling refers to nets towed from a boat, which usually sample hyper-benthic environments. The use of dragged or towed gears, which may damage the habitat, benthic environment or animals sampled (Feyrer *et al*. 2013), should be avoided or minimised (UFRC 2014). Alternative methods should also be sought where possible; for example, Feyrer *et al.* (2013) investigated the use of attaching a camera to the open cod-end of a trawl net, allowing fish to be recorded but to pass through the net unharmed, greatly reducing mortality.

Electrofishing: Electrofishing is one of the most effective and commonly employed sampling methods in shallow freshwater environments (Snyder 2003). While generally considered non-destructive, it can have negative impacts (Snyder 2003). In extreme cases, severe spinal injuries, internal haemorrhages, bleeding at the gills, physiological stress, asphyxiation and harmful effects on embryos have been recorded (Snyder 2003). It is therefore recommended that researchers familiarise themselves with, and apply, recommendations for international best practises regarding electrofishing (e.g. Beaumont *et al*. 2002; Goodchild 1991). Current strengths and settings and the electrofishing gear itself should be suited to the target species and conditions in the study area, such as conductivity, water depth and survey area (Bohlin *et al*. 1989). When sampling threatened or endangered fishes, electrofishing should be used with caution and alternative methods employed where possible (Ellender *et al*. 2012). If multiple electrofishing passes are intended, all fish captured should immediately be removed from the net and placed in aerated buckets, filled with ambient water, to avoid prolonged exposure. Bohlin *et al*. (1989) and Snyder (2003) provide comprehensive reviews on standardising electrofishing protocols and harmful effects of electrofishing.

Hook-and-line fishing: Hook-and-line fishing is commonly used in a range of research applications, such as quantifying abundance, assessing community structure and for tagging studies (Bennett & Attwood 1993; Dunlop, Mann & Van der Elst 2013; Willis *et al*. 2000). Catch-and-release is often employed, whereby fishes are released to the capture environment after undergoing procedures such as measuring or tagging, with minimal impact to the fishes and environment (Cooke *et al*. 2013a). However, excessive fight times or post-capture air exposure and high water temperatures have been shown to induce physiological stress responses in hook-and-line caught fishes, with implications for their successful release (Cooke & Suski 2004). In addition, fish captured by hook-and-line fishing may be subjected to stress as a result of barotrauma injuries if rapidly brought to the surface (Keniry *et al.* 1996), or damage to the gills or viscera through hook ingestion (Domeier, Dewar & Nasby-Lucas 2003). The potential impacts of hook-and-line fishing should thus be thoroughly considered before using this method to capture endangered species or in excessive water depths where barotrauma is likely.

Relevant mitigation measures, such as choice of hook design and size, optimal fishing times and minimised handling times need to be considered (see Cooke & Suski 2004). The use of circle hooks has been shown to decrease the incidence of gut- or deep-hooking (Cooke & Suski 2004) and post-release mortality (Prince *et al*. 2007) and the use of barbless hooks can reduce injury and facilitate hook removal (Schaeffer & Hoffman 2002). Brownscombe *et al*. (2016) provide guidelines for best practice in catch-and-release recreational fisheries.

Ichthyocides

Ichthyocides (poisons) and anaesthetics have been used extensively to determine fish species composition, particularly in freshwater and shallow reef environments (Ackerman & Bellwood 2000; Willis 2001). Rotenone, for example, is a commonly used ichthyocide, highly effective at killing most fish species (AFS 2014; Willis 2001). Although such techniques are effective for assessing cryptic species and provide greater species and family counts than non-destructive methods (Willis 2001), they can cause excessive mortality for sensitive species, are non-selective, vary widely in effectiveness, provide inefficient sampling of highly mobile species and cannot be used in sensitive areas or where sampling is to be repeated (Brock 1982). Furthermore, recent studies have highlighted that many non-target organisms are susceptible to ichthyocide concentrations used for fishes (e.g. Dalu *et al.* 2016). Careful consideration, especially of collateral impacts on non-target biota, is therefore necessary, prior to using ichthyocides. Clearwater, Hickey and Martin (2008) discuss several ichthyocides for pest control; however, a comprehensive review of ichthyocides and their use in fish research is lacking.

Anaesthesia and analgesia

Sedatives or anaesthetics are commonly prescribed and used during prolonged or invasive procedures, to immobilise fish and reduce pain and physiological stress related to capture, handling and processing, through depression of the nervous system (CCAC 2005; UFRC 2014). Minor procedures usually require mild sedation, whereas intrusive (major) procedures, such as laparotomy, usually require deep anaesthesia (Thorsteinsson 2002). Where anaesthetics or sedatives are used, only approved drugs should be used and administered at a concentration and duration appropriate to the size and species of fish and in accordance with established guidelines and relevant legislation (AFS 2014; UFRC 2014).

The SANS 10386 (SABS 2008) recommends four anaesthetics for use on fishes in South Africa, including tricaine methanesulfonate (MS-222), benzocaine (and benzocaine hydrochloride), metomidate and ketamine hydrochloride. However, none of these is registered in Australia, New Zealand, the United States, Canada or Europe (Javahery & Moradlu 2012). Other anaesthetics common in fish research include 2-phenoxyethanol, clove oil, AQUI-S™ (active ingredient isoeugenol) and quinaldine sulphate (Javahery & Moradlu 2012; Wagner & Cooke 2005). However, many of these are regarded as hazardous (Javahery & Moradlu 2012), particularly if fish are released and subsequently consumed by humans (Thorsteinsson 2002). Of these anaesthetics, only MS-222 is approved by the United States Food and Drug Administration (FDA) for use on animals in the United States (AFS 2014), but has a 21-day withdrawal period before fish can be released into the wild, negating its use in field studies. Eugenol (a derivative of clove oil) and carbon dioxide are the only immediate-release drugs approved by the FDA (AFS 2014) – yet neither is recommended for use in South Africa (SABS 2008), and carbon dioxide allows considerable trauma at shallow sedation and is not suitable for heavy sedation required in invasive procedures (Javahery & Moradlu 2012). Therefore, identifying an appropriate drug and concentration, particularly for fish intended for immediate release, is often a challenging task and should be based on thorough research of contemporary literature and legislation prior to any implementation (see Thorsteinsson 2002 for comprehensive review of anaesthetics).

Anaesthetics can induce prolonged negative metabolic effects and stress responses in fishes (CCAC 2005), as well as hypoxia from reduced respiration and vascular activity (Thorsteinsson 2002). In certain cases, this may lead to post-release mortality or increased predation risk (Cooke *et al*. 2005). Numerous studies on fishes, involving handling and even invasive procedures such as laparotomy, have deemed the use of anaesthesia inappropriate (Wagner & Cooke 2005). For large elasmobranchs, anaesthesia would require specialised lifting equipment, considerable risk to the animals and excessive quantities of chemicals; therefore, such studies usually rely on tonic immobility (e.g. Holland *et al*. 1999). Therefore, the use of anaesthetics should be evaluated prior to any study (Thorsteinsson 2002), particularly when fish are planned for release into the wild and in some cases may not be appropriate at all (Cooke *et al*. 2013b, 2016).

Collecting tissue and blood samples

Tissue collection: A variety of methods have been used to successfully collect tissue or blood samples from anaesthetised fish without significant effects on their survival. Non-lethal tissue sampling includes fin-clipping, excision of scales and gill, muscle or skin biopsies (McCormick 1993; UFRC 2014). Fin-clipping is common for genetic sampling and its deleterious effects have been extensively studied. Many studies reported no negative effects and complete regeneration of the affected fins in weeks (Woodall, Koldewey & Shaw 2011), while others reported some decrease in survival or growth of fin-clipped fish (O'Grady 1984). The appropriate site to sample is species specific. For example, partial clipping of the dorsal fin was successfully used for genetic sampling of seahorses with no significant effect on mortality (Woodall *et al*. 2011).

Blood collection: Fish blood is collected for various reasons, such as haematology and clinical chemistry tests (Satheeshkumar *et al.* 2012), parasitological investigations (Shahi *et al.* 2013) and investigations on adaptations to subzero temperatures (Miya *et al.* 2014). Such studies have demonstrated that blood samples can be successfully obtained, even from fish less than 100 g in mass, without compromising their survival. Plastic syringes with a small volume of anticoagulant such as sodium or ammonium heparin or sodium citrate can be used to avoid the rapid clotting that can occur with the use of glass syringes (Stoskopf 1993). The size of the syringe and diameter of needle should be appropriate to the size of fish. The preferred site for blood sampling from anaesthetised fish is the caudal vein running beneath the vertebrae, using a lateral or ventral approach. Study objectives will determine the required blood volume and concentration of anticoagulant. Other methods used to collect blood from fishes include cardiac puncture and caudal bleeding (UFRC 2014). These methods, however, can compromise the survival of the fish. Needles are most effective when new and sharp and should be replaced or sterilised after each sample to avoid contamination and unnecessary injuries to the fish.

Marking and tagging of fishes

Marking (using dyes or fin-clipping) and tagging of fishes with external or internal tags has been used extensively to estimate population sizes, discriminate stocks, identify migration rates and movement patterns and assess fish growth and survival (McFarlane, Wydoski & Prince 1990). Numerous marking and tagging methods are used, although most are intrusive to some degree (Thorsteinsson 2002). The capture, handling and marking/tagging process can affect the behaviour, physiology, swimming capacity, predator avoidance, feeding, growth rate and ultimate survival of tagged organisms (Naef-Daenzer *et al*. 2005; Thorsteinsson 2002). Studies should therefore aim to minimise these impacts, for the welfare of the study animal, to ensure that the process provides scientifically valuable data, and to maximise the value from every individual that has been subjected to the stress of capture, handling and processing (Cooke *et al*. 2013b).

Marking: Marking techniques for fishes, such as external dyes, fin-clipping, hot branding (e.g. lasers) and freeze branding (e.g. liquid nitrogen), do not breach the skin or musculature and are thus generally considered to have minimal impact on fishes (Murray & Fuller 2000). However, such marks usually exhibit short-term retention, and brightly coloured external marks or dyes (and any external part of a tag) should be avoided, as these can affect social structure or interactions of the fish or reduce its predator avoidance or hunting capabilities (Guy, Blankenship & Nielsen 1996; Murray & Fuller 2000). Chemical tags are another form of marking and may be natural (i.e. accumulation from the local environment) or induced, through immersion, injection or ingestion of chemicals, which become incorporated into tissue or calcified structures (Nielsen 1992). Chemical tagging is long lasting and allows large numbers of individuals to be tagged and does not necessarily require anaesthetic or even handling (Guy *et al*. 1996), but often requires sacrifice of the animal at a later stage.

General tagging considerations: To minimise the effects of tags on fish and maximise data collection and tag retention, tag size and weight should be appropriate for the size, weight and species of fish (Cooke *et al*. 2011; Stasko & Pincock 1977). The tag placement and attachment method must also be appropriate for the study species (Naef-Daenzer *et al*. 2005; SABS 2008) and have minimal influence on the posture, buoyancy, locomotion (Stasko & Pincock 1977), behaviour, growth and survival of the fish (Bradford *et al*. 2009). Where necessary, the suitability of a chosen tag type, size and attachment method should be assessed prior to tagging studies on a new species (CCAC 2005; Murray & Fuller 2000).

External tag attachment: External attachment is used for simple plastic or wire tags and electronic transmitters and requires that an external or trailing part of the tag be anchored somehow within the musculature or skeletal structure of the fish. A common type of external tagging, particularly in southern Africa, is dart tagging (Maggs & Cowley 2016). Dart tags are inserted using a sharp, sterile tag applicator, usually at the base of the dorsal fin and anchored behind the pterygiophores (Attwood 1998). Electronic tags can also be attached externally in a similar manner or with wires or nylon cords inserted through the dorsal musculature of the fish and secured with a plastic or metal plate (Liedtke & Rub 2012; Thorstad *et al*. 2013). Sharks and large pelagic fishes are often tagged *in situ* in the water, by means of a dart tag or electronic tag attached to the end of a sharpened pole. For pelagic sharks, tags (particularly satellite tags) are also commonly attached by means of plastic or metal bolts through the dorsal fin (Thorsteinsson 2002).

External tagging is simple and does not necessarily require animals to be anaesthetised, or even brought out of the water (Thorsteinsson 2002). External tags, however, can have negative impacts, as they may increase drag and impede normal swimming ability and reduce growth or survival (Murray & Fuller 2000). The attachment mechanisms breach the skin and musculature and could lead to infection, tissue damage or necrosis, reduced growth or reduced reproductive capacity (Thorsteinsson 2002). External tags, particularly larger volume electronic tags, should thus be shaped and attached in such a way that minimises drag, entanglement in aquatic vegetation, irritation and constriction (CCAC 2005; Murray & Fuller 2000). Owing to the disadvantages of external tag attachment, a number of internal tag implantation techniques have been used.

Gastric insertion: Gastric insertion of devices involves voluntary ingestion of the device embedded in bait or the forcing of the device down the pharynx, past the cardiac sphincter and into the stomach by means of a glass or plastic rod (Liedtke & Rub 2012; Thorsteinsson 2002). The process is less invasive than surgical implantation (Winger & Walsh 2001), and the internal device overcomes many of the problems associated with external tags, such as entanglement, drag or wound development (Stasko & Pincock 1977). However, gastric insertion can only be used when fish are not feeding and there are several drawbacks to the technique. There is a risk of tag loss through regurgitation, the presence of the device may affect the ability of the fish to feed and injury may be caused to the oesophagus or stomach (Thorstad *et al*. 2013; Winter 1996). Furthermore, gastric insertion cannot be used on fishes with a food-crushing pharyngeal apparatus, and the process does not allow for long-term tag retention (Stasko & Pincock 1977).

Surgical implantation of tags or devices: Laparotomy (the surgical implantation of a device through an incision in the body wall) is becoming recognised as a superior technique to external attachment and gastric insertion, particularly for long-term tag retention, reduced long-term physiological stress and data that may be more representative of normal behaviour (Cooke *et al.* 2011; Thorstad *et al*. 2013). The technique is considered to have little or no methodological bias in the long-term, and the internal placement of the tags removes the potential entanglement and hydrodynamic interference that may result from external tags (Jepsen *et al*. 2002; Thorstad *et al*. 2013). However, the recovery time after surgical implantation may be greater than that for external attachment or gastric insertion (Thorsteinsson 2002). Furthermore, this process requires suitably trained or qualified personnel and is governed in certain states by formal legislation. In South Africa, surgical procedures are regulated under the *Veterinary and Para-Veterinary Professions Act* (SA Government, Act No. 19 of 1982). For all tagging procedures, aseptic technique should be maintained, although this is difficult in most field settings.

Antibiotic powder (topical) or liquid (topical or by injection) can be applied post-tagging to prevent secondary infection. Before releasing tagged fish back into their natural environment, they should be monitored until having recovered from the effects of anaesthesia and surgery.

Euthanasia

Where the sacrifice of study fish cannot be avoided (e.g. voucher specimen collection or physiological studies), humane euthanasia techniques should be used (SABS 2008). Animals may be euthanised by chemical or physical methods, and the selected methods should be predictable, minimise pain and stress, produce rapid loss of consciousness and be compatible with the scientific aims (American Veterinary Medical Association 2013; SABS 2008). Fishes to be preserved should be euthanised prior to immersion in formalin or other preservatives (CCAC 2005). Where feasible, euthanasia should consist of a two-step process with initial anaesthesia until loss of equilibrium, followed by a physical or chemical method to cause brain death (CCAC 2005). Rapid cooling, followed by prolonged exposure in ice-cooled water, is recommended for small-bodied fishes (AVMA 2013). Overdosing with anaesthetics, such as MS-222, is an accepted method (Neiffer & Stamper 2009), whereas the use of carbon dioxide, suffocation (removal from water) and decapitation alone are generally not. The AVMA (2013) provides comprehensive guidelines on euthanasia of animals.

Reporting

Equally important as study design, animal welfare and appropriate methodologies in animal research, are the accurate, comprehensive reporting and communication of procedures, protocols and results, to facilitate knowledge transfer (Filipecki *et al*. 2011). To this end, the ARRIVE guidelines were developed to ensure that all relevant information is included in publications, to maximise the value of publications and render them useful for scientific advancement or policy making (Kilkenny *et al*. 2010). Such information may include *(inter alia*) numbers, demographics and sources of study subjects; sampling localities and times; detailed processing methods; reagent concentrations; holding conditions; statistical tests; positive and negative outcomes; limitations; new discoveries; and applicability of results beyond the specific study (Kilkenny *et al*. 2010).

Conclusion

The review of journal ethical requirements revealed considerable variability in the level of ethical reporting for research involving animals to be accepted for publication. While publishers, research institutions and government agencies may vary in their requirements for demonstrated ethical behaviour, such inconsistency should not deter investigators from taking individual responsibility for their ethical duties in sampling or manipulating fishes in the field. The principles of animal welfare and biodiversity conservation should always guide the development of research project methods, regardless of whether they are required by the journal in which an investigator wishes to publish.

To aid this principle, this review highlights both the common guiding documents on the ethical treatment of animals (particularly fishes) and common, important ethical considerations for managers, researchers, technicians and students, as they apply to commonly used methods for field-based research on fishes. Primary ethical considerations are that (1) all field, capture, sampling and processing activities must adhere to relevant legislation and should be approved by at least an institutional AEC, (2) the welfare of the study animals should be of primary concern and all efforts should be made to prevent or reduce pain, suffering and distress, (3) sample sizes should be kept to the minimum, but sufficient to achieve the objectives of the study, (4) the conservation status of the target organism should be considered, (5) destructive or invasive gears and processes should be applied with caution and (6) standard and accepted procedures should be followed. The review will provide a useful resource for journal editorial committees developing ethical guidelines for publication and for potential investigators designing and conducting field research on fishes. Application of these ethical requirements in field sampling studies will improve fish welfare and the conservation of fishes, especially rare and critically endangered species.

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Authors' contributions

R.H.B. was the lead author and O.L.F.W. was the project leader. R.H.B., B.R.E., P.P., T. Mäkinen, T. Miya, R.J.W., D.J.W. and O.L.F.W. contributed to conceptualisation, analyses, writing and editing the manuscript.

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Appendix 1

TABLE 1-A1: Categorisation, based on animal ethical requirements, of 250 ISI-rated, peer-reviewed, scientific journals with the most articles relating to fish research, revealed through a Web of Science (Thomson Reuters) search.

Journal	Category
ACTA ICHTHYOLOGICA ET PISCATORIA	4
ACTA PARASITOLOGICA	3
AFRICAN JOURNAL OF AQUATIC SCIENCE	3
AFRICAN JOURNAL OF ECOLOGY	4
AFRICAN JOURNAL OF MARINE SCIENCE	3
AFRICAN ZOOLOGY	4
AMBIO	4
AMERICAN MIDLAND NATURALIST	4
AMERICAN NATURALIST	1
AMERICAN ZOOLOGIST	4
ANIMAL BEHAVIOUR	1
ANNALES ZOOLOGICI FENNICI	4
ANNALS OF THE NEW YORK ACADEMY OF SCIENCES	4
ANTARCTIC SCIENCE	4
APPLIED AND ENVIRONMENTAL MICROBIOLOGY	1
AQUACULTURE	1
AQUACULTURE RESEARCH	1
AQUATIC BIOLOGY	2
AQUATIC CONSERVATION MARINE AND FRESHWATER ECOSYSTEMS	4
AQUATIC ECOLOGY	3
AQUATIC ECOSYSTEM HEALTH MANAGEMENT	4
AQUATIC INVASIONS	2
AQUATIC LIVING RESOURCES	4
AQUATIC SCIENCES	2
AQUATIC TOXICOLOGY	1
ARCHIV FUR FÜR HYDROBIOLOGIE/FUNDAMENTAL AND APPLIED LIMNOLOGY	4
ARCHIVES OF ENVIRONMENTAL AND OCCUPATIONAL HEALTH	2
ARCTIC	4
ARDEA	4
AUSTRALIAN JOURNAL OF ECOLOGY	2
AUSTRALIAN JOURNAL OF MARINE AND FRESHWATER RESEARCH	2
AUSTRALIAN JOURNAL OF ZOOLOGY	2
BEHAVIORAL ECOLOGY	1
BEHAVIORAL ECOLOGY AND SOCIOBIOLOGY	4
BEHAVIOUR	2
BIODIVERSITY AND CONSERVATION	2
BIOLOGICAL CONSERVATION	2
BIOLOGICAL INVASIONS	3
BIOLOGICAL JOURNAL OF THE LINNEAN SOCIETY	2
BIOLOGY LETTERS	1
BIOLOGY OF REPRODUCTION	4
BIOSCIENCE	4
BIOTA NEOTROPICA	4
BMC EVOLUTIONARY BIOLOGY	1
BMC GENOMICS	1
BRAIN BEHAVIOR AND EVOLUTION	3
BRAZILIAN ARCHIVES OF BIOLOGY AND TECHNOLOGY	2
BRAZILIAN JOURNAL OF BIOLOGY	4
BRAZILIAN JOURNAL OF OCEANOGRAPHY	4
BULLETIN FRANCAIS DE LA PECHE ET DE LA PISCICULTURE/ KNOWLEDGE AND MANAGEMENT OF AQUATIC ECOSYSTEMS	4
BULLETIN OF MARINE SCIENCE	4
CAHIERS DE BIOLOGIE MARINE	4
CALIFORNIA COOPERATIVE OCEANIC FISHERIES INVESTIGATIONS REPORTS	4
CANADIAN FIELD NATURALIST	3

Appendix Table 1-A1 continues →

TABLE 1-A1 (Continues…): Categorisation, based on animal ethical requirements, of 250 ISI-rated, peer-reviewed, scientific journals with the most articles relating to fish research, revealed through a Web of Science (Thomson Reuters) search.

Journal	Category
CANADIAN JOURNAL OF FISHERIES AND AQUATIC SCIENCES	1
CANADIAN JOURNAL OF ZOOLOGY REVUE CANADIENNE DE ZOOLOGIE	1
CANCER GENETICS AND CYTOGENETICS	3
CIENCIAS MARINAS	4
COLONIAL WATERBIRDS	4
COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY A MOLECULAR INTEGRATIVE PHYSIOLOGY	1
COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY C TOXICOLOGY AND PHARMACOLOGY	1
COMPARATIVE PARASITOLOGY	2
CONDOR	3
CONSERVATION BIOLOGY	4
CONSERVATION GENETICS	3
COPEIA	2
CORAL REEFS	4
CRUSTACEANA	2
CYBIUM	4
DEEP SEA RESEARCH PART I OCEANOGRAPHIC RESEARCH PAPERS	4
DEEP SEA RESEARCH PART II TOPICAL STUDIES IN OCEANOGRAPHY	4
DISEASES OF AQUATIC ORGANISMS	3
DIVERSITY AND DISTRIBUTIONS	4
ECOGRAPHY	4
ECOHYDROLOGY	4
ECOLOGICAL APPLICATIONS	3
ECOLOGICAL ENGINEERING	3
ECOLOGICAL INDICATORS	3
ECOLOGICAL MODELLING	2
ECOLOGICAL MONOGRAPHS	3
ECOLOGY	2
ECOLOGY AND EVOLUTION	2
ECOLOGY AND SOCIETY	4
ECOLOGY LETTERS	4
ECOLOGY OF FRESHWATER FISH	4
ECOSPHERE	1
ECOSYSTEMS	4
ECOTOXICOLOGY	2
EMU	4
ENVIRONMENTAL BIOLOGY OF FISHES	2
ENVIRONMENTAL MANAGEMENT	1
ENVIRONMENTAL MICROBIOLOGY	4
ENVIRONMENTAL MONITORING AND ASSESSMENT	4
ENVIRONMENTAL POLLUTION	2
ENVIRONMENTAL SCIENCE TECHNOLOGY	4
ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY	1
ESTUARIES	4
ESTUARIES AND COASTS	4
ESTUARINE COASTAL AND SHELF SCIENCE	4
EVOLUTION	4
EVOLUTIONARY ECOLOGY	3
EVOLUTIONARY ECOLOGY RESEARCH	4
FEMS MICROBIOLOGY ECOLOGY	4
FISH AND FISHERIES	4
FISH PHYSIOLOGY AND BIOCHEMISTRY	2
FISHERIES	4
FISHERIES MANAGEMENT AND ECOLOGY	1
FISHERIES OCEANOGRAPHY	4

Appendix Table 1-A1 continues next page →

TABLE 1-A1 (Continues…): Categorisation, based on animal ethical requirements, of 250 ISI-rated, peer-reviewed, scientific journals with the most articles relating to fish research, revealed through a Web of Science (Thomson Reuters) search.

Journal	Category
FISHERIES RESEARCH	1
FISHERIES SCIENCE	3
FISHERY BULLETIN	4
FOLIA PARASITOLOGICA	4
FOLIA ZOOLOGICA	2
FRESHWATER BIOLOGY	1
FRESHWATER SCIENCE	3
FUNCTIONAL ECOLOGY	1
GENE	2
GENERAL AND COMPARATIVE ENDOCRINOLOGY	2
GENES CHROMOSOMES CANCER	2
GLOBAL CHANGE BIOLOGY	1
GLOBAL ECOLOGY AND BIOGEOGRAPHY	4
HELGOLAND MARINE RESEARCH	2
HEREDITY	1
HUMAN ECOLOGY	4
HYDROBIOLOGIA	3
IBIS	1
ICES JOURNAL OF MARINE SCIENCE	1
ICHTHYOLOGICAL RESEARCH	2
IHERINGIA SERIE ZOOLOGIA	4
INDIAN JOURNAL OF FISHERIES	3
INTEGRATIVE AND COMPARATIVE BIOLOGY	1
INTERCIENCIA	4
INTERNATIONAL JOURNAL FOR PARASITOLOGY	3
INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY	2
INTERNATIONAL REVIEW OF HYDROBIOLOGY	3
IRANIAN JOURNAL OF FISHERIES SCIENCES	4
ITALIAN JOURNAL OF ZOOLOGY	4
JOURNAL OF ANIMAL ECOLOGY	2
JOURNAL OF APPLIED ECOLOGY	1
JOURNAL OF APPLIED ICHTHYOLOGY	4
JOURNAL OF ARCHAEOLOGICAL SCIENCE	3
JOURNAL OF BIOGEOGRAPHY	4
JOURNAL OF CHEMICAL ECOLOGY	2
JOURNAL OF COMPARATIVE PHYSIOLOGY B BIOCHEMICAL SYSTEMIC AND ENVIRONMENTAL PHYSIOLOGY	2
JOURNAL OF CRUSTACEAN BIOLOGY	4
JOURNAL OF EVOLUTIONARY BIOLOGY	4
JOURNAL OF EXPERIMENTAL BIOLOGY	1
JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY	4
JOURNAL OF EXPERIMENTAL ZOOLOGY	4
JOURNAL OF FISH BIOLOGY	1
JOURNAL OF FISH DISEASES	4
JOURNAL OF FRESHWATER ECOLOGY	4
JOURNAL OF GREAT LAKES RESEARCH	2
JOURNAL OF HELMINTHOLOGY	3
JOURNAL OF HEREDITY	1
JOURNAL OF HERPETOLOGY	1
JOURNAL OF MARINE SYSTEMS	2
JOURNAL OF MORPHOLOGY	2
JOURNAL OF NATURAL HISTORY	4
JOURNAL OF PARASITOLOGY	2
JOURNAL OF PLANKTON RESEARCH	4
JOURNAL OF RAPTOR RESEARCH	4
JOURNAL OF SEA RESEARCH	2
JOURNAL OF SHELLFISH RESEARCH	4
JOURNAL OF THE AMERICAN WATER RESOURCES ASSOCIATION	4
JOURNAL OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM	4

Appendix Table 1-A1 continues →

TABLE 1-A1 (Continues…): Categorisation, based on animal ethical requirements, of 250 ISI-rated, peer-reviewed, scientific journals with the most articles relating to fish research, revealed through a Web of Science (Thomson Reuters) search.

Journal	Category
JOURNAL OF THE NORTH AMERICAN BENTHOLOGICAL SOCIETY	3
JOURNAL OF THEORETICAL BIOLOGY	2
JOURNAL OF TROPICAL ECOLOGY	4
JOURNAL OF ZOOLOGY	1
KNOWLEDGE AND MANAGEMENT OF AQUATIC ECOSYSTEMS	4
LANDSCAPE ECOLOGY	2
LATIN AMERICAN JOURNAL OF AQUATIC RESEARCH	4
LIMNOLOGICA	3
LIMNOLOGY AND OCEANOGRAPHY	4
MARINE AND COASTAL FISHERIES	4
MARINE AND FRESHWATER BEHAVIOUR AND PHYSIOLOGY	4
MARINE AND FRESHWATER RESEARCH	2
MARINE BIOLOGY	3
MARINE BIOLOGY RESEARCH	4
MARINE ECOLOGY AN EVOLUTIONARY PERSPECTIVE	4
MARINE ECOLOGY PROGRESS SERIES	3
MARINE ECOLOGY PUBBLICAZIONI DELLA STAZIONE ZOOLOGICA DI NAPOLI I	4
MARINE ENVIRONMENTAL RESEARCH	2
MARINE GENOMICS	1
MARINE MAMMAL SCIENCE	4
MARINE POLICY	2
MARINE POLLUTION BULLETIN	4
MEMORIAS DO INSTITUTO OSWALDO CRUZ	2
METHODS IN ECOLOGY AND EVOLUTION	2
MOLECULAR BIOLOGY AND EVOLUTION	4
MOLECULAR ECOLOGY	1
MOLECULAR ECOLOGY RESOURCES	1
MOLECULAR PHYLOGENETICS AND EVOLUTION	3
NATURE	1
NATURWISSENSCHAFTEN	3
NEOTROPICAL ICHTHYOLOGY	4
NETHERLANDS JOURNAL OF SEA RESEARCH	1
NEW ZEALAND JOURNAL OF MARINE AND FRESHWATER RESEARCH	4
NORTH AMERICAN JOURNAL OF AQUACULTURE	1
NORTH AMERICAN JOURNAL OF FISHERIES MANAGEMENT	1
NORTHWEST SCIENCE	4
OCEAN COASTAL MANAGEMENT	2
OCEANOGRAPHY AND MARINE BIOLOGY	4
OECOLOGIA	3
OIKOS	4
PAKISTAN JOURNAL OF ZOOLOGY	4
PARASITOLOGY	1
PARASITOLOGY RESEARCH	2
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES	2
PLOS ONE	2
POLAR BIOLOGY	3
PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA	2
PROCEEDINGS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES	1
REGULATED RIVERS RESEARCH MANAGEMENT	4
RESTORATION ECOLOGY	3
REVIEWS IN FISH BIOLOGY AND FISHERIES	1
REVIEWS IN FISHERIES SCIENCE	4
REVISTA BRASILEIRA DE PARASITOLOGIA VETERINARIA	1
REVISTA BRASILEIRA DE ZOOLOGIA	4
REVISTA CHILENA DE HISTORIA NATURAL	3
REVISTA DE BIOLOGIA MARINA Y OCEANOGRAFIA	4

Appendix Table 1-A1 continues next page →

TABLE 1-A1 (Continues…): Categorisation, based on animal ethical requirements, of 250 ISI-rated, peer-reviewed, scientific journals with the most articles relating to fish research, revealed through a Web of Science (Thomson Reuters) search.

Journal	Category
REVISTA DE BIOLOGIA TROPICAL	4
RIVER RESEARCH AND APPLICATIONS	4
SARSIA	4
SCIENCE OF THE TOTAL ENVIRONMENT	2
SCIENTIA MARINA	4
SCIENTIFIC REPORTS	2
SOUTH AFRICAN JOURNAL OF ZOOLOGY	4
SOUTHEASTERN NATURALIST	4
SOUTHWESTERN NATURALIST	4
STUDIES ON NEOTROPICAL FAUNA AND ENVIRONMENT	1
SYSTEMATIC BIOLOGY	4
SYSTEMATIC PARASITOLOGY	2
THERIOGENOLOGY	1
TRANSACTIONS OF THE AMERICAN FISHERIES SOCIETY	2
TURKISH JOURNAL OF FISHERIES AND AQUATIC SCIENCES	4
TURKISH JOURNAL OF VETERINARY ANIMAL SCIENCES	2
VETERINARY PARASITOLOGY	1
VIE ET MILIEU LIFE AND ENVIRONMENT	4
WATER SCIENCE AND TECHNOLOGY	4
WATERBIRDS	4
WESTERN NORTH AMERICAN NATURALIST	4
WETLANDS	4
WETLANDS ECOLOGY AND MANAGEMENT	3
WILDLIFE RESEARCH	4
ZEBRAFISH	4
ZOOLOGIA	4
ZOOLOGICAL STUDIES	4
ZOOTAXA	4

Category 1, journal/publisher specifies a particular set (or sets) of animal ethics/care guidelines to which the manuscript is required to adhere; Category 2, journal/publisher requires for publication that the research was approved by an institutional, national or international animal ethics committee; Category 3, the journal/publisher requires that the author makes a statement on the welfare of the study animals; Category 4, journal/publisher has no mention of ethics, or treatment of animals, or ethical requirements.

Appendix 2

TABLE 1-A2: Web links to guiding documents, policies and legislation for the ethical treatment of animals in research, identified by 45 ISI-rated journals, in their instructions to authors.

Guiding document/policy/legislation	Link to resource
National Centre for the Replacement, Refinement and Reduction of Animals in Research: ARRIVE (Animal Research: Reporting of in Vivo Experiments) Guidelines	https://www.nc3rs.org.uk/arrive-guidelines
Animal Behaviour Journal: Guidelines for the treatment of animals in behavioural research and teaching	https://www.elsevier.com/__data/promis_misc/ASAB2006.pdf
World Medical Association: Declaration of Helsinki	http://www.wma.net/en/30publications/10policies/b3/
Council for International Organization of Medical Sciences/International Council for Laboratory Animal Science: International Guiding Principles for Biomedical Research Involving Animals	https://grants.nih.gov/grants/olaw/Guiding_Principles_2012.pdf
Basel Declaration Society: Basel Declaration (2010)	http://www.basel-declaration.org/
European Commission: Directive 2010/63/EU on the protection of animals used for scientific purposes	http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm
United Kingdom Government: Animals (Scientific Procedures) Act 1986 (revised 2013)	https://www.gov.uk/guidance/research-and-testing-using-animals
Canadian Council on Animal Care: Guidelines on: the care and use of fish in research, teaching and testing	http://www.ccac.ca/en_/
Society of Environmental Toxicology and Chemistry: Code of Ethics	https://www.setac.org/?page=SETACEthics
National Cancer Institute, Frederick National Laboratory for Cancer Research: Animal Care and Use Committee Guidelines	https://ncifrederick.cancer.gov/lasp/Acuc/Frederick/GuidelinesFnl.aspx
Journal of Fish Biology: Ethical justification for the use and treatment of fishes in research	https://www.editorialmanager.com/jfb/account/2011%20Ethics%20update%20j.1095-8649.2010.02900.x.pdf
American Fisheries Society: Guidelines for the use of Fishes in Research	http://fisheries.org/docs/wp/Guidelines-for-Use-of-Fishes.pdf
The National Academies: The Brazilian Legal Framework on the Scientific Use of Animals	http://www.fiocruz.br/omsambiental/media/ArtigoILARv5201eFilipecki.pdf
Council of Europe: European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes	https://rm.coe.int/CoERMPublicCommonSearchServices/DisplayDCTMContent?documentId=090000168007a67b
National Research Council: Guide for the Care and Use of Laboratory Animals	https://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-use-of-laboratory-animals.pdf