UWS Academic Portal

Ethical considerations in fish research

Sloman, K.A.; Bouyoucos, I.A.; Brooks, E.J.; Sneddon, L.U.

Published in:
Journal of Fish Biology

DOI:
[10.1111/jfb.13946](https://doi.org/10.1111/jfb.13946)

Published: 11/04/2019

Document Version
Peer reviewed version

[Link to publication on the UWS Academic Portal](#)

Citation for published version (APA):

Sloman, K. A., Bouyoucos, I. A., Brooks, E. J., & Sneddon, L. U. (2019). Ethical considerations in fish research. *Journal of Fish Biology*, 94(4), 556-577. [13946]. <https://doi.org/10.1111/jfb.13946>

General rights

Copyright and moral rights for the publications made accessible in the UWS Academic Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

If you believe that this document breaches copyright please contact pure@uws.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

"This is the peer reviewed version of the following article: Sloman, K. A., Bouyoucos, I. A., Brooks, E. J., & Sneddon, L. U. (2019). Ethical considerations in fish research. *Journal of Fish Biology.*, which has been published in final form at <https://doi.org/10.1111/jfb.13946>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions."

<https://authorservices.wiley.com/author-resources/Journal-Authors/licensing/self-archiving.html>

Ethical Considerations in Fish Research

Sloman, K.A.¹, Bouyoucos, I.A.^{2,3}, Brooks, E.J.⁴ & Sneddon, L.U.⁵

¹School of Health and Life Sciences, University of the West of Scotland, Paisley PA1 2BE

²Australian Research Council Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, Queensland, 4811, Australia

³PSL Research University, EPHE-UPVD-CNRS, USR 3278 CRIOBE, Université de Perpignan, 58 Avenue Paul Alduy, 66860 Perpignan Cedex, France

⁴Cape Eleuthera Island School, Rock Sound, Eleuthera, The Bahamas

⁵Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB

15

16 **Abstract**

17 Fishes are used in a wide range of scientific studies, from conservation research with
18 potential benefits to the species used to biomedical research with potential human benefits.
19 Fish research can take place in both laboratories and field environments and methods used
20 represent a continuum from non-invasive observations, handling, through to experimental
21 manipulation. While some countries have legislation or guidance regarding the use of fish in
22 research, many do not and there exists a diversity of scientific opinions on the sentience of
23 fish and how we determine welfare. Nevertheless, there is a growing pressure on the
24 scientific community to take more responsibility for the animals they work with through
25 maximising the benefits of their research to humans or animals while minimising welfare or
26 survival costs to their study animals. In this review, we focus primarily on the refinement of
27 common methods used in fish research based on emerging knowledge with the aim of
28 improving the welfare of fish used in scientific studies. We consider the use of anaesthetics
29 and analgesics, and how we mark individuals for identification purposes. We highlight the
30 main ethical concerns facing researchers in both laboratory and field environments and
31 identify areas which need urgent future research. We hope that this review will help inform
32 those who wish to refine their ethical practices and stimulate thought among fish researchers
33 for further avenues of refinement. Improved ethics and welfare of fishes will inevitably lead
34 to increased scientific rigour and is in the best interests of both fishes and scientists.

35

36

37

38 **Key Words:** welfare, nociception, 3Rs, anaesthesia, analgesia, refinement

40 **Fish Welfare**

41

42 The application of animal welfare concepts to fishes has lagged behind that of mammals.
43 However, fish welfare has received a considerable amount of attention in recent years, both in
44 relation to research and to commercial practices such as aquaculture and the ornamental fish
45 trade. The definition of ‘welfare’ will almost certainly depend on an individual researcher’s
46 contemplation but can be approached from three perspectives (Huntingford *et al.*, 2006),
47 which are feelings-based, function-based or nature-based. Good welfare from a feelings-
48 based perspective means that the animal should feel well, and should be free from pain or
49 fear, and have access to positive experiences. From a function-based perspective, an animal
50 should be in good health and be in an environment that does not require it to function beyond
51 its capacity. Nature-based good welfare requires that an animal can lead a natural life and
52 express natural behaviour. Previous reviews on fish welfare have adopted feelings-based
53 (Huntingford *et al.*, 2006) and function-based (Arlinghaus *et al.*, 2007) approaches. Despite
54 conflict between these attitudes, under many circumstances they may come to the same
55 conclusions regarding the best approaches to fish welfare. One of the key controversies in the
56 field of fish welfare is whether or not fishes experience pain (Sneddon, 2015). While
57 acceptance or rejection of the idea that fishes are sentient animals may influence a
58 researcher’s use of some welfare refinements (e.g. analgesia), opportunities to improve fish
59 welfare can often be justified regardless of a researcher’s stance on fish pain (Browman *et al.*,
60 2018). Therefore, the aim of this review is to bring together existing information on fish
61 welfare to allow the reader to make informed decisions around the methods used in fish
62 research. In many countries there is legislation or guidance surrounding the use of fishes in
63 scientific research, but for many countries no guidelines exist. As scientists we have a moral
64 responsibility to consider the impacts of our research methods from a variety of perspectives
65 including the impact on the individual animal and implications for its natural environment,
66 while also ensuring that the best scientific methods for validity of results are used.

67

68 In considering fish welfare in captivity, the five freedoms used by the UK Farm Animal
69 Welfare Council (2005) are often quoted and were specifically applied to fishes by

Huntingford *et al.* (2006). These state that animals should be free from (1) water and food deprivation, malnutrition; (2) environmental challenge; (3) disease, injury and functional impairment; (4) behavioural/interactive restriction and (5) mental and physical suffering. Some of these freedoms are intuitive and easy to interpret, such as the avoidance of disease and injury. However, others such as freedom from environmental challenge, or from mental suffering are more difficult to interpret. If we are to refine scientific methods of working with fishes to improve welfare then it is necessary to know how to assess fish welfare.

The natural stress response of fishes is often used in our assessment of welfare (Conte, 2004; Sneddon *et al.*, 2016) not least because our understanding of the physiological response of fishes to a variety of stressors is extensive, with many books and reviews written on the subject (e.g. Iwama *et al.*, 1997; Schreck, 2000; Barton, 2002; Schreck *et al.*, 2016).

However, we cannot assume that there is always a direct relationship between stress and welfare. For example, there are instances when physiological stress may be beneficial (Mommsen *et al.*, 1999; Love *et al.*, 2013; eustress, Schreck & Tort, 2016) and examples of adverse conditions that do not cause measureable activation of the stress axis (Huntingford *et al.*, 2006; Schreck & Tort, 2016).

Behaviour, or behavioural deficits are often used in the assessment of animal welfare (Mench & Mason, 1997) including choice tests (Dawkins, 1998, 2004), although these are based on the assumption that the animal will choose what is best for its own welfare which may not necessarily be the case. In fishes, a variety of welfare indicators have been suggested including changes in colour, ventilation rate, swimming behaviour, reduced food intake, loss of condition, slow growth, morphological abnormalities, injury, disease outbreaks and reduced reproductive output (Huntingford *et al.*, 2006; Sneddon *et al.*, 2016; Wilson *et al.*, 2018). In reality, combined measures are likely to be the best way of assessing welfare (Huntingford *et al.*, 2006) to account for intra-and inter-individual variation in specific responses (Mason & Mendl, 1993).

Legislation and Guidelines

Concern for fish welfare has led to legislation and guidance in some countries and the adoption of ethical guidelines by some scientific journals. While some countries have their

own extensive guidance documents for the use of animals in research (e.g. Canada: <http://www.ccac.ca/>; USA: <https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf>; Australia: <https://nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes>) or legislation (UK: <http://www.homeoffice.gov.uk/science-research/animal-research/>; EU: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32010L0063>), it should be recognised that a large number of countries do not have such guidance. As well as legislation surrounding experimentation on animals, working with fishes, particularly in the field, may require permits to work and sample fishes. It should also be recognised that just because something is permitted, or not mentioned, within such guidelines and legislation does not automatically make it ethical. Therefore, there is an onus on the individual researcher and research group to assess the ethics of their research based on our current understanding of fish biology, and to update this as knowledge changes. There are many guidance documents written for those working with animals used for scientific purposes, some of which are specifically tailored for fish. Examples include *Guidelines for the treatment of animals in behavioural research and teaching*, published by the journal *Animal Behaviour* (ASAB, 2012, 2018), *Ethical justification for the use and treatment of fishes in research* (Journal of Fish Biology, 2006; Metcalfe & Craig, 2011) published by the *Journal of Fish Biology* and *Guidelines for the use of fishes in research* (AFS, 2014) published by the American Fisheries Society. In 2010 the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines were published as part of the National Centre for the Replacement, Refinement and Reduction of Animal Research (NC3Rs) in *PLoS Biology*, although these focus more on experimental design and reporting of experiments involving laboratory animals rather than specific welfare issues (Kilkenny *et al.*, 2010). Recently (2017), the complimentary PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines were published to aid researchers in planning experiments with live animals, providing a checklist for designing studies and also potentially for those in the process of evaluating proposals for animal studies (Smith *et al.*, 2018).

A survey by Bennett *et al.* (2016), which considered the top 250 peer-reviewed ISI-rated journals for fish research (defined by numbers of fish papers), discovered that while 54% made some mention of animal ethics in their author guidelines, only 18% required adherence to a specific guiding document for successful submission. 46% of journals surveyed made no

mention of animal ethics requirements in their instructions to authors or publication policies. It is obviously not possible to tell from this survey, whether those journals not mentioning ethics in their policies still scrutinise papers for ethical concerns, however, their survey does highlight an area where perhaps more could be done to raise awareness of fish welfare, by guiding researchers to relevant information and/or training, and questioning unethical treatment of fishes.

The application of the 3Rs in fish research

Much of the legislation around animal experimentation is based on the ‘3Rs’. The 3Rs were originally put forward by Russell & Burch (1959) to prevent and reduce pain and suffering of animals in research. They are *Replacement*, *Reduction* and *Refinement*. In conforming to the 3Rs, we should, therefore, first ask ourselves the question ‘Do we need to use live animals for our research?’ or is there an alternative that would allow us to *replace* animal experimentation. If the answer is yes, that there is no suitable alternative for our scientific objectives, then the second question we must ask is ‘How do we minimise the number of animals used without compromising gain in scientific knowledge?’ i.e. how can we *reduce* the number of animals used in experimentation. Finally, we need to ask ‘How can we minimise the amount of pain or lasting harm experienced by an animal during experimentation?’ as a way to *refine* our experimental approach. Curzer *et al.* (2013) advocate the addition of a fourth ‘R’, *Refusal*, where research plans that are unlikely to provide any scientific gain are rejected before initiation.

Traditionally, the 3Rs have been applied to laboratory animal models where focus is on the individual animals used in an experiment. As fish researchers, much of our work is not limited to laboratory experiments and thus our ethical approach must transcend the laboratory boundaries. Curzer *et al.* (2013) promote the expansion of the 3Rs/4Rs approach to whole ecosystems, where there is a need to consider not only the particular species of interest, but also the impact of our research on the ecosystem as a whole. They extend the principle of the ‘R’s beyond individual animals to the populations and ecosystems to which they belong. Although, their intention is primarily to translate the 3Rs into principles for researchers

whose focus is ecosystem rather than individual based, we believe their paper provides interesting questions for field studies on fishes. Additional questions then become ‘Can our research ecosystem be replaced with a different ecosystem that will be harmed less without decreasing the amount of knowledge gained?’ (Replacement), ‘Are there ways we can reduce harm to the ecosystem by sampling different parts which will harm the ecosystem less but achieve the same scientific outcomes?’ for example spatial, temporal or ontogenetic variants (Reduction) and ‘Can we change the research procedure in order to decrease the amount of harm to the ecosystem as a whole?’ e.g. sampling methods. (Refinement). Of course the fourth R proposed by Curzer *et al.* (2013) remains the same: if the harm to the ecosystem outweighs any scientific contribution, or no scientific contribution will be gained, the experimental plan should simply be rejected.

Animal alternatives (Replacement)

For some scientific questions, it may be possible to use animal alternatives to inform fish biology. As ‘replacement’ has become an increased focus for mammalian studies, there has been an increase in fundamental research in fishes as replacements for mammalian model organisms. However, under the laws of many countries, legislation applies equally to fishes as it does mammals and only young pre-feeding fishes are considered replacement. Nevertheless, full replacement of fishes in experimental research can include living *in vitro* cultures or computer models which still achieve the scientific objectives and answer the scientific question but without the use of live fishes (reviewed by Schaeck *et al.*, 2013). A large number of cell lines are available for a range of species (Lakra *et al.*, 2011), with the first continuous cell line (RTG-2) dating back to the 1960s (Wolf & Quimby, 1962). Fish cell lines have been used widely as a replacement for live fishes, to investigate effects of toxicants such as metals and endocrine disruptors (Bols *et al.*, 2005; Navas & Segner, 2006; Bopp *et al.*, 2008). Cell culture can also be used to address our fundamental understanding of fish physiology (Wood *et al.*, 2002).

The term ‘replacement’ may not always refer to the full replacement of animals, but is used when live animals are not experimented on. Thus, organ preparations, where the fish is killed

and its organs maintained *in situ* or in culture, can be considered as ‘replacement’ for the use of live fishes in experimentation. Organ preparations provide a link between cell culture techniques and whole organism effects, providing an additional element of tissue complexity. Examples of organ cultures include intestinal perfusion to investigate the effects of toxicants or to further our understanding of fish osmoregulation (Sundell & Björnsson, 1988; Hoyle & Handy, 2005). Methods for *in vitro* perfusion of the brain have also been used to study the fundamentals of fish endocrinology. Once a sufficient biological understanding is achieved, it may also be possible to replace specific *in vitro* techniques with computer modelling to remove the need for animal tissue. It seems unlikely that computer modelling will ever fully replace animal experimentation but computer modelling may also allow better targeting of experimental work. Computer models that have been successfully used to determine the toxicity of compounds include Quantity Structure-Activity Relationship (QSAR) models (Moore *et al.*, 2003), the Biotic Ligand Model (BLM) and related models (Paquin *et al.*, 2002; Brix *et al.*, 2017) and Physiologically-based Pharmacokinetic (PBPK) models (Abbas & Hayton, 1997; Thomann *et al.*, 1997).

Partial replacement is where vertebrate animals are replaced with embryonic life-stages or invertebrate animals (<https://www.nc3rs.org.uk/the-3rs>), under the assumption that they are less sentient. This in itself raises moral and ethical questions as to how we determine which animals should be afforded ethical treatment (Bateson, 1991; Brown, 2015) as ‘less sentient’ could easily be replaced with ‘less researched’ or ‘less understood’. Animal legislation may also vary from country to country in terms of what is an ‘animal’ when used in scientific experiments. UK law considers ‘fishes’ in terms of use in scientific procedures to be those which are capable of independent feeding. Similarly, Canadian guidelines do not require ethical clearance for yolk-sac dependent embryos (see Box 1 Sneddon *et al.*, 2017 for details on other countries). The time to independent feeding from fertilisation varies considerably depending on the species. In zebrafish *Danio rerio* (Hamilton 1822), feeding starts around 4-5 days post-fertilisation but for other species such as salmonids this period may take several months. The use of fish embryos instead of later stages is therefore considered partial replacement and has been used extensively in toxicity studies with a move to use the fish embryo test (FET) as a replacement for the acute fish toxicity (AFT) test (Belanger *et al.*, 2013; Schaeck *et al.*, 2013; Sneddon *et al.*, 2017). This arbitrary cut-off point in guidance and legislation should be considered from an ethical standpoint with caution. To some it seems

counter-intuitive to think of the use of embryos instead of adults as animal ‘replacement’ and recent evidence suggests that larval zebrafish respond in much the same way to noxious stimuli as adults (Lopez-Luna *et al.*, 2017a). Whether or not we believe that embryos should be afforded the same ethical considerations as adults, the ways in which we treat embryos, including euthanasia and husbandry, merit consideration.

Sample Size (Reduction)

It is imperative when experiments are designed to use live fishes, that we minimise the number of animals used, but maintain the scientific rigour with which we test hypotheses. On the one hand, sampling more individuals than is necessary to robustly statistically test a hypothesis may subject more animals than necessary to potential pain and suffering and would be deemed unethical. On the other hand, not sampling enough individuals, could lead to a data set that cannot adequately address a hypothesis, rendering the data set unusable. From an ethical perspective, this results in a number of animals being used with no scientific gain, which perhaps fits more with the *Reject* definition of ‘*R*’ described above.

Various resources can be used to estimate required sample sizes prior to the start of an experiment. Power analysis is a common method for determining the smallest sample size that can be used to detect an effect at a specific level of significance (Festing & Altman, 2002) and for simple experimental designs can make use of freely available software. Power calculations for more complex experimental designs are likely to require input from a statistician. Power analyses generally rely on availability of previous data or pilot data; thus the provision of raw data by researchers can increase the usefulness of experiments as it can be used for future power analyses, meta analyses or further statistical analysis (Festing & Altman, 2002). However, power analyses are closely related to the P value, and it has been argued that interpretation of data based on P values can lead to incorrect conclusions. Therefore, consideration of the effect size is also important (Sneddon *et al.*, 2017). Additionally, it is not uncommon to see comments within manuscripts where samples have been lost due to equipment malfunction; thus a perfectly designed experiment based entirely on power analysis, may fall short if a few samples are lost through technical or human error.

Experiments with early life stages may also be subject to high natural mortality (Wilson *et al.*, 2018). Thus while every effort should always be used to minimise the number of fishes used in live experiments, there may also be circumstances where it is necessary to use more animals than predicted by power analyses. Where research is feeding into management of fisheries, it may be necessary to have large sample sizes to meet legal regulatory requirements and/or to be of use to management strategies (Cooke *et al.*, 2016a). Non-significant results may often be disregarded through academic pressures, yet the publication of these data may be of benefit to the design of future animal experiments by other researchers (Fanelli, 2010; Dwan *et al.*, 2013).

Improving experiments on live fishes from a welfare perspective (*Refinement*)

There are many instances when it is not possible to replace live fishes in experiments, therefore, the remainder of this review focuses on refinement. Compromised welfare will be reflected in behavioural and physiological alterations, thus a fish whose welfare is compromised is likely to yield unreliable data. Therefore, it is imperative for both fishes and researchers that we consider how to refine fish welfare within our experiments.

As fishes are the most speciose of all the vertebrates, the range of fish species that could potentially be used for experimental research is vast. That said, the majority of lab-based fish work focuses around a handful of species. In terms of scientific rigour, the fish species chosen must be appropriate to the study objectives. Therefore, choice of species requires knowledge of their life-history, behaviour, physiology and genetics along with husbandry requirements for laboratory studies (ASAB, 2012, 2018). Within certain model species, there are also genetic strains which may differ phenotypically (e.g. Séguret *et al.*, 2016; van den Bos *et al.*, 2017) and the most appropriate strain for the experimental question must be considered.

Some studies, particularly field studies, may involve work with endangered or protected fish species. Lethal studies involving endangered species are controversial (Heupel &

Simpfendorfer, 2010; Minter *et al.*, 2014) and are likely only to be justified if the conservation benefit to the species as a whole significantly out-weighs the cost to the individuals and the population affected by sampling. Ethical decisions regarding lethal sampling or removal from the wild are often influenced by public, media and political pressures making decisions based on scientific evidence important (Heupel & Simpfendorfer, 2010). Lethal sampling or collection for laboratory studies are not the only factors which require ethical consideration; simple observation also has the potential to disrupt habitats and behaviours of threatened species, so attention must be paid to both the individual and environmental cost.

When removing fishes from the wild or purchasing fishes for laboratory studies, regulations exist for species threatened with extinction (Appendix I cites) and those that are not necessarily now threatened with extinction, but may become so unless trade is regulated (Appendix II cites; <https://www.cites.org/>). Movement of fishes also has the potential to introduce 'invasive' species to naïve ecosystems, indeed many exotic species have been historically introduced on a global scale. Unintentional release of both non-native and native fishes can lead to the spread of disease in wild populations and in fishes, unlike in most vertebrates, domestic sources of infection in wild populations, rather than wild sources have been identified as the most important driver of emerging infectious diseases (Tompkins *et al.*, 2015). While a large proportion of unintentional releases can be attributed to aquaculture and the ornamental fish trade, researchers must take care that they are not contributing to this problem. It should also be remembered that 'invasive' fishes are no 'less' of a fish thus even when it is necessary to carry out targeted removal from a non-native habitat this should be done as humanely as possible.

Do Fishes Experience Pain?

In choosing refinements to apply, as fish researchers we are likely to at least have an opinion on whether fishes experience pain. The ability to react to potentially damaging stimuli is seen in all animals and is mediated through nociception, which is innervated by sensory receptors that convey information on injury to the central nervous system (Sneddon, 2018). In humans

it is accepted that nociception leads to the sensation or feeling of pain, which is often defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (IASP, 1979). Noxious stimuli are extremes of temperature, high mechanical pressure and chemicals (e.g. acids, venoms) that excite nociceptors, receptors that preferentially detect injurious stimuli. In pain assessment, humans self-report their feelings of the intensity, type and duration of pain but animals do not share our language. Therefore, assessing pain in non-human animals is based upon detecting changes in behaviour and physiology when applying a damaging stimulus that would cause pain to humans (Sneddon *et al.*, 2014).

Nociceptors have been identified in non-human mammals and other vertebrate groups such as amphibian, reptilian and avian species but can also be found in invertebrates (reviews in Sneddon *et al.*, 2014; Sneddon, 2015, 2018). Empirical studies in fishes have also confirmed the presence of nociceptors, demonstrating their properties are very similar to those found in humans and other mammals (Sneddon, 2002, 2003a, 2018; Ashley *et al.*, 2006, 2007; Mettam *et al.*, 2012). Studies in fishes have demonstrated changes in brain activity during noxious stimuli (Dunlop & Laming, 2005; Nordgreen *et al.*, 2007; Reilly *et al.*, 2008a; Sneddon, 2011) and recorded adverse changes in behaviour and physiology during noxious treatments (Sneddon *et al.*, 2003a,b; Sneddon, 2003b; Dunlop *et al.*, 2006; Reilly *et al.*, 2008b; Roques *et al.*, 2010; Mettam *et al.*, 2011; Maximino, 2011; White *et al.*, 2017) all of which can be prevented by analgesics known to be effective in relieving pain in mammals (Sneddon, 2003b, 2012; Newby *et al.*, 2009; Nordgreen *et al.*, 2009; Mettam *et al.*, 2011; Schroeder & Sneddon, 2017; Lopez-Luna *et al.*, 2017a,b,c,d; Taylor *et al.*, 2017). This empirical evidence taken together supports the idea that fishes may experience pain.

Whole Animal Responses to Noxious Stimuli

The rainbow trout *Oncorhynchus mykiss* (Walbaum 1792), was the first teleost model shown to possess nociceptors in 2002 (Sneddon, 2002, 2003a): these nociceptors are A-delta and C fibres that act as nociceptors in humans and other mammals. Nociceptors have also been identified in agnathans (Matthews & Wicklegren, 1978) but studies have not yet found

nociceptors in elasmobranchs where a lack of C fibres has been reported (e.g. Snow *et al.*, 1996). The rainbow trout has three types of nociceptors including polymodal (mechanical, thermal and chemical stimuli), mechanothermal (no response to chemicals) and mechanochemical (no response to temperature) (Sneddon, 2003a; Ashley *et al.*, 2006; 2007; Mettam *et al.*, 2012). A relatively lower percentage of trout nociceptors are C fibres (4-5%, Sneddon, 2002; Roques *et al.*, 2010) compared with terrestrial vertebrates (50%; Young, 1977) although reptiles also have a low percentage of C fibres (Terashima & Liang, 1994). Some researchers suggest that the small number of C fibres means teleosts cannot experience pain (Rose *et al.*, 2014), however, it could be argued that A-delta fibres conduct more rapidly, so the fish system may react faster. The trout A-delta fibres perform the same function as mammalian C fibres reacting to different types of noxious stimuli and many are polymodal nociceptors (Sneddon, 2002, 2003a; Ashley *et al.*, 2006, 2007; Roques *et al.*, 2010; Mettam *et al.*, 2012).

Fish sensory neuroanatomical pathways are fairly conserved from an evolutionary perspective when compared with mammals (Sneddon, 2004). Within the teleost brain there are various connections to important processing areas including the thalamus and cortical areas (Rink & Wulliman, 2004) that in mammals are used for pain responses. Higher brain areas rather than just the spinal cord and hindbrain reflex centres respond during noxious events in teleost fishes (Dunlop & Laming, 2005; Nordgreen *et al.*, 2007; Reilly *et al.*, 2008a; Sneddon, 2011) thus the response to noxious stimuli is not restricted to the reflex centres as once suggested (Rose, 2002). Fishes learn to avoid potentially painful stimuli usually in one or a few trials (e.g. Yoshida & Hirano, 2010) and avoidance behaviour can continue for several days (Dunlop *et al.*, 2006; Millsopp & Laming, 2008). Protracted, complicated responses are exhibited by a variety of fish species during a noxious event (reviewed in Sneddon, 2009, 2015). Normal behaviours such as feeding and swimming can be suspended during noxious events (Sneddon, 2003b; Reilly *et al.*, 2008b; Correia *et al.*, 2011a; Roques *et al.*, 2012). Guarding behaviour is also seen in response to noxious stimuli, where a fish will avoid using an area where a noxious stimulus has been applied. For example, rainbow trout suspend eating after an acid injection to the lips for up to 3 h, unlike control individuals and those treated with analgesic (Sneddon, 2003b). Other physiological responses to noxious stimuli include elevated opercular beat rate and elevated stress hormone (cortisol) concentrations (Sneddon, 2003b; Ashley *et al.*, 2009; Roques *et al.*, 2012).

385

386 *Motivational Change After Noxious Stimuli*

387

388 If an experience alters future behaviour and decision making as well as motivational state
389 then it must be important to the animal. Thus if a potentially painful treatment results in long-
390 term behavioural alterations then this provides insight into the significance and importance of
391 pain. In mammals, self-administration paradigms involve gauging the propensity to self-
392 medicate with analgesics. Here, food or water is medicated and animals can self-select this
393 analgesia-laden water or food to reduce their pain, suggesting an internal mental experience
394 or affective component to pain (e.g. Pham *et al.*, 2010). Unfortunately this approach does not
395 work with fishes as they suspend feeding for the duration of noxious stimuli (Sneddon, 2009).
396 An alternative is to determine whether the fish will endure a cost to accessing analgesia. If
397 pain is a negative internal state then fishes should pay a cost in either added physical effort or
398 relinquish access to a resource or preferred area to obtain access to analgesia. For example,
399 zebrafish, subjected to a noxious stimuli lost their preference for favourable enrichment and
400 instead chose unfavourable barren areas with dissolved analgesic (Sneddon, 2011) suggesting
401 that zebrafish will seek substances known to induce pain-relief in mammals, and are willing
402 to endure a cost to access analgesia in an unfavourable environment.

403

404 *The Debate Surrounding Pain in Fish*

405

406 Those that do not believe fishes experience pain have argued that animals must have a human
407 cortical structure to consciously experience pain. Because fishes lack a multi-layered, human-
408 like cortex, it is argued that they are not aware of pain and do not feel discomfort or suffer
409 (Rose, 2002). However, as described above there is growing empirical evidence that supports
410 the concept of pain in fishes, and that this is a significant and negative state (Broom, 2007,
411 2014; Brown, 2015; Chandroo *et al.*, 2004; Sneddon, 2009, 2011, 2015; Sneddon *et al.*,
412 2014). The hypothesis that a function such as pain suddenly arises in humans without any
413 precursor challenges evolutionary laws (Sneddon, 2009). Recent commentaries argue for
414 (Merker, 2016) and against (Key, 2016) a fish's ability to perceive pain and have stimulated
415 much debate (Animal Sentience, 2016). These debates can become semantic arguments and

systematic review of the evidence by independent parties has been suggested as a way forward (Cooke, 2016). Regardless, we cannot communicate directly with fishes thus self-reporting of pain is impossible. If we believe that empirical evidence suggests that fish responses fulfil the definition of animal pain, then from an ethical and moral perspective we should err on the side of caution to protect their health and welfare.

Common Considerations for Laboratory and Field Studies

Anaesthesia

The use of anaesthetics in fishes has been researched extensively, particularly with regard to the physiological effects they cause (reviewed by Ross & Ross, 2008; Matthews & Varga, 2012). However, the first decision a researcher has to address is when is it appropriate to use anaesthetic? Anaesthesia is generally recommended for any invasive procedure where the fish needs to be immobilised, but there are variations on what this means. Some researchers recommend anaesthesia for routine husbandry procedures such as weighing fishes, as struggling by the fish has the potential to cause injury both to the fish and the researcher. Inaccuracy of measurements due to movement of the fish may also occur. However, many researchers would argue that anaesthesia itself is stressful, with subsequent physiological effects, and so where handling is minimal and procedures are non-invasive or minimally invasive, the then use of anaesthesia may be counterintuitive (Cooke *et al.*, 2016a). For field studies there is the added complication that fishes will be released with some anaesthetic agents requiring a withdrawal period before fishes can be released into the wild (Wargo Rub *et al.*, 2014; Bennett *et al.*, 2016), for example if the fish may be caught for subsequent human consumption (Ross & Ross, 2008). Anaesthesia may also alter physiology and behaviour such that the likelihood of post-release predation increases (Cooke *et al.* 2016a). Therefore, deciding whether anaesthetics should be used must be assessed on a case-by-case basis, with regard to the welfare of the fish and the circumstances under which it will be used.

For some larger fish species where it is impractical to use anaesthesia e.g. sharks, it may be possible to use alternatives to anaesthesia such as tonic immobility (Kessel *et al.*, 2015). Holland *et al.* (1999) used tonic immobility for external and internal (surgical implantation) attachment of acoustic transmitters and found that within a few seconds to a few minutes, tiger sharks *Galeocerdo cuvier* (Péron & Lesueur 1822) showed tonic immobility after inversion. However, while tonic immobility [and other similar techniques such as atonic immobility (Wells *et al.*, 2005)] may reduce fish movement and therefore risk of injury to the fish and researcher, more research is needed to understand the stress associated with tonic immobility itself. In lemon sharks *Negaprion brevirostris* (Poey 1868), tonic immobility was found to be a stressful experience which magnified changes in blood chemistry parameters (Brooks *et al.*, 2011a).

If anaesthesia is to be used, the decision of which anaesthetic to use is often influenced by availability, cost effectiveness, ease of use, nature of study and safety for user (Cho & Heath, 2000). For fishes, the usual route of anaesthesia is *via* immersion, although injection is also used. Periods of immersion necessary to induce anaesthesia will depend on the ventilatory mechanism of the fish, for example air breathing fishes may require a much longer immersion period than fishes which rely entirely on dissolved oxygen from the water (Ross & Ross 2008; Neiffer & Stamper, 2009). There are a variety of different anaesthetics available which can be added to the water and work through different physiological mechanisms. Commonly used compounds include MS222 (tricaine methanesulfonate; ethyl 3-aminobenzoate methanesulfonate), benzocaine (ethyl-4-aminobenzoate), etomidate, eugenol (4-allyl-2-methoxyphenol; the active ingredient of clove oil), AQUI-S™ (active ingredient isoeugenol), metomidate and 2-phenoxyethanol (Neiffer & Stamper, 2009). Carbon dioxide has been used as an anaesthetic for fishes, and results in cerebral hypoxia. However, its use is controversial for several reasons, including observed struggling of fishes as anaesthesia is induced and then removed (Wargo Rub *et al.*, 2014) and the difficulty in achieving controlled anaesthesia compared to other drugs (Ross & Ross, 2008). The use of non-chemical methods of anaesthesia such as cooling and electro-immobilisation are also controversial as anaesthetics. While cooling may immobilise the fish, it does not completely block nerve conduction (Matthews & Varga, 2012) and it is unlikely that true anaesthesia and analgesia are achieved (Ross & Ross, 2008). Hence, cooling is generally not an accepted method of anaesthesia in Europe (Lidster *et al.*, 2017). Electro-immobilisation is not recommended due

to its narrow safety margin between anaesthesia and injury (Wargo Rub *et al.*, 2014) and the level of anaesthesia and analgesia achieved remains uncertain (Ross & Ross, 2008). The choice of which chemical anaesthetic to be used will be influenced by a range of factors, not least which can be legally purchased by the researcher. From an ethical perspective, it is important that the researcher understands how the chosen anaesthetic works and any potential lasting physiological changes that may be induced. For example, addition of MS222 to fresh water usually results in a lowered pH, and therefore requires buffering, or it can cause plasma acidosis in the fish. Anaesthetic agents can also alter the physiology of fishes in different ways, thus potentially confounding comparisons between physiological studies that have used different chemical routes of anaesthesia (Small, 2003; Holloway *et al.*, 2004).

Often absent from the list of things to consider when choosing an anaesthetic chemical is the welfare of the fish itself in response to the anaesthetic agent. Recent work has highlighted that the way fishes respond behaviourally to anaesthetic exposure is species-specific. Readman *et al.* (2017) argue that in addition to assessing the ease of delivery, and rate and stability of anaesthesia, researchers should also consider the way a fish responds to anaesthesia when initially exposed to the chemical. Zebrafish demonstrate a negative behavioural response when exposed to benzocaine and MS222 (Readman *et al.*, 2013) and appear to remember exposure to some anaesthetics (Wong *et al.*, 2014). Medaka *Oryzias latipes* (Temminck & Schlegel 1846) also show a negative behavioural response to these chemicals, whereas carp *Cyprinus carpio* L. 1758 and rainbow trout do not (Readman *et al.*, 2017). Clearly further research is warranted on the welfare of anaesthesia practice in fishes, along with discussions at regulatory levels to ensure that chemicals with the greatest welfare potential are accessible. Reporting of anaesthesia details, including concentrations, buffering, duration and mention of behavioural responses would allow for better comparison between studies and may contribute to enhancing fish welfare in this area.

Analgesia

If we accept that a potentially painful event substantially alters behaviour and physiology in fishes we should attempt to minimise or alleviate this. Drugs with analgesic properties could

be employed during any invasive procedures such as invasive tagging, surgery, cannulation, exposure to noxious chemicals or after damage to the fish occurs *via* factors such as net abrasion or injuries from aggressive encounters or diseases. The major classes of analgesic drugs are opioids, nonsteroidal anti-inflammatory drugs (NSAIDs), local anaesthetics, and miscellaneous drugs that have pain-relieving properties (e.g. anti-depressants). Opioids, NSAIDs and local anaesthetics have been investigated with regards to analgesia in fishes (Table 1; Sneddon, 2012).

Opioids act on the three opioid receptors (μ , δ and κ) located on neuronal cell membranes to produce analgesia. A classic example of such a drug is morphine. Opioids inhibit neurotransmitter release presynaptically thereby blocking nociceptors but these drugs also block central transmission. Several studies have shown morphine is an effective analgesic in rainbow trout (Sneddon, 2003b; Baker *et al.*, 2013; Jones *et al.*, 2012) and other species (e.g. goldfish *Carassius auratus* (L. 1758), Nordgreen *et al.*, 2009; zebrafish, Lopez-Luna *et al.*, 2017a,b,c,d; Magalhaes *et al.*, 2017; Schroeder & Sneddon, 2017; Taylor *et al.*, 2017). Morphine has a pharmacokinetically similar action in fishes compared with mammals, however, morphine persists for a prolonged period after administration due to slower excretion rates (half-life 37 h; Newby *et al.*, 2006, 2009). Thus it may be preferable to use morphine in long term situations and peri- or post-operatively since one dose may be all that is required. Other opioids have been studied. For example, tramadol, dermorphine and β -casomorphin reduced the magnitude of response to electric shock in cod *Gadus morhua* L. 1758, carp and rainbow trout (Chervova & Lapshin, 2000; Chervova *et al.*, 1994). Butorphanol has been explored in the chain dogfish *Scyliorhinus rotifer* (Garman 1881) and carp as part of a combined anaesthetic protocol but appeared to have limited effectiveness (Davis *et al.*, 2006; Harms *et al.*, 2005). Buprenorphine also has poor analgesic properties in rainbow trout (Mettam *et al.*, 2011; Baker *et al.*, 2013), with studies showing a depressive effect on the cardioventilatory system (Gräns *et al.*, 2014). However, Curtwright *et al.* (2015) used buprenorphine to effectively reduce responses to noxious heat in larval zebrafish when they were immersed in 0.3 mg ml⁻¹.

NSAIDs inhibit arachidonate cyclo-oxygenase (COX) enzymes to reduce the production of thromboxanes and prostaglandins providing anti-inflammatory, anti-pyretic and analgesic

properties (Rang *et al.*, 2003). Ketoprofen was found to be ineffective in the chain dogfish to determine the minimum anaesthetic concentration of MS-222 required to prevent a response to an acute noxious stimulus (Davis *et al.*, 2006). Ketoprofen did reduce some indicators of muscle damage after surgery in carp but did not prevent behavioural changes (Harms *et al.*, 2005). Carprofen had some efficacy in rainbow trout during noxious stimulation, with trout resuming normal feeding more quickly than trout with no carprofen. However, this drug depressed activity even in control trout which may be an undesirable side effect (Mettam *et al.*, 2011). Rizzo *et al.* (2017) tested the impact of flunixin, ketorolac and ketoprofen in rainbow trout, none of which appeared to be effective at ameliorating an increase in opercular beat rate 2 h after surgery at the doses employed in the study. Indomethacin injected intraperitoneally (20 μ l of 0.2 mg ml⁻¹) did reduce pain related responses in adult zebrafish treated with formalin (Magalhaes *et al.*, 2017). Aspirin administered via immersion prevented the reduced activity seen in adult and larval zebrafish when subjected to noxious events (Lopez-Luna *et al.*, 2017a,b,c,d; Schroeder & Sneddon, 2017). Future studies need to investigate NSAIDs further to determine the minimum effective dose and explore any unwanted side-effects since long term use of these drugs in mammals, birds and reptiles can result in gastric ulcers or renal disease.

Local anaesthetics act by inhibiting the propagation of action potentials *via* blockade of sodium channels and by affecting membrane function (Rang *et al.*, 2003). Therefore, they prevent pain sensation by blocking transmission of nociceptive signals. Relatively few studies have explored local anaesthetics, however, novocaine is known to reduce reflex responses to electric shock in cod (Chervova, 1997) but more information is required before it can be recommended. Lidocaine effectively prevents behavioural and physiological alterations in rainbow trout during subcutaneous injection of acetic acid (Mettam *et al.*, 2011). Exposure to lidocaine by emersion also ameliorates substantial changes in the behaviour of adult zebrafish subject to caudal fin clipping (Schroeder & Sneddon, 2017) and was also effective when used with larval zebrafish exposed to noxious chemical and thermal stimuli (Lopez-Luna *et al.*, 2017a,b,c,d). More research is necessary to test the wide range of local anaesthetics on a range of fish species to construct reliable analgesic protocols. As the use of analgesics increases in fish research, it is important that more fundamental studies investigate both the potential benefits of analgesic use for fish welfare, but also any adverse effects.

Tagging

Visual identification

Phenotypic methods represent the least invasive methods of identifying individuals and have been used in many studies. These can be simple uses of visual assessment such as scale patterns in carp (Huntingford *et al.*, 2013) or the use of software to generate identification of white-spotted eagle rays, *Aetobatus narinari* (Euphrasen 1790) (Flowers *et al.*, 2017). However, for a large number of species individual identification is not possible by eye, and methods of marking individuals are needed. Colour marks are commonly used, either as small pieces of coloured material attached to the fish or temporary colour marks applied onto or just beneath the skin. Attachment of small coloured labels (e.g. coloured beads, discs) to fishes has been used successfully in a number of species (Sloman *et al.*, 2005; Webster & Laland, 2009). Traditional methods of marking the skin include dyes such as alcian blue (Kelly, 1967), and freeze-branding with liquid nitrogen (Nahhas & Jones, 1980; Bangs *et al.*, 2013) but more recently, fluorescent dyes such as VIE tags (Visible Implant Elastomer; <http://www.nmt.us/products/vie/vie.shtml>) have been developed. VIE tags are likely to cause minimal harm to the fish and have been used and evaluated extensively (Malone *et al.*, 1999; Reeves & Buckmeier, 2011; Hohn & Petrie-Hanson, 2013; White & Brown, 2013). However, there has been a relatively limited number of studies focussed on understanding the behavioural implications of colour tagging where the addition of colour has the potential to alter natural behaviours (Frommen *et al.*, 2015). Fishes can also be tagged with marks not visible to the naked human eye. For example, immersion in the fluorochrome dye, calcein, produces a distinct calcein mark on the fin rays and scales which are visible under a fluorescent microscope (Leips *et al.*, 2001; Mohler, 2003). This is particularly useful for batch-marking or tracking cohorts of fishes. Another way of marking individuals is fin clipping, where a portion of the fin, usually the caudal fin, is removed. Adipose fin removal in salmonids has been widely used for many years to distinguish hatchery-reared fishes from wild salmonids. However, some ethical considerations of fin clipping both for identification and genetic analyses are given in Table 2.

External and internal tags

Beyond visually tagging individuals, devices can be attached to, or implanted within individuals to keep track of their identity and movements. There are a variety of different factors which will determine the type of tag used and individual validation studies are important for determining whether the intended tag is suitable for the target animal. Validation studies are typically designed to observe the effects of tags on swimming kinematics, swimming performance, energy expenditure (measured as oxygen uptake rates) while swimming or at rest, behaviour, or survivorship (Ross & McCormick, 1981; Lowe, 1996; Lefrançois *et al.*, 2001; Makiguchi & Ueda, 2009; Bouyoucos *et al.*, 2017). It is unclear, however, what constitutes an acceptable level of impairment to the aforementioned metrics due to tagging. Indeed, tag burden will differ based on the size/life stage of the fish. Ultimately, validation should be conducted to determine, firstly, whether the intended tag can be deployed on the target individuals and whether the tag or target species/life stage can be refined. If the tag is deployed, any consequences of tag bearing should be documented.

The so-called “2 % rule” that is often referenced suggests that tags (both external and internal) within 2 % of the target individual’s mass should not produce negative consequences (Jepsen *et al.*, 2015). This should not be considered definitive as some studies that have adhered to the 2 % rule have observed tagging effects (e.g. Brown *et al.*, 1999) and some fishes are able to endure tag burdens greater than 2 %. For example, tags of 5-10% body mass were successfully used to track the migration of 2-year-old sockeye salmon smolts *Oncorhynchus nerka* (Walbaum 1792) from freshwater natal grounds to the open ocean (Clark *et al.*, 2016). Indeed, adding a tag will increase the mass of an animal, but it is arguably more relevant to an aquatic organism to consider changes in buoyancy (i.e. submerged *weight*), rather than changes in absolute *mass* for establishing acceptable characteristics of external tags to be applied across species. For instance, two tag packages with similar masses (e.g. floating *versus* neutral or negatively buoyant tags) would have very different consequences for buoyancy compensation. Furthermore, for external tags, the 2 % rule neglects acceptable limits for induced hydrodynamic drag, which will have profound effects on energy expenditure, as drag scales exponentially with swimming speed.

636

637 Popular telemetry devices, such as pop-up satellite archival tags (PSAT) or acoustic
638 transmitters, can be applied both externally and internally. Telemetry devices, rather than
639 logging devices that must be physically retrieved to collect data, afford researchers the
640 opportunity to remotely observe an animal in its habitat with minimal disturbance (Cooke *et*
641 *al.*, 2004, 2016b; Block *et al.*, 2011; Hussey *et al.*, 2015). Telemetry studies using external
642 tags must consider removal or retrieval of inert tags or tag attachment gear (e.g. bridles) to
643 avoid lifetime consequences for tag-bearing animals. A popular approach for external tags is
644 the use of corrodible galvanized components of the tag package, the corrosion of which is
645 triggered by the device itself under predefined conditions, or as a function of the ambient
646 water's physical conditions (e.g. Speed *et al.*, 2013; Whitmore *et al.*, 2016). However, this
647 method comes with the risk of tag packages not detaching (with long-term consequences for
648 the animal), or detaching prematurely (with consequences for data collection). Tags can be
649 permanently affixed to eliminate the chance of premature detachment, but strategies to
650 recapture animals and remove tags ought to be considered.

651

652 *External Tags*

653

654 More substantial external tags have the potential to affect buoyancy and increase effort to
655 overcome hydrodynamic drag while swimming (Jepsen *et al.*, 2015). When alterations in
656 buoyancy and drag become excessive, external tags can confer negative sub-lethal or lethal
657 consequences to the tag-bearing individual. For example, external tags can affect the
658 swimming kinematics, activity levels, and swimming performance of the target species
659 (Methling *et al.*, 2011; Bouyoucos *et al.*, 2017). Biofouling in the field and laboratory can
660 further alter the physical properties of external tags (Thorstad *et al.*, 2001). Swimming with
661 increased hydrodynamic drag, and compensating for changes in buoyancy can increase a
662 tagged fish's energy expenditure (Lowe *et al.*, 1998; Lefrançois *et al.*, 2001; Steinhausen *et*
663 *al.*, 2006; Methling *et al.*, 2011) leading to reduced growth rates, delayed maturation
664 (McFarlane & Beamish, 1990; Manire & Gruber, 1991; Sykes *et al.*, 2012) reduced foraging
665 ability and ability to escape predators (Ross & McCormick, 1981; Feltham & MacLean,
666 1996; Adams *et al.*, 1998). As such, the inappropriate application of external tags to fishes
667 can have direct consequences for individuals.

668

669 If externally-attached tags are used over long periods of time, new tissue may grow around
670 the tag, thereby enveloping the device (Lucas, 1989; Bauer & Loupal, 2007). Alternatively,
671 fishes can expel tags from the tissue site of attachment and abrasions, loss of scales, or
672 wounds associated with tagging may have pathological outcomes (Makiguchi & Ueda, 2009).
673 External tags also come with the risk of entanglement, either trapping the animal, or getting
674 torn from the tissue, possibly resulting in a wound. However, in many cases the animal may
675 be able to persist indefinitely with relatively minor impairments associated with bearing an
676 external tag (especially as they grow).

677

678 *Internal Tags*

679

680 An alternative to external attachment is to implant tags into the fish, either within fish tissues
681 or more usually within the peritoneal cavity. Internal tags can vary in size quite considerably
682 and while implantation avoids the potential issues with hydrodynamic drag, large internal
683 tags can still affect buoyancy control. Small implantable tags, such as passive integrated
684 transponder (PIT) tags for identification, can be quickly inserted through a needle into muscle
685 or the peritoneal cavity but surgery may be necessary to implant larger tags, which usually
686 requires anaesthesia (but see alternatives, e.g. tonic immobility in elasmobranchs; Kessel *et*
687 *al.*, 2015) and a recovery period (Cooke *et al.*, 2005). If there is a need to retrieve tags,
688 animals must undergo a second surgical procedure and recovery, unless the second sampling
689 is terminal. Indeed, some implantable tags, such as acoustic transmitters, can last for years
690 with little to no impact on the animal (Smukall *et al.*, 2018); under these circumstances, a
691 second capture event and surgical procedure to remove the tag would be far more invasive
692 than simply leaving the implanted tag. Tags can be inserted gastrically, but are likely to be
693 passed by the fish, unless the target species is no longer eating, as is the case for semelparous
694 salmon during their terminal spawning migrations (Ramstad & Woody, 2003;
695 Brunnschweiler, 2009; Neely *et al.*, 2009).

696

697 Whether external or internal tags are used, it is important that researchers confirm that any
698 given fish is in suitable condition for deployment. Collecting fishes in the field for tag

deployment can be an inherently stressful experience and even fishes in relatively good condition post-release may still become another's meal if it experiences a number of impairments to performance (Raby *et al.*, 2014; Tolentino *et al.*, 2017). Fortuitously, tremendous research effort has gone into defining best-practice approaches to improve the condition of fishes post-release, especially Pacific salmon (*Oncorhynchus* spp.) (Farrell *et al.*, 2001; Cooke *et al.*, 2012; Cook *et al.*, 2019). Successful use of tagging therefore relies on proper validation of the tag or tag package and consideration of impacts of capture and handling on the fish (Jepsen *et al.*, 2015; Cook *et al.*, 2019).

Fate at the End of the Experiment

Release

Where possible, many researchers seek to release wild-caught fishes back to their native habitat when they are no longer required for scientific research. Fishes should obviously be in good health when they are released and should be displaying 'normal' behaviour and physiology (Bennett *et al.*, 2016). Animals released in poor physiological condition or exhibiting unusual behaviours are likely to be more vulnerable to predation. While return to its natural environment may be beneficial to the individual fish, the risk to the ecosystem of returning these individuals must be carefully assessed. Primary considerations include whether the fish could have contracted disease or parasites while in captivity and whether they have been exposed to chemical compounds which may be retained in their tissues (e.g. anaesthetics, toxicants) (Wargo Rub *et al.*, 2014; Costello *et al.*, 2016). Some experiments may seek to release fishes reared in captivity to the wild; such releases are likely to be subject to local legislation to ensure no negative effects on native populations. The behaviour of fish held in captivity for long periods may vary markedly from the behaviour of wild fishes, thus care should be taken to enhance the viability of these fishes prior to release (reviewed by Brown & Laland, 2001).

Rehoming

Where ornamental fishes are used, particularly if they were originally purchased from the ornamental fish trade, it may be possible to rehome fishes following experimental work if they are in full health and without any effects from the experimental work they have been used for. If this is to happen, care should be taken to ensure that their new home is suitable, for example, in line with OATA guidance for fishes purchased within the retail trade (OATA, 2015). The researcher should be confident that the new owner possesses an aquarium or pond that is well established and suitable for the species and it is good practice to provide the new owner with care instructions for their new fishes. Where appropriate, if the social environment is likely to be very different in their new home it may be possible to acclimate the fish, for example to a lower stocking density, before they leave the research facility. Consideration should also be given to the welfare of the fish during transport to their new home (see *Transport* section below).

Euthanasia

Euthanasia, the induction of humane death of an animal, may be the only suitable or permissible option at the end of an experiment. There are two main approaches to euthanasia in fishes, physical or chemical. Physical methods include stunning (i.e. a sharp blow to the head) and chemical methods include overdose with anaesthesia (Ross & Ross, 2008). Whether physical or chemical methods are used, confirmation of death by decapitation or pithing (i.e. destruction of the brain and anterior spinal cord) are recommended (Ross & Ross, 2008). UK and EU legislation (ASPA, 2013; EU, 2010) favours overdose of anaesthetic or percussion stunning followed by destruction of the brain (Metcalf & Craig, 2011). For percussion stunning, prior use of an anaesthetic is advised as long as any distress caused by administration of the anaesthetic is less than that likely to be caused by killing without anaesthetic. Extensive guidelines on euthanasia are also provided by the American Veterinary Medical Association (AVMA, 2007). For larvae where percussion stunning is not appropriate, this is replaced with overdose of anaesthetic followed by killing by emersion in iced water or a fixative (Metcalf & Craig, 2011). In many instances, the impact of the

method chosen (e.g. anaesthesia) may not be resolved within the scientific literature (Hawkins *et al.*, 2016). Therefore it is important that researchers continue to consult the literature regarding the ethics of euthanasia so that best practice is followed.

Holding fishes in the Laboratory

Transport to the lab

If fishes are to be used in laboratory experiments then, unless they are bred in-house, they first have to be transported to the lab. Given the range of experimental fish species, the source of fishes will be diverse but there is an ethical responsibility that lies with the researcher to understand where the fish they purchased originated from, for example, ensuring that suppliers of ornamental fishes are not supporting destructive practices of collection (e.g. dynamiting, use of cyanide). Purchasing fishes from a reputable supplier with concern for fish welfare is important and in the UK, the Home Office state that zebrafish used for scientific research should have been bred for that purpose (ASPA, 2013). The main challenges fishes face during transport include stress and injury during handling and preparation for transport, deterioration of water quality during transport leading to increased disease susceptibility, stress and metabolic shock following transport (Pickering *et al.*, 1982; Portz *et al.*, 2006; Sampaio & Freire, 2016). Handling and transport stressors have the potential to influence later experimental results so a thorough understanding of the history of experimental fishes is important. If fishes are in good health prior to transport they are more likely to cope with the stress of transport and to be free from disease. In addition to fishes obtained from a supplier (commercial or non-commercial), fishes may be wild caught and then transferred to the lab. Here, the stress of capture also needs to be considered as it is likely to affect their ability to cope with transport stress and the ethics of capture techniques for wild fishes are discussed below (see *Catch and Release* below).

The welfare of fishes transported live for food aquaculture and also in the ornamental fish industry has been studied extensively (e.g. Walster, 2008; European Commission, 2017).

Fishes supplied for experimental purposes are often transported much shorter distances, but may have previously experienced longer transport times. Maintenance of good water quality during transport is essential. For very short durations, ensuring sufficient oxygen levels and appropriate temperature are the primary concerns as these are the parameters that will change the quickest. Although many fish species can tolerate a period of hypoxia, hypoxia will induce physiological changes (Montpetit & Perry, 1998). Fishes are often transported in bags and for short periods of transport, adding oxygen or air to the bag is common practice (Amend *et al.*, 1982; Froese, 1988). Other alternatives for avoiding problems with oxygen demand include reducing metabolic rate of the fish *via* sedation or reduced water temperatures. Both of these methods will cause physiological changes. During transport, accumulation of carbon dioxide leads to a decrease in water pH (Sampaio & Freire, 2016). If the duration of transport is long enough for this to occur, buffers can be added to the water to maintain pH such as Tris buffer (Amend *et al.*, 1982; Singh *et al.*, 2004), pH buffer 8.3 Trizma® (Stuart *et al.*, 2013), sodium bicarbonate and sodium carbonate (Correia *et al.*, 2011b).

Ammonia is the main nitrogenous waste product of fishes and is excreted primarily *via* the gills. Total ammonia nitrogen (TAN) comprises un-ionised (NH₃) and ionised ammonia (NH₄⁺) with high levels of the former causing convulsion, coma and death in fishes (Wright & Wood, 2012). Confinement in a small space, whether this be in tanks or in transport bags, allows water ammonia concentrations to rise, with TAN composition depending on temperature and pH (Portz *et al.*, 2006). For a transport period of short duration with good water quality at the start of transport, ammonia toxicity is unlikely to be an issue. For longer transport periods there are two main strategies for mitigating the effects of elevated TAN. Withdrawal of food prior to transport reduces ammonia excretion and has the additional benefits of reducing metabolic rate (Lim *et al.*, 2003; Walster, 2008) and preventing regurgitation of food during transport. Alternatively chemicals such as zeolites or AmQuel® can be added to the water to bind ammonia.

In addition to maintaining good water quality, there are a plethora of chemicals and commercial products that have been investigated for their potential to alleviate stress during transport (Vanderzwalmen *et al.*, 2018). While further research is needed for many of these

compounds in terms of their benefits, we also need to consider the effects of exposure to these chemicals on following experimental studies. Exposure to any chemicals during transport, which induce an altered physiological state in the fish could have concomitant effects on experimental results. Therefore, an understanding of physiological effects and clearance/recovery rates of any chemicals used during transport is needed.

Once fishes arrive at the laboratory, it is important to avoid a sudden change in water quality when they are introduced into a new experimental system. Common arrival protocols involve slowly mixing system water with the water the fish are transported in over a period of time. Prior to experiments starting, fishes need to be given sufficient time to recover from the stress of transport during which time they are closely monitored for any signs of ill-health or abnormal behaviours. Quarantine periods should be considered, particularly when new fishes will be added to recirculating systems containing existing stock.

Quality of Water and Food

It is accepted that fishes require good water quality for good health; fishes are in direct contact with the water and deviations from appropriate water quality can quickly lead to health deterioration. Some of the main concerns over water quality are discussed above in relation to transport. The diversity of waters that fishes inhabit is astounding, including ion-poor acidic waters of the Amazon and high salinity and high alkalinity lakes. Thus it is vital to understand the requirements of the species being worked with; in the case of wild caught fishes, the water chemistry of their natural environment may need to be replicated in the laboratory. The optimal pH in which to keep freshwater fishes can range from 5.0 to 10.0 (Portz *et al.*, 2006). Generally speaking, oxygen, temperature, pH and salinity are the key parameters of importance and the build-up of waste products such as ammonia, nitrite and nitrate requires monitoring as all can quickly reach toxic levels (Matthews, 2004; Stevens *et al.*, 2017). Tank materials may also alter water quality, and care must be taken to avoid materials which leach harmful chemicals into the water. Unhealthy fishes will of course not provide robust scientific data. Reporting of water parameters and ranges allows other researchers to ensure that fishes were held in optional conditions and can often help in comparisons between laboratories. Water parameters that can change very quickly (pH, temperature, DO) are usually monitored frequently (e.g. daily), while in an established

aquarium water parameters which are slower to change (ammonia, nitrate, nitrite) may be monitored less frequently (e.g. weekly). There are automated ways of monitoring water quality available (e.g. www.seneye.com) which are increasing in popularity and allow for more frequent monitoring and faster response to suboptimal conditions.

Poor water quality often leads to outbreaks of disease. Acute outbreaks of disease cause obvious detriment to welfare and chronic disease conditions, particularly those which may go unnoticed for a period of time, may confound research results (Kent *et al.*, 2008). The majority of research into fish diseases has focused on aquaculture species, and until recently research into diseases of other common research species was lacking. Common diseases of zebrafish have received the most attention and include systemic and dermal bacterial infections, mycobacteriosis, microsporidiosis and *Ichthyophthirius* infections (Matthews, 2004). Mycobacteriosis is the most commonly researched disease of warm-water research species with different species of *Mycobacterium* exhibiting different levels of virulence (Kent *et al.*, 2008). Indeed *Mycobacterium* is often found in fishes in the absence of disease (Beran *et al.*, 2006). Strategies for avoiding introduction of pathogens to research facilities put forward by Kent *et al.* (2008) include sourcing pathogen-free fishes, quarantining new stock, prophylactic treatment with therapeutic agents, avoiding introduction of contaminated water or food and using eggs only for new stock. Routine monitoring and culling of moribund, old or anorexic fishes may help prevent spread of chronic diseases.

While water quality as a factor in fish welfare is well recognised, less attention has been paid to the importance of diet for welfare. This is perhaps surprising given that nutritional deficiencies are likely to cause stress and affect disease resistance (Oliva-Teles, 2012). The natural diet of fishes is incredibly diverse and yet most commercial fish diets were originally formulated for aquaculture, and are aimed at carnivorous species of fish with requirements of up to 50% protein and 20% lipid (Halver & Hardy, 2002). While some fish food manufacturers will take into account the different requirements of particular fish species, most commercially available diets are unlikely to be suitable for all fish species (O'Brine *et al.*, 2015). Additionally, in their natural environment fishes can show seasonal changes in food intake which is rarely considered under laboratory conditions. Live food may be given to experimental fishes (Lawrence, 2007) and can count as enrichment (see *Quality of Housing*

below) but there are ethical issues with feeding live vertebrate prey (e.g. fishes) (see Table 2). The potential for live foods to act as vectors for pathogenic organisms is also an important consideration (Watts *et al.*, 2016).

Feeding fishes a diet with a protein level higher than required can result in excess ammonia production and therefore decreased water quality (Ballestrazzi *et al.*, 1994; Yang *et al.*, 2002) and feeding fishes a diet with the wrong type or content of lipid can lead to reduced growth (Watanabe, 1982; Oliva-Teles, 2012). While most commercial diets contain sufficient vitamins and minerals for healthy growth, some forms of diet may leach vitamins into the water if they are not consumed immediately (Pannevis & Earle, 1994). In comparison with mammalian work, it is notable that no standardised diet for model fish species exists. For example, very little is known about zebrafish nutrition and yet it is one of the most widely used fish models of human health and disease (Lawrence, 2007; Watts *et al.*, 2016). Variability in diets across zebrafish labs, in addition to variable feeding frequencies, has the potential to influence experimental repeatability, and was recently identified as one of the most critical areas for refinement of *D. rerio* care (Lidster *et al.*, 2017).

Quality of Housing

Correct water quality parameters and a nutritious diet will likely maintain health, but if we strive to allow fishes to perform more natural behaviours when in captivity then we need to consider how they are housed. Housing conditions in terms of tank design and water flow must be considered relative to the species, a subject which has been extensively reviewed by Portz *et al.* (2006). The great difficulties associated with defining ‘natural behaviours’ for fish species, particularly those that are under-researched, has been documented many times previous (Huntingford & Kadri, 2008), along with the idea that animals bred in captivity may have altered ‘natural’ behavioural phenotypes compared to wild caught animals (Duncan & Fraser, 1997). Nevertheless, there are several considerations which must be applied here.

There are a variety of circumstances under which fishes may be held for research purposes. Breeding colonies for fishes such as zebrafish are common-place where complete life-cycles are held within the laboratory. Other fish species, such as salmonids, are more commonly purchased as juveniles and held only for a part of their life-cycle. The presence of stock tanks, where fishes are usually held before being transferred to experimental tanks is usual, and it is not unusual for the housing conditions to be different between stock and experimental tanks. When considering housing of fishes for research, the two factors which have received most attention are stocking density and environmental enrichment. Both are important not only from an ethical standpoint to ensure welfare is maximised, but also because they have the potential to alter the behaviour and physiology of the fish with consequences for scientific data collected.

The effects of stocking density on salmonid welfare have been reviewed extensively, particularly in relation to aquaculture (e.g. Ellis *et al.*, 2002) where high densities can reduce growth and increase fin erosion. Low densities, on the other hand, can lead to excessive aggressive behaviour (Ellis *et al.*, 2002), and the establishment of dominance hierarchies (Turnbull *et al.*, 2005) with the potential for increased aggression and physiological consequences (Sloman & Armstrong, 2000). Appropriate stocking densities for other species requires species-specific knowledge and particular attention must be paid to stocking densities where fish are sequentially removed during the course of the experiment, thus providing variable stocking densities. Levels of aggressive behaviour will also be species and life-stage specific. In zebrafish, stocking density has the potential to affect sex determination with higher stocking densities producing greater prevalence of males (Ribas *et al.*, 2017). Other husbandry factors which can influence sex determination in zebrafish include temperature, hypoxia and food ration (Santos *et al.*, 2017).

Näslund & Johnson (2014) define environmental enrichment in their comprehensive review of fish enrichment techniques as “a deliberate increase in environmental complexity with the aim to reduce maladaptive and aberrant traits in fish reared in otherwise stimuli-deprived environments.” Environmental enrichment for fishes, is usually an increase in structural complexity, but also for fishes commonly includes dietary enrichment (Brown *et al.*, 2003) and social enrichment. For animals kept in captivity the EU directive on the protection of

animals used for scientific purposes (EU, 2010) recommends enrichment such as hiding places or bottom substrate but recognises that this needs to be considered on a species by species basis. The addition of enrichment to tanks is often done to improve welfare, but the evidence that environmental enrichment promotes welfare in fishes is controversial, with many effects being species-specific (Kistler *et al.*, 2011). In a recent survey of zebrafish facilities, less than 25% of facilities used environmental enrichment in the form of plants or substrate and were not considered practical by many of the laboratories surveyed (Lidster *et al.*, 2017). Motivational and/or preference tests are often used and can indicate if a species values the presence of enrichment (e.g. Sullivan *et al.*, 2015). Value may also vary depending on stocking density and species composition (Sloman *et al.*, 2011). For a list of types of enrichment in relation to the types of species that may benefit, see Table 2 in Williams *et al.* (2009).

One of the most common structural enrichments for fish tanks is the addition of a shelter, or an area of vegetation, particularly to stock tanks. These provide places to hide and can allow individuals to escape from conspecific aggression. In salmonid species, the addition of physical enrichment can reduce basal plasma cortisol (Näslund *et al.*, 2013) and aid recovery from a stressful event (Pounder *et al.*, 2016). Physiological benefits such as forebrain cell proliferation have been associated with enrichment (Kihlslinger & Nevitt, 2006; von Krogh *et al.*, 2010), and the presence of enrichment has been linked to increased learning in some species (Strand *et al.*, 2010; Carbia & Brown, 2019) but not others (Brydges & Braithwaite, 2009). Therefore, it becomes important for research purposes, not only to consider whether enrichment is positive for welfare, but also any differences in physiology that result from enriched *versus* barren holding conditions.

Another type of structural enrichment includes the addition of substrate, such as sand or gravel, which has been shown to increase foraging behaviour in goldfish (Smith & Gray, 2011). Batzina & Karakatsouli (2012) found that gilthead seabream *Sparus aurata* L. 1758 grew better and were less aggressive with blue or red/brown glass gravel, compared with control (no gravel) or green glass. Benefits of substrate enrichment have been noted in flatfishes (McVicar, 1987; Ellis *et al.*, 1997; Ottesen *et al.*, 2007). However, one of the major drawbacks to the use of substrate, and also other physical structures, is the potential for

pathogenic bacteria to grow on the increased surface area, although this has not been extensively researched (Tuckey & Smith, 2001). Batzina & Karakatsouli (2012) found higher water nitrite levels in tanks with substrate, which they potentially attributed to the increased substrate-particle surface area facilitating retention of fish waste products. These potential negative effects of environmental enrichment warrant further attention.

There are also concerns with the use of enrichment for toxicity trials, as the nature of regulatory test guidelines is such that many common forms of aquatic enrichment are not possible (Williams *et al.*, 2009). If the guidelines are not met the study is considered invalid. For toxicity tests, microbial growth can break down chemicals in the water and microbial growth itself may be promoted by the addition of solvents used to dissolve the test chemical (Williams *et al.*, 2009). An additional problem with enrichment in toxicity testing is that some types of material will adsorb chemicals, meaning fishes are not exposed to a constant concentration and some forms of enrichment may leach chemicals into the water. A small amount of research has considered appropriate forms of environment enrichment for fishes during toxicity trials (Wilkes *et al.*, 2012) but this is clearly something that warrants further attention.

There are some important questions regarding the methods of housing fishes for experimental work which we believe are still often over-looked and require more thorough investigation. Often, only brief mention of the conditions in which stock fishes are held are provided in scientific manuscripts and yet these conditions are likely to influence scientific results found. The ARRIVE guidelines suggest that information on both housing and husbandry conditions should be provided; we suggest that this should be for both stocking and experimental conditions. Indeed, providing information on why enrichment was not used for a particular species or life-stage can be useful to other researchers in planning their experiments. Researchers should consider in particular, how the transfer of animals from stocking conditions to experimental conditions may alter the physiology or behaviour of their experimental animals and also whether each ‘replicate’ animal receives the same treatment. For example, if stock tanks contain environmental enrichment but experimental tanks do not, does this change in habitat complexity induce stress prior to the start of an experiment?

Common Scientific Procedures

Once within an experimental system, there are a variety of different scientific procedures which a fish may be exposed to, and as discussed above, the effects of these protocols must always be weighed against the scientific advancement that they provide. It is not possible here to cover all of the different procedures, and many will vary from experiment to experiment, but ethical considerations of some of the most common scientific procedures used on fishes are given in Table 2.

Field Studies

Non-invasive field observations

Snorkelling or SCUBA diving are often used for observing fish behaviour or conducting visual census along with video recordings. Although generally considered non-invasive, there may be instances when the presence of divers or equipment has the potential to alter natural behaviours and minimising the impact of these types of surveys on the environment should be prioritised. The effects of flash photography where still images are used for individual identification has also been tested in some species (Harasti & Gladstone, 2013). Whether direct observation or video is more appropriate may depend on the behaviours being analysed (Branconi *et al.*, 2019).

Baited remote underwater video surveys (BRUVS)

Baited remote underwater video surveys (BRUVS) are a non-invasive alternative to fishing surveys for generating relative abundance indices (Brooks *et al.*, 2011b; Whitmarsh *et al.*, 2017; Sherman *et al.*, 2018). The efficacy of BRUVS in generating relative abundance indices has been validated against many traditional fishing gears, suggesting that BRUVS produce comparable estimates under certain circumstances (Brooks *et al.*, 2011b; Santana-

Garcon *et al.*, 2014; McLean *et al.*, 2015). In general, BRUVS are considered to have minimal negative impacts on the target population, sampling location, or environment (Whitmarsh *et al.*, 2017). Furthermore, BRUVS can be more resource efficient and cost effective per replicate than maintaining fishing surveys, can detect animals that would otherwise be excluded by the selectivity of fishing gears, and generate a permanent record of fishes present including an ethogram of behaviours (Brooks *et al.*, 2011b; Kilfoil *et al.*, 2017). The use of BRUVS is not without shortcomings. Baiting will alter the behaviour of fishes and in extreme cases, inter- and intraspecific competition for the bait lead to agonistic interactions between animals (O'Shea *et al.*, 2015). Possible abrasions from fishes bumping into the BRUVS unit or bait bag/box may cause minimal harm and larger fishes (e.g. sharks) may be able to remove, ingest, or retain part of the BRUVS unit (Brooks *et al.*, 2011b). Nevertheless, BRUVS are considerably less invasive relative to fishing techniques and their use to survey wild fish populations, from coral reefs to the deep sea, has become commonplace over the past 20 years (Whitmarsh *et al.*, 2017).

Catch and release of wild animals

The capture of wild animals is an inherently stressful process and techniques to mitigate stress and mortality have received much attention in fishes. The perception of a threat upon capture can induce a stress response in the form of increased levels of circulating stress hormones (catecholamines and corticosteroids), and exercise associated with struggling during capture can induce a physiological response of metabolic (e.g. accumulation of lactic acid) and/or respiratory (e.g. accumulation of CO₂) origin (Wood, 1991; Wendelaar Bonga, 1997). Handling fishes after capture can cause further stress; the duration and nature of handling (e.g. how long fishes are held out of water) can affect the stress response (Gingerich *et al.*, 2007; Cook *et al.*, 2015). Collecting fishes in extreme conditions (e.g. high water temperatures, low dissolved oxygen), or exposing fishes to extreme environmental gradients (e.g. temperature and pressure change from capture at depth) can also have physiological consequences (Pankhurst, 2011; Wilson *et al.*, 2014; Talwar *et al.*, 2017). Physiological stress associated with capture may ultimately be sufficient to induce immediate or post-release mortality for some species (Wood *et al.*, 1983).

Fishes can experience physical injury during capture and handling. Foul-hooking or ingesting hooks can be quite serious; the risk of these injuries can be minimized by modifying fishing gear, such as switching from “J” hooks to circle hooks (Skomal *et al.*, 2002; Reinhardt *et al.*, 2018). Depending on the nature of the injury, a fish should either be released immediately to minimize additional handling stress, or euthanised (Fobert *et al.*, 2009). Some species, however, are demonstrably resilient to injuries (Chin *et al.*, 2015; Kessel *et al.*, 2017). Physical injuries (e.g. scale loss or cuts) can also arise from entanglement and abrasion, crushing injuries from net fishing gears, and barotrauma following rapid ascent; severe injuries can quickly lead to critical failure of vital tissues (Cook *et al.*, 2019).

Captured fishes are unable to escape predators and can also be rendered unable to do so upon release. Indeed, depredation is a serious issue to consider for catch and release of wild fishes (Raby *et al.*, 2014). Techniques to recover captured fishes in recreational and commercial fisheries have been validated for many fisheries (Brownscombe *et al.* 2017a; Cook *et al.* 2019), such as retaining fishes to allow partial or full recovery to improve condition, performance, and the odds of survival upon release (Farrell *et al.*, 2001; Suski *et al.*, 2007; Brownscombe *et al.*, 2013). However, some techniques previously thought to improve condition (e.g., assisted ventilation) may be of little benefit in some species or given the context-specific nature of capture (Brownscombe *et al.*, 2017b; Cook *et al.* 2019). The chronic effects associated with stressful events are receiving considerable attention in fishes, with the overall body of research suggesting that a fish that survives capture to be released may take hours to days to resume normal behaviour and physiology (Kieffer, 2000; Wilson *et al.*, 2014). The best recommendations from the available literature for mitigating a fish’s stress response resulting from capture are to minimize capture and handling duration, and to minimize the duration of air exposure (Cooke *et al.*, 2013; Cook *et al.*, 2019). Indeed, social programs have even been developed to encourage anglers to follow these simple “best-practice” guidelines, such as handling fishes in the water (Danylchuk *et al.*, 2018).

Field studies must also account for the capture of non-target species, i.e. bycatch. Gear and technique modifications can improve the selectivity of fishing gear to minimize bycatch. Physical attributes of gear, including the timing and location of fishing, can affect the likelihood of encountering bycatch species. For instance, hook type and size can have effects

on capture rates of certain species (Reinhardt *et al.*, 2018). In addition, fishing depth, bait, time of day, season, and environmental conditions (e.g. sea surface temperature) can all influence the likelihood of encountering bycatch (Watson & Kerstetter, 2006). Implementing bycatch reduction devices can (theoretically) reduce the incidence of bycatch species without affecting that of the target species (Jordan *et al.*, 2013). Some studies, for example, have tested the efficacy of devices generating electromagnetic fields to deter electroreceptive fishes (O'Connell & He, 2014). It should be noted that fishing in aquatic environments can attract more than just fish bycatch; avian, reptilian, and possibly mammalian bycatch must also be considered (Lewison *et al.*, 2004).

The combined effects of capture, handling, and environmental conditions must be considered when choosing the best methods to ensure high post-release survivorship (PRS) during catch-and-release fishing. Fortuitously, determining the magnitude of physiological stress experienced by the target species using a given fishing gear can be assessed using simple assays and commercially available equipment (Cooke *et al.*, 2013; Madliger *et al.*, 2018). Simple phlebotomy techniques paired with species-validated point-of-care analytical devices can provide insights into an animal's physiological status (e.g. whole-blood acid-base status or metabolite concentrations) within minutes of blood sampling (Stoot *et al.*, 2014). Similarly, simple behavioural indices, such as reflexes to various stimuli (e.g. a vigorous escape when released or inducing a biting response) can be powerful predictors of an animal's PRS (Davis, 2010). As such, advances in field-based behaviour and physiology studies of fishes have revealed simple diagnostic techniques that can be introduced to a study to improve confidence that animals are being returned to the wild in a condition that will not compromise their survival.

CONCLUSION

Most fish biologists share the consensus that it is the best interests of their research that the fishes they work with are not stressed, are healthy and that their welfare is carefully considered, regardless of whether or not they believe fishes can experience pain. Definitions of welfare spark controversy, yet again there is much consensus in suggested measures.

Regardless of these controversies, improved ethics and welfare of fishes, almost inevitably results in improved scientific rigour as it challenges us to think about the natural habitat and life histories of the species we work with. Current dilemmas facing fish researchers include when to use anaesthetics, whether to use analgesics and the lack of a standardised diet for many laboratory-held species. Many non-significant results go unreported and many studies fail to provide sufficient information on how fishes were treated.

Regulations and guidelines can differ quite dramatically from country to country, with what is acceptable for one fish researcher considered an illegal practice for another. There is similar inconsistency in the prominence of ethical consideration by fish-related academic journals. Here there seems to be capacity for journals to contribute to improved welfare through education, debate and careful consideration of ethical requirements. The emphasis on fish welfare both out-with and amongst the scientific community seems unlikely to wane and it is in our best interest to strive to refine our research practices both for the fishes and environments we work with and for our scientific outcomes.

Acknowledgements

The authors would like to thank three anonymous reviewers and Myriam Vanderzwalmen for their thoughtful comments on previous drafts.

References

- Abbas, R. & Hayton, W.L. (1997). A physiologically based pharmacokinetic and pharmacodynamics model for paraoxon in rainbow trout. *Toxicology and Applied Pharmacology* **145**, 192-201.
- Adams, N.S., Rondorf, D.W., Evans, S.D., Kelly, J.E. & Perry, R.W. (1998). Effects of surgically and gastrically implanted radio transmitters on swimming performance and predator avoidance of juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 781-787.
- Aiello, B.R., Stewart, T.A. & Hale, M.E. (2016). Mechanosensation in an adipose fin. *Proceedings of the Royal Society B* **283**, 20152794.
- AFS (2014). Use of Fishes in Research Committee (joint committee of the American Fisheries Society, the American Institute of Fishery Research Biologists, and the American Society of Ichthyologists and Herpetologists) (2014). Guidelines for the use of fishes in research. American Fisheries Society, Bethesda, Maryland.

- Amend, D.F., Croy, T.R., Goven, B.A., Johnson, K.A. & McCarthy, D.H. (1982).
Transportation of fish in closed systems: methods to control ammonia, carbon dioxide,
pH, and bacterial growth. *Transactions of the American Fisheries Society* **111**, 603-
611.
- Animal Sentience (2016). <http://animalstudiesrepository.org/animsent/vol1/iss3/>
- Arlinghaus, R., Cooke, S.J., Schwab, A. & Cowx, I.G. (2007). Fish welfare: a challenge to
the feelings based approach, with implications for recreational fishing. *Fish and
Fisheries* **8**, 57-71.
- ASAB (2012). Guidelines for the treatment of animals in behavioural research and teaching.
Animal Behaviour **83**, 301-309.
- ASAB (2018). Guidelines for the treatment of animals in behavioural research and teaching.
Animal Behaviour **135**, in press.
- ASPA (2013).
[https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment
_data/file/619140/ConsolidatedASPA1Jan2013.pdf](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/619140/ConsolidatedASPA1Jan2013.pdf)
- Ashley, P.J., Sneddon, L.U. & McCrohan, C.R. (2006). Properties of corneal receptors in
teleost fish. *Neuroscience Letters* **410**, 165-168.
- Ashley, P.J., Sneddon, L.U. & McCrohan, C.R. (2007). Nociception in fish: stimulus-
response properties of receptors on the head of trout *Oncorhynchus mykiss*. *Brain
Research* **1166**, 47-54.
- Ashley, P.J., Ringrose, S., Edwards, K.L., McCrohan, C.R. & Sneddon, L.U. (2009). Effect
of noxious stimulation upon antipredator responses and dominance status in rainbow
trout. *Animal Behaviour* **77**, 403-410.
- AVMA (2007). <https://grants.nih.gov/grants/olaw/Euthanasia2007.pdf>
- Baker, T.R., Baker, B.B., Johnson, S.M. & Sladky, K.K. (2013). Comparative analgesic
efficacy of morphine sulfate and butorphanol tartrate in koi (*Cyprinus carpio*)
undergoing unilateral gonadectomy. *JAVMA-Journal of The American Veterinary
Medical Association* **243**, 882-890.
- Ballestrazzi, R., Lanari, D., D'Agaro, E. & Mion, A. (1994). The effect of dietary protein
level and source on growth, body composition, total ammonia and reactive phosphate
excretion of growing sea bass (*Dicentrarchus labrax*). *Aquaculture* **127**, 197-206.
- Balzarini, V., Taborsky, M., Wanner, S., Koch, F. & Frommen, J.G. (2014). Mirror, mirror on
the wall: the predictive value of mirror tests for measuring aggression in fish.
Behaviour Ecology and Sociobiology **68**, 871-878.
- Bangs, B.L., Falcy, M.R., Scheerer, P.D. & Clements, S. (2013). Comparison of three
methods for marking a small floodplain minnow. *Animal Biotelemetry* **1**, 18.
- Barton, B.A. (2002). Stress in fishes: a diversity of responses with particular reference to
changes in circulating corticosteroids. *Integrative and Comparative Biology* **42**, 517-
525.
- Bateson, P. (1991). Assessment of pain in animals. *Animal Behaviour* **42**, 827-839.
- Batzina, A. & Karakatsouli, N. (2012). The presence of substrate as a means of
environmental enrichment in intensively reared gilthead seabream *Sparus aurata*:
Growth and behavioral effects. *Aquaculture* **370-371**, 54-60.
- Bauer, C. & Loupal, G. (2007). Common carp tissue reactions to surgically implanted radio
tags with external antennas. *Journal of Fish Biology* **70**, 292-297.
- Belanger, S.E., Rawlings, J.M. & Carr, G.J. (2013). Use of fish embryo toxicity tests for the
prediction of acute fish toxicity to chemicals. *Environmental Toxicology and Chemistry*
32, 1768-1783.

1217 Bennett, R.H., Ellender, B.R., Mäkinen, T., Miya, T., Pattrick, P., Wasserman, R.J.,
1218 Woodford, D.J. & Weyl, O.L.F. (2016). Ethical considerations for field research on
1219 fishes. *Koedoe* **58**, a1353.

1220 Beran, V., Matlova, I., Dvorska, L., Svastova, P. & Palik, I. (2006). Distribution of
1221 mycobacteria in clinically healthy ornamental fish and their aquarium environment.
1222 *Journal of Fish Diseases* **29**, 383-393.

1223 Block, B.A., Jonsen, I.D., Jorgensen, S.J., Winship, A.J., Shaffer, S.A., Bograd, S.J., Hazen,
1224 E.L., Foley, D.G., Breed, G.A., Harrison, A.L., Ganong, J.E., Swithenbank, A., Castleton,
1225 M., Dewar, H., Mate, B.R., Shilinger, G.L., Schaefer, K.M., Benson, S.R., Weise, M.J.,
1226 Henry, R.W. & Costa, D.P. (2011). Tracking apex marine predator movements in a
1227 dynamic ocean. *Nature* **475**, 86-90.

1228 Bols, N.C., Dayeh, V.R., Lee, L.E.J. & Schirmer, K. (2005). Use of fish cell lines in the
1229 toxicology and ecotoxicology of fish. Piscine cell lines in environmental toxicology.
1230 *Biochemistry & Molecular Biology of Fishes* **6**, 43-84.

1231 Bopp, S.K., Abicht, H.K. & Knauer, K. (2008). Copper-induced oxidative stress in rainbow
1232 trout gill cells. *Aquatic Toxicology* **86**, 197-204.

1233 Bouyoucos, I.A., Suski, C.D., Mandelman, J.W. & Brooks, E.J. (2017). Effect of weight and
1234 frontal area of external telemetry packages on the kinematics, activity levels and
1235 swimming performance of small-bodied sharks. *Journal of Fish Biology* **90**, 2097-
1236 2110.

1237 Branconi, R., Wong, M.Y.L. & Buston, P.M. (2019). Comparison of efficiency of direct
1238 observations by SCUBA diver and indirect observations via video camera for
1239 measuring reef fish behaviour. *Journal of Fish Biology* in press.

1240 Breacker, C., Barber, I., Norton, W.H.J., McDermid, J.R. & Tilley, C.A. (2017). A low-cost
1241 method of skin swabbing for the collection of DNA samples from small laboratory fish.
1242 *Zebrafish* **14**, 35-41.

1243 Brix, K.V., DeForest, D.K., Tear, L., Grosell, M. & Adams, W.J. (2017). Use of multiple
1244 linear regression models for setting water quality criteria for copper: A complimentary
1245 approach to the Biotic Ligand Model. *Environmental Science & Technology* **51**, 5182-
1246 5192.

1247 Brooks, E.J., Sloman, K.A., Liss, S., Hassan-Hassanein, L., Danylchuk, A.J., Cooke, S.J.,
1248 Mandelman, J.W., Skomal, G.B., Sims, D.W. & Suski, C.D. (2011a). The stress
1249 physiology of extended duration tonic immobility in the juvenile lemon shark,
1250 *Negaprion brevirostris* (Poey 1868). *Journal of Experimental Marine Biology and*
1251 *Ecology* **409**, 351-360.

1252 Brooks, E., Sloman, K., Sims, D. & Danylchuk, A. (2011b). Validating the use of baited
1253 remote underwater video surveys for assessing the diversity, distribution and abundance
1254 of sharks in the Bahamas. *Endangered Species Research* **13**, 231-243.

1255 Broom, D. M. (2007). Cognitive ability and sentience: which aquatic animals should be
1256 protected? *Diseases of Aquatic Animals* **75**, 99-108.

1257 Broom, D.M. (2014). *Sentience and Animal Welfare*. 185 pp. Wallingford: CABI
1258 International.

1259 Browman, H.I., Cooke, S.J., Cowx, I.G., Derbyshire, S.W.G., Kasumyan, A., Key, B., Rose,
1260 J.D., Schwab, A., Skiftesvik, A.B., Stevens, D., Watson, C.A. & Arlinghaus, R. (2018).
1261 Welfare of aquatic animals: where things are, where they are going, and what it means
1262 for research, aquaculture, recreational angling, and commercial fishing. *ICES Journal*
1263 *of Marine Science* in press.

1264 Brown, C. (2015). Fish intelligence, sentience and ethics. *Animal Cognition* **18**, 1-17.

- Brown, C., Davidson, T. & Laland, K. (2003). Environmental enrichment and prior experience of live prey improve foraging behaviour in hatchery-reared Atlantic salmon. *Journal of Fish Biology* **63**, 187-196.
- Brown, C. & Laland, K. (2001). Social learning and life skills training for hatchery reared fish. *Journal of Fish Biology* **59**, 471-493.
- Brown, R.S., Cooke, S.J., Anderson, W.G. & McKinley, R.S. (1999). Evidence to challenge the “2% Rule” for biotelemetry. *North American Journal of Fisheries Management* **19**, 867-871.
- Brownscombe, J.W., Thiem, J.D., Hatry, C., Cull, F., Haak, C.R., Danylchuk, A.J. & Cooke, S.J. (2013). Recovery bags reduce post-release impairments in locomotory activity and behavior of bonefish (*Albula* spp.) following exposure to angling-related stressors. *Journal of Experimental Marine Biology and Ecology* **440**, 207-215.
- Brownscombe, J.W., Parmar, T.P., Almeida, J., Giesbrecht, E., Batson, J., Chen, X., Wesch, S., Ward, T.D., O'Connor, C.M. & Cooke, S.J. (2017b). The efficacy of assisted ventilation techniques for facilitating the recovery of fish that are exhausted from simulated angling stress. *Fisheries Research* **186**, 619-624.
- Brownscombe, J.W., Danylchuk, A.J., Chapman, J.M., Gutowsky, L.F. & Cooke, S.J. (2017a). Best practices for catch-and-release recreational fisheries – angling tools and tactics. *Fisheries Research* **186**, 693-705.
- Brunnschweiler, J.M. (2009). Tracking free-ranging sharks with hand-fed intra-gastric acoustic transmitters. *Marine and Freshwater Behavior and Physiology* **42**, 201-209.
- Brydges, N.M. & Braithwaite, V.A. (2009). Does environmental enrichment affect the behaviour of fish commonly used in laboratory work? *Applied Animal Behaviour Science* **118**, 137-143.
- Buckland-Nicks, J.A. (2016). New details of the neural architecture of the salmonid adipose fin. *Journal of Fish Biology* **89**, 1991-2003.
- Buckland-Nicks, J.A., Gillis, M. & Reimchen, T.E. (2012). Neural network detected in a presumed vestigial trait: ultrastructure of the salmonid adipose fin. *Proceedings of the Royal Society B* **279**, 553-563.
- Campanella, J.J. & Smalley, J.V. (2006). A minimally invasive method of piscine tissue collection and an analysis of long-term field-storage conditions for samples. *BMC Genetics* **7**, 32.
- Carbia, P.S. & Brown, C. (2019). Environmental enrichment influences spatial learning ability in captive-reared intertidal gobies (*Bathygobius cocosensis*). *Animal Cognition* **22**, 89-98.
- Champagne, C.E., Austin, J.D., Jelks, H.L. & Jordan, F. (2008). Effects of fin clipping on survival and position-holding behaviour of brown darters, *Etheostoma edwini*. *Copeia* **2008**, 916-919.
- Chandroo, K.P., Duncan, I.J.H. & Moccia, R.D. (2004). Can fish suffer?: perspectives on sentience, pain, fear and stress. *Applied Animal Behaviour Science* **86**, 225-250.
- Chervova, L.S. (1997). Pain sensitivity and behavior of fishes. *Journal of Ichthyology* **37**, 98-102.
- Chervova, L.S. & Lapshin, D.N. (2000). Opioid modulation of pain threshold in fish. *Doklady Biological Sciences* **375**, 590-591.
- Chervova, L.S., Lapshin, D.N. & Kamenskii, A.A. (1994). Pain sensitivity of trout and analgesia induced by intranasal administration of dermorphine. *Doklady Biological Sciences* **338**, 424-425.
- Chin, A., Mourier, J. & Rummer, J.L. (2015). Blacktip reef sharks (*Carcharhinus melanopterus*) show high capacity for wound healing and recovery following injury. *Conservation Physiology* **3**, cov062.

- Cho, G.K. & Heath, D.D. (2000). Comparison of tricaine methanesulphonate (MS222) and clove oil anaesthesia effects on the physiology of juvenile chinook salmon *Oncorhynchus tshawytscha* (Walbaum). *Aquaculture Research* **31**, 537-546.
- Clark, T.D., Furey, N.B., Rechisky, E.L., Gale, M.K., Jeffries, K.M., Porter, A.D., Casselman, M.T., Lotto, A.G., Patterson, D.A., Cooke, S.J., Farrell, A.P., Welch, D.W. & Hinch, S.G. (2016). Tracking wild sockeye salmon smolts to the ocean reveals distinct regions of nocturnal movement and high mortality. *Ecological Applications* **26**, 959–978.
- Cook, K.V., Lennox, R.J., Hinch, S.G. & Cooke, S.J. (2015). Fish out of water: How much air is too much? *Fisheries* **40**, 452-461.
- Cook, K.V., Reid, A.J., Patterson, D.A., Robinson, K.A., Chapman, J.M., Hinch, S.G. & Cooke, S.J. (2019). A synthesis to understand responses to capture stressors among fish discarded from commercial fisheries and options for mitigating their severity. *Fish and Fisheries* **20**, 25-43.
- Cooke, S.J. (2016). Spinning our wheels and deepening the divide: Call for an evidence-based approach to the fish pain debate. *Animal Sentience* **3**(42).
- Cooke, S.J., Hinch, S.G., Wikelski, M., Andrews, R.D., Kuchel, L.J., Wolcott, T.G. & Butler, P.J. (2004). Biotelemetry: A mechanistic approach to ecology. *Trends in Ecology and Evolution* **19**, 334–343.
- Cooke, S.J., Crossin, G.T., Patterson, D.A., English, K.K., Hinch, S.G., Young, J.L., Alexander, R.F., Healey, M.C., Kraak, G. van der & Farrell, A.P. (2005). Coupling non-invasive physiological assessments with telemetry to understand inter-individual variation in behaviour and survivorship of sockeye salmon: development and validation of a technique. *Journal of Fish Biology* **67**, 1342–1358.
- Cooke, S. J., Hinch, S. G., Donaldson, M. R., Clark, T. D., Eliason, E. J., Crossin, G. T., Raby, G. D., Jeffries, K. M., Lapointe, M., Miller, K., Patterson, D.A. & Farrell, A.P. (2012). Conservation physiology in practice: How physiological knowledge has improved our ability to sustainably manage Pacific salmon during up-river migration. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **367**, 1757–1769.
- Cooke, S.J., Donaldson, M.R., O'Connor, C.M., Raby, G.D., Arlinghaus, R., Danylchuk, A.J., Hanson, K.C., Hinch, S.G., Clark, T.D., Patterson, D.A. & Suski, C.D. (2013). The physiological consequences of catch-and-release angling: perspectives on experimental design, interpretation, extrapolation and relevance to stakeholders. *Fisheries Management and Ecology* **20**, 268–287.
- Cooke, S.J., Wilson, A.D.M., Elvidge, C.K., Lennox, R.J., Jepsen, N., Colotelo, A.H. & Brown, R.S. (2016a). Ten practical realities for institutional animal care and use committees when evaluating protocols dealing with fish in the field. *Reviews in Fish Biology and Fisheries* **26**, 123-133.
- Cooke, S.J., Brownscombe, J.W., Raby, G.D., Broell, F., Hinch, S.G., Clark, T.D. & Semmens, J.M. (2016b). Remote bioenergetics measurements in wild fish: Opportunities and challenges. *Comparative Biochemistry and Physiology A* **202**, 23–37.
- Conte, F.S. (2004). Stress and the welfare of cultured fish. *Applied Animal Behaviour Science* **86**, 205-223.
- Correia, A.D., Cunha, S.R., Scholze, M. & Stevens, E.D. (2011a). A novel behavioral fish model of nociception for testing analgesics. *Pharmaceuticals* **4**, 665-680.
- Correia, J.P.S., Graça, J.T.C., Hirofumi, M. & Kube, N. (2011b). Long-term transportation, by road and air, of chub mackerel (*Scomber japonicas*) and Atlantic bonito (*Sarda sarda*). *Zoo Biology* **30**, 459-472.

- Costello, M.J., Beard, K.H., Corlett, R.T., Cumming, G.S., Devictor, V., Loyola, R., Maas, B., Miller-Rushing, A.J., Pakeman, R. & Primack, R.B. (2016). Field work ethics in biological research. *Biological Conservation* **203**, 268-271.
- Curtright, A., Rosser, M., Goh, S., Keown, B., Wagner, E., Sharifi, J., Raible, D.W. & Dhaka, A. (2015). Modeling nociception in zebrafish: a way forward for unbiased analgesic discovery. *PLoS One* **10**, e0116766.
- Curzer, H.J., Wallace, M., Perry, G., Muhlburger, P. & Perry, D. (2013). Environmental research ethics. *Environmental Ethics* **35**, 95-114.
- Danylchuk, A.J., Danylchuk, S.C., Kosiarski, A., Cooke, S.J. & Huskey, B. (2018) Keepemwet Fishing—An emerging social brand for disseminating best practices for catch-and-release in recreational fisheries. *Fisheries Research* **205**, 52–56.
- Davis, M.W. (2010). Fish stress and mortality can be predicted using reflex impairment. *Fish and Fisheries* **11**, 1–11.
- Davis, M.R., Mylniczenko, N., Storms, T., Raymond, F. & Dunn, J.L. (2006). Evaluation of intramuscular ketoprofen and butorphanol as analgesics in chain dogfish (*Scyliorhinus retifer*). *Zoo Biology* **25**, 491-500.
- Dawkins, M.S. (1998). Evolution and animal welfare. *Quarterly Review of Biology* **73**, 305-328.
- Dawkins, M.S. (2004). Using behaviour to assess animal welfare. *Animal Welfare* **13**, 3-7.
- Deters, K. A., Brown, R.S., Carter, K.M., Boyd, J.W. & Eppard, M.B. (2010). Performance assessment of suture type, water temperature, and surgeon skill in juvenile Chinook salmon surgically implanted with acoustic transmitters. *Transactions of the American Fisheries Society* **139**, 888–899.
- Duncan, I.J.H. & Fraser, D. (1997). Understanding animal welfare. In *Animal Welfare* (Appleby, M.C. & Hughes, B.O., eds), pp. 19-31. Wallingford: CABI Publishing.
- Dunlop, R. & Laming, P. (2005). Mechanoreceptive and nociceptive responses in the central nervous system of goldfish (*Carassius auratus*) and trout (*Oncorhynchus mykiss*). *Journal of Pain* **6**, 561–568.
- Dunlop, R., Millsopp, S. & Laming, P. (2006). Avoidance learning in goldfish (*Carassius auratus*) and trout (*Oncorhynchus mykiss*) and implications for pain perception. *Applied Animal Behaviour Science* **97**, 255-271.
- Dwan, K., Gamble, C., Williamson, P.R. & Kirkham, J.J. (2013). Systematic review of the empirical evidence of study publication bias and outcome reporting bias- An updated review. *PLoS ONE* **8**, e66844.
- Ellis, T., Howell, B.R. & Hughes, R.N. (1997). The cryptic responses of hatchery-reared sole to a natural sand substratum. *Journal of Fish Biology* **51**, 389-401.
- Ellis, T., North, B., Scott, A.P., Bromage, N.R., Porter, M. & Gadd, D. (2002). The relationships between stocking density and welfare in farmed rainbow trout. *Journal of Fish Biology* **61**, 493-531.
- Elwood, R.W., Stoilova, V., McDonnell, A., Earley, R.L. & Arnott, G. (2014). Do mirrors reflect reality in agonistic encounters? A test of mutual cooperation in displays. *Animal Behaviour* **97**, 63-67.
- EU Directive (2010). <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32010L0063>
- European Commission (2017). Report Welfare of farmed fish: Common practices during transport and at slaughter SANTE/2016/G2/009, September 2017. https://ec.europa.eu/food/sites/food/files/animals/docs/aw_platform_20180621_pres05.pdf
- Fanelli, D. (2010). Do pressures to publish increase scientists' bias? An empirical support from US States data. *PLoS One* **5**, e10271.

- Farrell, A.P., Gallagher, P.E., Fraser, J., Pike, D., Bowering, P., Hadwin, A.K.M., Parkhouse, W. & Routledge, R. (2001). Successful recovery of the physiological status of coho salmon on board a commercial gillnet vessel by means of a newly designed revival box. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 1932–1946.
- Feltham, M.J. & MacLean, J.C. (1996). Carlin tag recoveries as an indicator of predation on salmon smolts by goosanders and red-breasted mergansers. *Journal of Fish Biology* **48**, 270–282.
- Festing, M.F.W. & Altman, D.G. (2002). Guidelines for the design and statistical analysis of experiments using laboratory animals. *ILAR Journal* **43**, 244–258.
- Flowers, K.I., Henderson, A.C., Lupton, J.L. & Chapman, D.D. (2017). Site affinity of whitespotted eagle rays *Aetobatus narinari* assessed using photographic identification. *Journal of Fish Biology* **91**, 1337–1349.
- Fobert, E., Meining, P., Colotelo, A., O'Connor, C. & Cooke, S.J. (2009). Cut the line or remove the hook? An evaluation of sublethal and lethal endpoints for deeply hooked bluegill. *Fisheries Research* **99**, 38–46.
- Froese, R. (1988). Relationship between body weight and loading densities in fish transport using the plastic bag method. *Aquaculture and Fisheries Management* **19**, 275–281.
- Frommen, J.G., Hanak, S., Schmidl, C.A. & Thünken, T. (2015). Visible implant elastomer tagging influences social preferences of zebrafish (*Danio rerio*). *Behaviour* **152**, 1765–1777.
- Fu, C., Cao, Z.D. & Fu, S.-J. (2013). The effects of caudal fin amputation on metabolic interaction between digestion and locomotion in juveniles of three cyprinid fish species with different metabolic modes. *Comparative Biochemistry and Physiology A* **164**, 456–465.
- Gill, A.B. (2003). The dynamics of prey choice in fish: the importance for prey size and satiation. *Journal of Fish Biology* **63**, 105–116.
- Gingerich, A.J., Cooke, S.J., Hanson, K.C., Donaldson, M.R., Hasler, C.T., Suski, C.D. & Arlinghaus, R. (2007). Evaluation of the interactive effects of air exposure duration and water temperature on the condition and survival of angled and released fish. *Fisheries Research* **86**, 169–178.
- Gräns, A., Sandblom, E., Kiessling, A. & Axelsson, M. (2014). Post-surgical analgesia in rainbow trout: Is reduced cardioventilatory activity a sign of improved animal welfare or the adverse effects of an opioid drug? *PLoS ONE* **9**, e95283.
- Halver, J.E. & Hardy, R.W. (2002). *Fish Nutrition*. San Diego, CA: Academic Press.
- Harasti, D. & Gladstone, W. (2013). Does underwater flash photography affect the behaviour, movement and site persistence of seahorses? *Journal of Fish Biology* **83**, 1344–1353.
- Harms, C.A., Lewbart, G.A., Swanson, C.R., Kishimori, J.M. & Boylan, S.M. (2005). Behavioral and clinical pathology changes in koi carp (*Cyprinus carpio*) subjected to anesthesia and surgery with and without intra-operative analgesics. *Comparative Medicine* **55**, 221–226.
- Hart, P.J.B. (1997). Foraging tactics. In *Behavioural Ecology of Teleost Fishes* (Godin, J.G.J., ed.), pp. 104–133. Oxford: Oxford University Press.
- Hawkins, P., Prescott, M.J., Carbone, L., Dennison, N., Johnson, C., Makowska, I.J., Marquardt, N., Readman, G., Weary, D. & Colledge, H.D.R. (2016). A good death? Report of the second Newcastle meeting on laboratory animal euthanasia. *Animals* **6**, E50.
- Heupel, M.R. & Simpendorfer, C.A. (2010). Science or slaughter: need for lethal sampling of sharks. *Conservation Biology* **24**, 1212–1218.
- Hohn, C. & Petrie-Hanson, L. (2013). Evaluation of visible implant elastomer tags in zebrafish (*Danio rerio*). *Biology Open* **2**, 1397–1401.

1464 Holland, K.N., Wetherbee, B.M., Lowe, C.G. & Meyer, C.G. (1999). Movements of tiger
 1465 sharks (*Galeocerdo cuvier*) in coastal Hawaiian waters. *Marine Biology* **134**, 665-673.
 1466 Holloway, A.C., Keene, J.L., Noakes, D.G. & Moccia, R.D. (2004). Effects of clove oil and
 1467 MS-222 on blood hormone profiles in rainbow trout *Oncorhynchus mykiss*, Walbaum.
 1468 *Aquaculture* **35**, 1025-1030.
 1469 Hoyle, I. & Handy, R.D. (2005). Dose-dependent inorganic mercury absorption by isolated
 1470 perfused intestine of rainbow trout, *Oncorhynchus mykiss*, involves both amiloride-
 1471 sensitive and energy-dependent pathways. *Aquatic Toxicology* **72**, 147-159.
 1472 Huntingford, F.A., Adams, C., Braithwaite, F.A., Kadri, S., Pottinger, T.G., Sandøe, P. &
 1473 Turnbull, J.F. (2006). Current issues in fish welfare. *Journal of Fish Biology* **68**, 332-
 1474 372.
 1475 Huntingford, F.A. & Kadri, S. (2008). Welfare and fish. In *Fish Welfare* (Branson, E.J., ed.),
 1476 pp.19-31. Oxford: Blackwell.
 1477 Huntingford, F.A., Borcato, F.L. & Mesquita, F.O. (2013). Identifying individual common
 1478 carp *Cyprinus carpio* using scale pattern. *Journal of Fish Biology* **83**, 1453-1458.
 1479 Hussey, N. E., Kessel, S. T., Aarestrup, K., Cooke, S. J., Cowley, P. D., Fisk, A. T., Harcourt,
 1480 R. G., Holland, K. N., Iverson, S. J., Kocik, J. F., Mills Flemming, J.E. & Whoriskey,
 1481 F.G. (2015). Aquatic animal telemetry: A panoramic window into the underwater
 1482 world. *Science* **348**, 1255642.
 1483 IASP, (1979) *Pain*, **6**, 249-252.
 1484 Iwama, G.K., Pickering, A.D., Sumpter, J.P. & Schreck, C.P. (1997). *Fish Stress and Health*
 1485 *in Aquaculture*. Cambridge: Cambridge University Press.
 1486 Jepsen, N., Thorstad, E.B., Havn, T. & Lucas, M.C. (2015). The use of external electronic
 1487 tags on fish: an evaluation of tag retention and tagging effects. *Animal Biotelemetry* **3**,
 1488 49–71.
 1489 Jones, S.G., Kamunde, C., Lemke, K. & Stevens, E.D. (2012). The dose-response relation for
 1490 the antinociceptive effect of morphine in a fish, rainbow trout. *Journal of Veterinary*
 1491 *Pharmacology and Therapeutics* **35**, 563-570.
 1492 Jordan, L.K., Mandelman, J.W., McComb, D.M., Fordham, S.V., Carlson, J.K. & Werner
 1493 T.B. (2013). Linking sensory biology and fisheries bycatch reduction in elasmobranch
 1494 fishes: a review with new directions for research. *Conservation Physiology* **1**, cot002.
 1495 *Journal of Fish Biology* (2006). Ethical justification for the use and treatment of fishes in
 1496 research. *Journal of Fish Biology* **68**, 1-2.
 1497 Kelly, W.H. (1967). Marking freshwater and a marine fish by injected dyes. *Transactions of*
 1498 *the American Fisheries Society* **96**, 163-175.
 1499 Kent, M.L., Feist, S.W., Harper, C., Hoogstraten-Miller, S., Law, J.M., Sánchez-Morgado,
 1500 J.M., Tanguay, R.L., Sanders, G.E., Spitsbergen, J.M., Whipps, C.M. (2008).
 1501 Recommendations for control of pathogens and infectious diseases in fish research
 1502 facilities. *Comparative Biochemistry and Physiology C* **149**, 240-248.
 1503 Kessel, S.T., Fraser, J., Bonn, W.G. van, Brooks, J.L., Guttridge, T.L., Hussey, N.E. &
 1504 Gruber, S.H. (2017). Transcoelomic expulsion of an ingested foreign object by a
 1505 carcharhinid shark. *Marine and Freshwater Research* **68**, 2173.
 1506 Kessel, S.T., Hussey, N.E. & MacLatchey, D. (2015). Tonic immobility as an anaesthetic for
 1507 elasmobranchs during surgical implantation procedures. *Canadian Journal of Fisheries*
 1508 *and Aquatic Sciences* **72**, 1287–1291.
 1509 Key, B. (2016) Why fish do not feel pain. *Animal Sentience* **1**(1).
 1510 <http://animalstudiesrepository.org/animsent/vol1/iss3/1/>
 1511 Kieffer, J.D. (2000). Limits to exhaustive exercise in fish. *Comparative Biochemistry and*
 1512 *Physiology A* **126**, 161–179.

- Kihlslinger, R.L. & Nevitt, G.A. (2006). Early rearing environment impacts cellular growth in juvenile salmon. *Journal of Experimental Biology* **209**, 504-509.
- Kilfoil, J., Wirsing, A., Campbell, M., Kiszka, J., Gastrich, K., Heithaus, M., Zhang, Y. & Bond, M. (2017). Baited remote underwater video surveys undercount sharks at high densities: insights from full-spherical camera technologies. *Marine Ecology Progress Series* **585**, 113–121.
- Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M. & Altman, D.G. (2010). Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. *PLoS Biology* **8**, e1000412.
- Kistler, C., Hegglin, D., Wurbel, H. & König, B. (2011). Preference for structured environment in zebrafish (*Danio rerio*) and checker barbs (*Puntius oligolepis*). *Applied Animal Behaviour Science* **135**, 318-327.
- Lakra, W.S., Swaminathan, T.R. & Joy, K.P. (2011). Development, characterisation, conservation and storage of fish cell lines. A review. *Fish Physiology and Biochemistry* **37**, 1-20.
- Lauder, G.V. (2000). Function of the caudal fin during locomotion in fishes: Kinematics, flow visualisation, and evolutionary patterns. *American Zoologist* **40**, 101-122.
- Lawrence, C. (2007). The husbandry of zebrafish (*Danio rerio*): A review. *Aquaculture* **269**, 1-20.
- Lefrançois, C., Odion, M. & Claireaux, G. (2001). An experimental and theoretical analysis of the effect of added weight on the energetics and hydrostatic function of the swimbladder of European sea bass (*Dicentrarchus labrax*). *Marine Biology* **139**, 13–17.
- Leips, J., Baril, C.T., Rodd, F.H., Reznick, D.N., Bashey, F., Visser, G.J. & Travis, J. (2001). The suitability of calcein to mark poeciliid fish and a new method of detection. *Transactions of the American Fisheries Society* **130**, 501-507.
- Lewison, R., Crowder, L., Read, A. & Freeman, S. (2004). Understanding impacts of fisheries bycatch on marine megafauna. *Trends in Ecology and Evolution* **19**, 598–604.
- Lidster, K., Readman, G.D., Prescott, M.J. & Owen, S.F. (2017). International survey on the use and welfare of zebrafish *Danio rerio* in research. *Journal of Fish Biology* **90**, 1891-1905.
- Lim, L.C., Dhert, P. & Sorgeloos, P. (2003). Recent developments and improvements in ornamental fish packaging systems for air transport. *Aquaculture Research* **34**, 923-935.
- Lind, J. & Cresswell, W. (2005). Determining the fitness consequences of antipredation behaviour. *Behavioral Ecology* **16**, 945-956.
- Lopez-Luna, J., Al-Jubouri, Q., Al-Nuaimy, W. & Sneddon, L.U. (2017a). Activity reduced by noxious chemical stimulation is ameliorated by immersion in analgesic drugs in zebrafish. *Journal of Experimental Biology* **220**, 1451-1458.
- Lopez-Luna, J., Al-Jubouri, Q., Al-Nuaimy, W. & Sneddon, L.U. (2017b). Impact of analgesic drugs on the behavioural responses of larval zebrafish to potentially noxious temperatures. *Applied Animal Behaviour Science* **188**, 97-105.
- Lopez-Luna, J., Al-Jubouri, Q., Al-Nuaimy, W. & Sneddon, L.U. (2017c). Impact of stress, fear and anxiety on the nociceptive responses of larval zebrafish. *PLoS One* **12**, e0181010.
- Lopez-Luna, J., Canty, M.N., Al-Jubouri, Q., Al-Nuaimy, W. & Sneddon, L.U. (2017d). Behavioural responses of fish larvae modulated by analgesic drugs after a stress exposure. *Applied Animal Behaviour Science* **195**, 115–120.
- Love, O.P., McGowan, P.O. & Sheriff, M.L. (2013). Maternal adversity and ecological stressors in natural populations: the role of stress axis programming in individuals, with implications for populations and communities. *Functional Ecology* **27**, 81-92.

- Lowe, C.G., Holland, K.N. & Wolcott, T.G. (1998). A new acoustic tailbeat transmitter for fishes. *Fisheries Research* **36**, 275–283.
- Lowe, C.G. (1996). Kinematics and critical swimming speed of juvenile scalloped hammerhead sharks. *Journal of Experimental Biology* **199**, 2605–2610.
- Lucas, M.C. (1989). Effects of implanted dummy transmitters on mortality, growth and tissue reaction in rainbow trout, *Salmo gairdneri*. *Journal of Fish Biology* **35**, 577–587.
- Madliger, C.L., Love, O.P., Hultine, K.R. & Cooke, S.J. (2018). The conservation physiology toolbox: status and opportunities. *Conservation Physiology* **6**.
- Magalhaes, F.E.A., de Sousa, C.A.P.B., Santos, S.A.A.R., Menezes, R.B., Batista, F.L.A., Abreu, A.O., de Oliveira, M.V., Moura, L.F.W.G., Raposo, R.D. & Campos, A.R. (2017). Adult zebrafish (*Danio rerio*): An alternative behavioral model of formalin-induced nociception. *Zebrafish* **14**, 422–429.
- Makiguchi, Y. & Ueda, H. (2009). Effects of external and surgically implanted dummy radio transmitters on mortality, swimming performance and physiological status of juvenile masu salmon *Oncorhynchus masou*. *Journal of Fish Biology* **74**, 304–11.
- Manire, C.A. & Gruber, S.H. (1991). Effect of M-type dart tags on field growth of juvenile lemon sharks. *Transactions of the American Fisheries Society* **120**, 776–780.
- Malone, J.C., Forrester, G.E. & Steele, M.A. (1999). Effects of subcutaneous microtags on the growth, survival, and vulnerability to predation of small reef fishes. *Journal of Experimental Marine Biology and Ecology* **237**, 243–253.
- Mason, G. & Mendl, M. (1993). Why is there no simple way of measuring animal welfare? *Animal Welfare* **2**, 301–319.
- Matthews, G. & Wickelgren W.O. (1978). Trigeminal sensory neurons of the sea lamprey. *Journal of Comparative Physiology A* **123**, 329– 333.
- Matthews, J.L. (2004). Common diseases of laboratory zebrafish. *Methods in Cell Biology* **77**, 617–643.
- Matthews, M. & Varga, Z.M. (2012). Anesthesia and euthanasia in zebrafish. *ILAR Journal* **53**, 192–204.
- Maximino, C. (2011). Modulation of nociceptive-like behavior in zebrafish (*Danio rerio*) by environmental stressors. *Psychology and Neuroscience* **4**, 149 – 155
- McFarlane, G.A. & Beamish, R.I. (1990). Effect of an external tag on growth of sablefish (*Anoplopoma fimbria*), and consequences to mortality and age at maturity. *Canadian Journal of Fisheries and Aquatic Sciences* **47**, 1551–1557.
- McLean, D.L., Green, M., Harvey, E.S., Williams, A., Daley, R. & Graham, K.J. (2015). Comparison of baited longlines and baited underwater cameras for assessing the composition of continental slope deepwater fish assemblages off southeast Australia. *Deep Sea Research I* **98**, 10–20.
- McVicar, A.H. (1987). Black patch necrosis of the skin of *Solea solea* (L.): the role of sand in prophylaxis and treatment. *Journal of Fish Diseases* **10**, 59–63.
- Mench, J.A. & Mason, G.J. (1997). Behaviour. In *Animal Welfare* (Appleby, M.C. & Hughes, B.O., eds), pp. 127–142. New York, NY: CAB International.
- Merker, B.H. (2016). The line drawn on pain still holds. *Animal Sentience* **1**(46).
<http://animalstudiesrepository.org/animsent/vol1/iss3/46/>
- Metcalf, J.D. & Craig, J.F. (2011). Ethical justification for the use and treatment of fishes in research: an update. *Journal of Fish Biology* **78**, 393–394.
- Methling, C., Tudorache, C., Skov, P.V. & Steffensen, J.F. (2011). Pop up satellite tags impair swimming performance and energetics of the European eel (*Anguilla anguilla*). *PLoS One* **6**, e20797.

- Mettam, J.M., Oulton, L.J., McCrohan, C.R. & Sneddon, L.U. (2011). The efficacy of three types of analgesic drug in reducing pain in the rainbow trout, *Oncorhynchus mykiss*. *Applied Animal Behaviour Science* **133**, 265-274.
- Mettam J.J., McCrohan C.R. & Sneddon L.U. (2012). Characterisation of chemosensory trigeminal receptors in the rainbow trout (*Oncorhynchus mykiss*): responses to irritants and carbon dioxide. *Journal of Experimental Biology* **215**, 685-693.
- Millsopp, S. & Laming, P. (2008). Trade-offs between feeding and shock avoidance in goldfish (*Carassius auratus*). *Applied Animal Behaviour Science* **113**, 247-254.
- Minteer, B.A., Collins, J.P., Love, K.E. & Puschendorf, R. (2014). Avoiding (re)extinction. *Science* **344**, 260-261.
- Mohler, J.W. (2003). Producing fluorescent marks on Atlantic salmon fin rays and scales with calcein via osmotic induction. *North American Journal of Fisheries Management* **23**, 1108-1113.
- Mommsen, T.P., Vijayan, M.M. & Moon, T.W. (1999). Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Reviews in Fish Biology and Fisheries* **9**, 211-268.
- Montpetit, C.J. & Perry, S.F. (1998). The effects of chronic hypoxia on the acute adrenergic stress response in the rainbow trout (*Oncorhynchus mykiss*). *Physiological Zoology* **71**, 377-386.
- Moore, D.R.J., Breton, R.L. & MacDonald, D.B. (2003). A comparison of model performance for six quantitative structure-activity relationship packages that predict acute toxicity to fish. *Environmental Toxicology and Chemistry* **22**, 1799-1809.
- Nahhas, R. & Jones, N.V. (1980). The application of the freeze-branding technique to trout fry. *Aquaculture Research* **11**, 23-28.
- Näslund, J. & Johnson, J.I. (2014). Environmental enrichment for fish in captive environments: effects of physical structures and substrates. *Fish and Fisheries* **17**, 1-30.
- Näslund, J., Rosengren, M., Del Villar, D., Gansel, L., Norrgård, J.R., Persson, L., Winkowski, J.J. & Kvingedal, E. (2013). Hatchery tank enrichment affects cortisol levels and shelter-seeking in Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* **70**, 585-590.
- Navas, J.M. & Segner, H. (2006). Vitellogenin synthesis in primary cultures of fish liver cells as endpoint for in vitro screening of the (anti)oestrogenic activity of chemical substances. *Aquatic Toxicology* **80**, 1-22.
- Neely, B.C., Steffensen, K.D. & Pegg, M.A. (2009). A comparison of gastrically and surgically implanted telemetry transmitters in shovelnose sturgeon. *Fisheries Management and Ecology* **16**, 323-328.
- Neiffer, D.L. & Stamper, M.A. (2009). Fish sedation, anesthesia, analgesia, and euthanasia: considerations, methods, and types of drugs. *ILAR Journal* **50**, 343-360.
- Newby, N.C., Mendonca, P.C., Gamperl, K. & Stevens, E.D. (2006). Pharmacokinetics of morphine in fish: Winter flounder (*Pseudopleuronectes americanus*) and seawater-acclimated rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology C* **143**, 275-283.
- Newby, N. C., Wilkie, M. P. & Stevens, E. D. (2009). Morphine uptake, disposition, and analgesic efficacy in the common goldfish (*Carassius auratus*). *Canadian Journal of Zoology* **87**, 388-399.
- Nordgreen, J., Horsberg, T.E., Ranheim, B. & Chen, A.C.N. (2007). Somatosensory evoked potentials in the telencephalon of Atlantic salmon (*Salmo salar*) following galvanic stimulation of the tail. *Journal of Comparative Physiology A*, **193**, 1235-1242.
- Nordgreen, J., Garner, J.P., Janczak, A.M., Ranheim, B., Muir, W.M. & Horsberg, T.E. (2009). Thermoception in fish: Effects of two different doses of morphine on

- thermal threshold and post-test behaviour in goldfish (*Carassius auratus*). *Applied Animal Behaviour Science* **119**, 101-107.
- OATA (2015). <https://ornamentalfish.org/wp-content/uploads/2015/10/CODE-OF-CONDUCT-FINAL-OCT-2015.pdf>
- O’Brine, T.M., Vrětelová, J., Snellgrove, D.L., Davies, S.J. & Sloman, K.A. (2015). Growth, oxygen consumption, and behavioral responses of *Danio rerio* to variation in dietary protein and lipid levels. *Zebrafish* **12**, 296-304.
- O’Connell, C.P. & He, P. (2014). A large scale field analysis examining the effect of magnetically-treated baits and barriers on teleost and elasmobranch behavior. *Ocean and Coastal Management* **96**, 130–137.
- O’Shea, O.R., Mandelman, J., Talwar, B. & Brooks, E.J. (2015). Novel observations of an opportunistic predation event by four apex predatory sharks. *Marine and Freshwater Behaviour and Physiology* **48**, 374–380.
- Oliva-Teles, A. (2012). Nutrition and health of aquaculture fish. *Journal of Fish Diseases* **35**, 83-108.
- Ottesen, O.H., Noga, E.J. & Sandaa, W. (2007). Effect of substrate on progression and healing of skin erosions and epidermal papillomas of Atlantic halibut, *Hippoglossus hippoglossus* (L.). *Journal of Fish Diseases* **30**, 43-53.
- Pankhurst, N.W. (2011). The endocrinology of stress in fish: an environmental perspective. *General Comparative Endocrinology* **170**, 265-275.
- Pannevis, M.C. & Earle, K.E. (1994). Nutrition of ornamental fish: Water soluble vitamin leaching and growth of *Paracheirodon innesi*. *Journal of Nutrition* **124**, 2633S-2635S.
- Paquin, P.R., Gorsuch, J.W., Apte, S., Batley, G.E., Bowles, K.C., Campbell, P.G.C., Delos, C.G., Di Toro, D.M., Dwyer, R.L., Galvez, F., Gensemer, R.W., Goss, G.G., Hogstrand, C., Janssen, C.R., McGeer, J.C., Naddy, R.B., Playle, R.C., Santore, R.C., Schneider, U., Stubblefield, W.A., Wood, C.M. & Wu, K.B. (2002). The biotic ligand model: a historical overview. *Comparative Biochemistry and Physiology* **133C**, 3-35.
- Pham, T. M., Hagman, B., Codita, A., Van Loo, P. L. P., Strommer, L. & Baumans, V. (2010). Housing environment influences the need for pain relief during postoperative recovery in mice. *Physiology & Behavior* **99**, 663-668.
- Pickering, A.D., Pottinger, T.G. & Christie, P. (1982). Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: a time-course study. *Journal of Fish Biology* **20**, 229-244.
- Portz, D.E., Woodley, C.M. & Cech Jr, J.J. (2006). Stress associated impacts of short-term holding on fishes. *Reviews in Fish Biology and Fisheries* **16**, 125-170.
- Pounder, K.C., Mitchell, J.L., Thomson, J., Pottinger, T., Buckley, J. & Sneddon, L.U. (2016). Does environmental enrichment promote recovery from stress in rainbow trout? *Applied Animal Behaviour Science* **176**, 136-142.
- Raby, G.D., Packer, J.R., Danylchuk, A.J. & Cooke, S.J. (2014). The understudied and underappreciated role of predation in the mortality of fish released from fishing gears. *Fish and Fisheries* **15**, 489–505.
- Ramstad, K.M. & Woody, C.A. (2003). Radio tag retention and tag-related mortality among adult sockeye salmon. *North American Journal of Fisheries Management* **23**, 978–982.
- Rang, H.P., Dale, M.M., Ritter, J.M. & Moore, P.K. (2003). *Pharmacology*. 797 pp. Livingstone, London: Churchill.
- Readman, G.D., Owen, S.F., Murrell, J.C. & Knowles, T.G. (2013). Do fish perceive anaesthetics as adverse? *PLoS ONE* **8**, e73773.
- Readman, G.D., Owen, S.F., Knowles, T.G. & Murrell, J.C. (2017). Species specific anaesthetics for fish anaesthesia and euthanasia. *Nature Scientific Reports* **7**, 7102.

1709 Reeves, K.S. & Buckmeier, D.L. (2011). Mortality, predation and tag visibility of fish marked
1710 with visible implant elastomer tags. *North American Journal of Fisheries Management*
1711 **29**, 323-328.

1712 Reilly, S.C., Quinn, J.P., Cossins, A.R. & Sneddon, L.U. (2008a). Novel candidate genes
1713 identified in the brain during nociception in common carp (*Cyprinus carpio*) and
1714 rainbow trout (*Oncorhynchus mykiss*). *Neuroscience Letters* **437**, 135–138.

1715 Reilly, S.C., Quinn, J.P., Cossins, A.R. & Sneddon, L.U. (2008b). Behavioural analysis of a
1716 nociceptive event in fish: comparisons between three species demonstrate specific
1717 responses. *Applied Animal Behaviour Science* **114**, 248-259.

1718 Reinhardt, J.F., Weaver, J., Latham, P.J., Dell’Apa, A., Serafy, J.E., Browder, J.A.,
1719 Christman, M., Foster, D.G. & Blankinship, D.R. (2018). Catch rate and at-vessel
1720 mortality of circle hooks versus J-hooks in pelagic longline fisheries: A global meta-
1721 analysis. *Fish and Fisheries* **19**, 413-430.

1722 Ribas, L., Valdivieso, A., Díaz, N. & Piferrer, F. (2017). Appropriate rearing density in
1723 domesticated zebrafish to avoid masculinization: links with the stress response. *Journal*
1724 *of Experimental Biology* **220**, 1056-1064.

1725 Rink, E. & Wullimann, M.F. (2004). Connections of the ventral telencephalon (subpallium)
1726 in the zebrafish (*Danio rerio*). *Brain Research* **1011**, 206-220.

1727 Rizzo, A.L., Wooster, G.A., Guanzini, L.E., Peterson, C.M., Fenderson, K.S., Erb, H.N.,
1728 Bowser, P.R. & Martin, M.E. (2017). Biochemical, histopathologic, physiologic, and
1729 behavioral effects of nonsteroidal anti-inflammatory drugs in rainbow trout
1730 (*Oncorhynchus mykiss*). *Comparative Medicine* **67**, 106-111.

1731 Roques, J. A. C., Abbink, W., Geurds, F., van de Vis, H. & Flik, G. (2010). Tailfin clipping, a
1732 painful procedure: studies on Nile tilapia and common carp. *Physiology and Behavior*
1733 **101**, 533-540.

1734 Roques, J.A.C., Abbink, W., Chereau, G., Fourneyron, A., Spanings, T., Burggraaf, D., van
1735 de Bos, R., van de Vis, H. & Flik, G. (2012). Physiological and behavioral responses to
1736 an electrical stimulus in Mozambique tilapia (*Oreochromis mossambicus*). *Fish*
1737 *Physiology and Biochemistry* **38**, 1019–1028.

1738 Rose, J.D. (2002). The neurobehavioral nature of fishes and the question of awareness and
1739 pain. *Reviews in Fisheries Science* **10**, 1-38.

1740 Rose, J.D., Arlinghaus, R., Cooke, S.J., Diggles, B.K., Sawynok, W., Stevens, E.D. &
1741 Wynne, C. D. L. (2014). Can fish really feel pain? *Fish and Fisheries* **15**, 97–133.

1742 Ross, L.J. & Ross, B. (2008). *Anaesthetic and Sedative Techniques for Aquatic Animals*.
1743 Oxford: Blackwell Publishing.

1744 Ross, M.J. & McCormick, J.H. (1981). Effects of external radio transmitters on fish. *Progress*
1745 *Fish-Culturist* **43**, 67–72.

1746 Russell, W.M.S. & Burch, R.L. (1959). *The Principles of Humane Experimental Technique*.
1747 London: Methuen.

1748 Sampaio, F.D.F. & Freire, C.A. (2016). An overview of stress physiology of fish transport:
1749 changes in water quality as a function of transport duration. *Fish and Fisheries* **17**,
1750 1055-1072.

1751 Santana-Garcon, J., Braccini, M., Langlois, T.J., Newman, S.J., Mcauley, R.B. & Harvey,
1752 E.S. (2014). Calibration of pelagic stereo-BRUVs and scientific longline surveys for
1753 sampling sharks. *Methods in Ecology and Evolution* **5**, 824–833.

1754 Santos, D., Luzio, A. & Coimbra, A.M. (2017). Zebrafish sex differentiation and gonad
1755 development: a review on the impact of environmental factors. *Aquatic Toxicology* **191**,
1756 141-163.

1757 Schaeck, M., Van den Broeck, W., Hermans, K. & Decostere, A. (2013). Fish as research
1758 tools: alternatives to in vivo experiments. *ATLA* **41**, 219-229.

- Schreck, C.B. (2000). Accumulation and long-term effects of stress in fish. In *The Biology of Animal Stress: Basic Principles and Applications for Animal Welfare*. (Moberg, G.P. & Mench, J.A. Eds), pp. 147-158. CABI Publishing: Wallingford, U.K.
- Schreck, C.B. & Tort, L.T. (2016). The concept of stress in fish. In *Biology of Stress in Fish*, Fish Physiology Series, Volume 35 (Schreck, C.B., Tort, L.T., Farrell, A. & Brauner, C., eds), pp. 1-34. New York, NY: Academic Press.
- Schreck, C.B., Tort, L.T., Farrell, A. & Brauner, C. (2016). *Biology of Stress in Fish*, Fish Physiology Series, Volume 35. New York, NY: Academic Press.
- Schroeder, P. & Sneddon, L.U. (2017). Exploring the efficacy of immersion analgesics in zebrafish using an integrative approach. *Applied Animal Behaviour Science* **187**, 93-102.
- Séguret, A., Collignon, B. & Halloy, J. (2016). Strain differences in the collective behaviour of zebrafish (*Danio rerio*) in heterogeneous environments. *Royal Society Open Science* **3**, 160451.
- Sherman, C.S., Chin, A., Heupel, M.R. & Simpfendorfer, C.A. (2018). Are we underestimating elasmobranch abundances on baited remote underwater video systems (BRUVS) using traditional metrics? *Journal of Experimental Marine Biology and Ecology* **503**, 80-85.
- Singh, R.K., Vartak, V.R., Balange, A.K. & Ghughuskar, M.M. (2004). Water quality management during transportation of fry of Indian major carps, *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton). *Aquaculture* **235**, 297-302.
- Skomal, G.B., Chase, B.C. & Prince, E.D. (2002). A comparison of circle hook and straight hook performance in recreational fisheries for juvenile Atlantic bluefin tuna. *American Fisheries Society Symposium* **30**, 57-65.
- Sloman, K.A. & Armstrong, J.D. (2002). Physiological effects of dominance hierarchies: laboratory artefacts or natural phenomena? *Journal of Fish Biology* **61**, 1-23.
- Sloman, K.A. & Tamilselvan, P. (2017). Developmental social experience of parents affects behaviour of offspring in zebrafish. *Animal Behaviour* **133**, 153-160.
- Sloman, K.A., McDonald, M.D., Barimo, J.F., Lepage, O., Winberg, S., Wood, C.M. & Walsh, P.J. (2005). Does pulsatile urea excretion serve as a social signal in the Gulf toadfish *Opsanus beta*? *Physiological and Biochemical Zoology* **78**, 724-735.
- Sloman, K.A., Baldwin, L., McMahon, S. & Snellgrove, D. (2011). The effects of mixed-species assemblages on the behaviour and welfare of fish held in home aquaria. *Applied Animal Behaviour Science* **135**, 160-168.
- Small, B.C. (2003). Anaesthetic efficacy of metomidate and comparison of plasma cortisol responses to tricaine methane-sulfonate, quinaldine and clove oil anesthetised channel catfish *Ictalurus punctatus*. *Aquaculture* **218**, 177-185.
- Smith, A. & Gray, H. (2011). Goldfish in a tank: the effect of substrate on foraging behaviour in aquarium fish. *Animal Welfare* **20**, 311-319.
- Smith, A.J., Clutton, R.E., Lilley, E., Hansen, K.E.A. & Brattelid, T. (2018). PREPARE: guidelines for planning animal research and testing. *Laboratory Animals* **52**, 135-141.
- Smith, M.D., Asche, F., Guttormsen, A.G. & Wiener, J.B. (2010). Genetically modified salmon and full impact assessment. *Science* **330**, 1052-1053.
- Smukall, M.J., Kessel, S.T., Franks, B.R., Feldheim, K.A., Guttridge, T.L. & Gruber, S.H. (2018). No apparent negative tagging effects after 13 years at liberty for lemon shark, *Negaprion brevirostris* implanted with acoustic transmitter. *Journal of Fish Biology* in press.
- Sneddon, L.U. (2002). Anatomical and electrophysiological analysis of the trigeminal nerve in a teleost fish, *Oncorhynchus mykiss*. *Neuroscience Letters* **319**, 167-171.

1808 Sneddon, L.U. (2003a). The evidence for pain in fish: the use of morphine as an analgesic.
1809 *Applied Animal Behaviour Science* **83**, 153-162.

1810 Sneddon, L.U. (2003b). Trigeminal somatosensory innervation of the head of a teleost fish
1811 with particular reference to nociception. *Brain Research* **972**, 44-52.

1812 Sneddon, L.U. (2004). Evolution of nociception in vertebrates: comparative analysis of lower
1813 vertebrates. *Brain Research Reviews* **46**, 123-130.

1814 Sneddon, L.U. (2009). Pain perception in fish indicators and endpoints. *ILAR Journal* **50**,
1815 338-342.

1816 Sneddon, L.U. (2011). Pain perception in fish: Evidence and implications for the use of fish.
1817 *Journal of Consciousness Studies* **18**, 209-229.

1818 Sneddon, L.U. (2012). Clinical anaesthesia and analgesia in fish. *Journal of Exotic Pet*
1819 *Medicine* **21**, 32-43.

1820 Sneddon L.U. (2015). Pain in aquatic animals. *Journal of Experimental Biology* **218**, 967-
1821 976.

1822 Sneddon, L.U., Braithwaite, V.A. & Gentle, M.J. (2003a). Do fishes have nociceptors?
1823 Evidence for the evolution of a vertebrate sensory system. *Proceedings of the Royal*
1824 *Society of London Series B-Biological Sciences* **270**, 1115-1121.

1825 Sneddon, L.U., Braithwaite, V.A. & Gentle, M.J. (2003b). Novel object test: Examining
1826 nociception and fear in the rainbow trout. *Journal of Pain* **4**, 431-440.

1827 Sneddon, L.U. Elwood, R.W, Adamo, S. & Leach, M.C. (2014). Defining and assessing pain
1828 in animals. *Animal Behaviour* **97**, 201-212.

1829 Sneddon, L.U., Wolfenden, D.C.C. & Thomson, J.S. (2016). Stress management and welfare.
1830 In *Biology of Stress in Fish*, Fish Physiology Series, Volume 35 (Schreck, C.B., Tort,
1831 L., Farrell, A.P. & Brauner, C.J. eds), pp. 463-539. New York, NY: Academic Press.

1832 Sneddon, L.U., Halsey, L.G. & Bury, N.R. (2017). Considering aspects of the 3Rs principles
1833 within experimental animal biology. *Journal of Experimental Biology* **220**, 3007-3016.

1834 Sneddon, L.U. (2018). Comparative physiology of nociception and pain. *Physiology* **33**, 63-
1835 73.

1836 Snow, P.J., Renshaw, G.M.C. & Hamlin, K.E. (1996). Localization of enkephalin
1837 immunoreactivity in the spinal cord of the long-tailed ray *Himantura fai*. *Journal of*
1838 *Comparative Neurology* **367**, 264-273.

1839 Speed, C.W., O'Shea, O.R. & Meekan, M.G. (2013). Transmitter attachment and release
1840 methods for short-term shark and stingray tracking on coral reefs. *Marine Biology* **160**,
1841 1041-1050.

1842 Steinhausen, M.F., Andersen, N.G. & Steffensen, J.F. (2006). The effect of external dummy
1843 transmitters on oxygen consumption and performance of swimming Atlantic cod.
1844 *Journal of Fish Biology* **69**, 951-956.

1845 Stevens, C.H., Croft, D.P., Paull, G.C. & Tyler, C.R. (2017). Stress and welfare in
1846 ornamental fishes: what can be learned from aquaculture? *Journal of Fish Biology* **91**,
1847 409-428.

1848 Stoot, L.J., Cairns, N., Cull, F., Taylor, J.J., Jeffrey, J.D., Morin, F., Mandelman, J.W., Clark,
1849 T.D. & Cooke, S.J. (2014). Use of portable blood physiology point-of-care devices for
1850 basic and applied research on vertebrates: a review. *Conservation Physiology* **2**,
1851 cou011-cou011.

1852 Strand, D.A., Utne-Palm, A.C., Jakobsen, P.J., Braithwaite, V.A., Jensen, K.H. & Salvanes,
1853 A.G.V. (2010). Enrichment promotes learning in fish. *Marine Ecology Progress Series*
1854 **412**, 273-282.

1855 Stuart, K., Losordo, M., Olin, P. & Drawbridge, M. (2013). Effects of stocking density and
1856 water conditioners on yolk-sac larvae of two marine finfish during simulated air
1857 transport. *Aquaculture Research* **46**, 2124-2132.

- Sullivan, M., Lawrence, C. & Blache, D. (2015). Why did the fish cross the tank? Objectively measuring the value of enrichment for captive fish. *Applied Animal Behaviour Science* **174**, 181-188.
- Sundell, K. & Björnsson, B.T. (1988). Kinetics of calcium fluxes across the intestinal mucosa of the marine teleost, *Gadus morhua*, measured using an *in vitro* perfusion method. *Journal of Experimental Biology* **140**, 171-186.
- Sundström, L.F. & Devlin, R.H. (2016). Ecological implications of genetically modified fishes in freshwater fisheries, with a focus on salmonids. In *Freshwater Fisheries Ecology* (Craig, J. F. ed.), pp. 594-615. Wiley.
- Suski, C.D., Cooke, S.J., Danylchuk, A.J., O'Connor, C.M., Gravel, M.-A., Redpath, T., Hanson, K.C., Gingerich, A.J., Murchie, K.J., Danylchuk, S.E., Koppelman, J.B. & Goldberg, T.L. (2007). Physiological disturbance and recovery dynamics of bonefish (*Albula vulpes*), a tropical marine fish, in response to variable exercise and exposure to air. *Comparative Biochemistry and Physiology A* **148**, 664-73.
- Sykes, P.J., Stryhn, H., McClure, C., Brooking, C.L., Hammell, K.L. (2012). Evaluation of external operculum loop tags to individually identify cage-cultured Atlantic halibut *Hippoglossus hippoglossus* in commercial research trials. *Journal of Fish Biology* **80**, 2267-2280.
- Talwar, B., Brooks, E., Mandelman, J. & Grubbs, R. (2017). Stress, post-release mortality, and recovery of commonly discarded deep-sea sharks caught on longlines. *Marine Ecology Progress Series* **582**, 147-161.
- Taslina, K., Davie, A., McAndrew, B.J. & Penman, D.J. (2016). DNA sampling from mucus in the Nile tilapia, *Oreochromis niloticus*: minimally invasive sampling for aquaculture-related genetics research. *Aquaculture Research* **47**, 4032-4037.
- Taylor, J. C., Dewberry, L. S., Totsch, S. K., Yessick, L. R., DeBerry, J. J., Watts, S. A. & Sorge, R. E. (2017). A novel zebrafish-based model of nociception. *Physiology & Behavior* **174**, 83-88.
- Terashima, S.I. & Liang, Y.F. (1994). C mechanical nociceptive neurons in the crotaline trigeminal ganglia. *Neuroscience Letters* **179**, 33-36.
- Thomann, R.V., Shkreli, F. & Harrison, S. (1997). A pharmacokinetic model of cadmium in rainbow trout. *Environmental Toxicology and Chemistry* **16**, 2268-2274.
- Thorstad, E., Økland, F. & Heggberget, T. G. (2001). Are long term negative effects from external tags underestimated? Fouling of an externally attached telemetry transmitter. *Journal of Fish Biology* **59**, 1092-1094.
- Tolentino, E.R., Howey, R.P., Howey, L.A., Jordan, L.K.B., Grubbs, R.D., Brooks, A., Williams, S., Brooks, E.J. & Shipley, O.N. (2017). Was my science project eaten? A novel approach to validate consumption of marine biologging instruments. *Animal Biotelemetry* **5**, 3.
- Tompkins, D.M., Carver, S., Jones, M.E., Krkošek, M. & Skerratt, L.F. (2015). Emerging infectious diseases of wildlife: a critical perspective. *Trends in Parasitology* **31**, 149-159.
- Tuckey L.M. & Smith, T.I.J. (2001). Effects of photoperiod and substrate on larval development and substrate preference of juvenile southern flounder, *Paralichthys lethostigma*. *Journal of Applied Aquaculture* **11**, 1-20.
- Turnbull, J.F., Bell, A., Adams, C.E., Bron, J. & Huntingford, F.A. (2005). Stocking density and welfare of cage farmed Atlantic salmon: application of a multivariate analysis. *Aquaculture* **243**, 121-132.
- UK Farm Animal Welfare Council (2005). The Five Freedoms. Available at: <http://webarchive.nationalarchives.gov.uk/20121010012427/http://www.fawc.org.uk/freedoms.htm>

1908 van den Bos, R., Mes, W., Galligani, P., Heil, A., Zethof, A., Flik, G. & Gorissen, M. (2017).
1909 Further characterisation of differences between TL and AB zebrafish (*Danio rerio*):
1910 Gene expression, physiology and behaviour at day 5 of the larval stage. *PLoS One* **12**,
1911 e0175420.

1912 Vanderzwalmen, M., Eaton, L., Mullen, C., Henriquez, F., Carey, P., Snellgrove, D. &
1913 Sloman, K.A. (2018). The use of feed and water additives for live fish transport.
1914 *Aquaculture Reviews* <https://doi.org/10.1111/raq.12239>

1915 von Krogh, K., Sørensen, C., Nilsson, G.E. & Øverli, Ø. (2010). Forebrain cell proliferation,
1916 behaviour, and physiology of zebrafish, *Danio rerio*, kept in enriched or barren
1917 environments. *Physiology & Behavior* **101**, 32-39.

1918 Wagner, G.N., Cooke, S.J., Brown, R.S. & Deters, K.A. (2011). Surgical implantation
1919 techniques for electronic tags in fish. *Reviews in Fish Biology and Fisheries* **21**, 71-81.

1920 Walster, C. (2008) The welfare of ornamental fish. In *Fish Welfare* (Branson, E.J., ed.), pp.
1921 269-290. Oxford: Blackwell Publishing.

1922 Wargo Rub, A.M., Jepsen, N., Liedtke, T.L., Moser, M.L. & Weber, E.P.S. (2014). Surgical
1923 tagging and telemetry methods in fisheries research: promoting veterinary and research
1924 collaborations. *American Journal of Veterinary Research* **75**, 402-416.

1925 Watanabe, T. (1982). Lipid nutrition in fish. *Comparative Biochemistry and Physiology B* **73**,
1926 3-15.

1927 Watson, J.W. & Kerstetter, D.W. (2006). Pelagic longline fishing gear: A brief history and
1928 review of research efforts to improve selectivity. *Marine Technology Society Journal*
1929 **40**, 6-11.

1930 Watts, S.A., Lawrence, C., Powell, M. & D'Abramo, L.R. (2016). The vital relationship
1931 between nutrition and health in zebrafish. *Zebrafish* **13**, S72-S76.

1932 Webster, M.M. & Laland, K.N. (2009). Evaluation of a non-invasive tagging system for
1933 laboratory studies using three-spined sticklebacks *Gasterosteus aculeatus*. *Journal of*
1934 *Fish Biology* **75**, 1868-1873.

1935 Wells, R.M.G., McNeil, H. & MacDonald, J.A. (2005). Fish hypnosis: Induction of an atonic
1936 immobility reflex. *Marine and Freshwater Behaviour and Physiology* **38**, 71-78.

1937 Wendelaar Bonga, S.E. (1997). The stress response in fish. *Physiological Reviews* **77**, 591-
1938 625.

1939 White, G.E. & Brown, C. (2013). Site fidelity and homing behaviour in intertidal fishes.
1940 *Marine Biology* **160**, 1365-1372.

1941 White, L.J., Thomson, J.S., Pounder, K.C., Coleman R.C. & Sneddon, L.U. (2017). The
1942 impact of social context on behaviour and the recovery from welfare challenges in
1943 zebrafish, *Danio rerio*. *Animal Behaviour* **132**, 189-199.

1944 Whitmarsh, S.K., Fairweather, P.G. & Huveneers, C. (2017). What is Big BRUVver up to?
1945 Methods and uses of baited underwater video. *Reviews in Fish Biology and Fisheries*
1946 **27**, 53-73.

1947 Whitmore, B.M., White, C.F., Gleiss, A.C. & Whitney, N.M. (2016). A float-release package
1948 for recovering data-loggers from wild sharks. *Journal of Experimental Marine Biology*
1949 *and Ecology* **475**, 49-53.

1950 Wilkes, L., Owen, S.F., Readman, G.D., Sloman, K.A. & Wilson, R.W. (2012). Does
1951 structural enrichment for toxicology studies improve zebrafish welfare? *Applied Animal*
1952 *Behaviour Science* **139**, 143-150.

1953 Williams, T.D., Readman, G.D. & Owen, S.F. (2009). Key issues concerning environmental
1954 enrichment for laboratory-held fish species. *Laboratory Animals* **43**, 107-120.

1955 Wilson, S.M., Raby, G.D., Burnett, N.J., Hinch, S.G. & Cooke, S.J. (2014). Looking beyond
1956 the mortality of bycatch: sublethal effects of incidental capture on marine animals.
1957 *Biological Conservation* **171**, 61-72.

1958 Wilson, R.P., Holton, M., Wilson, V.L., Gunner, R., Tysse, B., Wilson, G.I., Quintana, F.,
1959 Duarte, C. & Scantlebury, D.M. (2018). Towards informed metrics for examining the
1960 role of human-induced animal responses in tag studies on wild animals. *Integrated*
1961 *Zoology* **10**, 267-281.

1962 Wolf, K. & Quimby, M.C. (1962). Established eurythermic line of fish cells *in vitro*. *Science*
1963 **135**, 1065-1066.

1964 Wong, D., von Keyserlingk, M.A.G., Richards, J.G. & Weary, D.M. (2014). Conditioned
1965 place avoidance of zebrafish (*Danio rerio*) to three chemicals used for euthanasia and
1966 anaesthesia. *PLoS ONE* **9**, e88030.

1967 Wood, C.M. (1991). Acid-base and ion balance, metabolism, and their interactions, after
1968 exhaustive exercise in fish. *Journal of Experimental Biology* **160**, 285–308.

1969 Wood, C.M., Turner, J.D. & Graham, M.S. (1983). Why do fish die after severe exercise?
1970 *Journal of Fish Biology* **22**, 189–201.

1971 Wood, C.M., Kelly, S.P., Zhou, B., Fletcher, M., O'Donnell, M., Elettib, B. & Pärt, P.
1972 (2002). Cultured gill epithelia as models for the freshwater fish gill. *Biochimica et*
1973 *Biophysica Acta* **1566**, 72-83.

1974 Wright, P.A. & Wood, C.M. (2012). Seven things fish know about ammonia and we don't.
1975 *Respiratory Physiology and Neurobiology* **184**, 231-240.

1976 Yang, S.D., Liou, C.H. & Liu, F.G. (2002). Effects of dietary protein level on growth
1977 performance, carcass composition and ammonia excretion in juvenile silver perch
1978 (*Bidyanus bidyanus*). *Aquaculture* **213**, 363-372.

1979 Yoshida, M. & Hirano, R. (2010). Effects of local anesthesia of the cerebellum on classical
1980 fear conditioning in goldfish. *Behavioral Brain Function* **6**, 20.

1981 Young R.F. (1977). Fiber spectrum of the trigeminal sensory root of frog, cat and man
1982 determined by electron microscopy. In: *Pain in the Trigeminal Region* (Anderson, D.L.
1983 & Matthews, D., eds), 137–160 pp. Amsterdam: Elsevier.

1984

1985 **Table 1.** The three classes of analgesic drugs tested in fishes, documenting the range of doses investigated, the species employed in the study,
1986 side effects including whether the analgesic prevented changes related to noxious stimuli and a comment on analgesic efficacy (based on data
1987 from Curtwright *et al.*, 2015; Davis *et al.*, 2006; Harms *et al.*, 2005; Lopez-Luna *et al.*, 2017a; Mettam *et al.*, 2011; Newby *et al.*, 2006; 2009;
1988 Nordgreen *et al.*, 2009; Rizzo *et al.*, 2017; Schroeder & Sneddon, 2017; Sneddon, 2003b; Taylor *et al.*, 2017). Where not effective is stated these
1989 drugs did not prevent the changes in behaviour associated with treatment with a noxious stimulus. The drugs were administered by injection at
1990 the site of damage, intramuscularly (i.m.), intraperitoneally (i.p.) or *via* immersion in tank water.

Analgesic	Dose	Species	Side effects	Efficacy
<i>Local Anaesthetics</i>				
Lidocaine	0.1-2 mg	Trout (at site)	None observed	Injection at 1 mg
	1-5 mg l ⁻¹	Zebrafish (immersion)	None observed	Immersion at 2.5-5 mg l ⁻¹
<i>Opioids</i>				
Morphine	2.5-50 mg kg ⁻¹	Trout (i.m.)	None observed	i.m. at 5 mg kg ⁻¹
		Flounder (i.p.)		
		Goldfish (i.m.)		
		Koi carp (i.m.)		

	20 µl of 0.2 mg ml ⁻¹	Zebrafish (i.p)	None observed	Effective
	1 & 48 mg l ⁻¹	Zebrafish (immersion)	None Observed	Immersion at 48 mg l ⁻¹
Buprenorphine	0.01-0.3 mg kg ⁻¹	Trout (i.m.)	Reduced activity No impact on feeding Depresses heart and ventilation rate	Not effective
		Zebrafish (immersion)	None observed	Immersion at 0.3 mg ml ⁻¹
Butorphanol	0.25-10 mg kg ⁻¹	Koi carp (i.m.)	Koi acute decrease in feeding	Not effective
		Dogfish (i.m.)		Koi – improved behaviour
NSAIDs				

Carprofen	1-5 mg kg ⁻¹	Trout (i.m.)	Depressed activity Increased ventilation	Reduced suspension of feeding using 2.5 mg kg ⁻¹
Ketoprofen	1-4 mg kg ⁻¹	Koi carp (i.m.) Dogfish (i.m.) Trout (i.m.)	No impact on behaviour in Koi and trout Healing and physiology unaffected in trout	Not effective
Aspirin	1-2.5 mg l ⁻¹	Zebrafish (Immersion)	None observed	Immersion at 2.5 mg l ⁻¹
Ketorolac	0.5 mg kg ⁻¹	Trout (i.m.)	No impact on behaviour, physiology or healing	Not effective
Flunixin	0.5 mg kg ⁻¹	Trout (i.m.)	No impact on behaviour, physiology or healing	Not effective
Indomethacin	20 µl of 0.2 mg ml ⁻¹	Zebrafish (i.p.)	None observed	Effective

Scientific Procedure	Ethical considerations
Fin Clipping	<p>Fin clipping is a common technique used to acquire a DNA sample, where a small portion of the fin is removed, usually the caudal fin. Fin clipping is carried out in a variety of species, including small species such as zebrafish and three-spined stickleback <i>Gasterosteus aculeatus</i> L. 1758 where the ‘clip’ may represent quite a large proportion of the fin. Fin clipping is usually carried out under anaesthesia, but can potentially cause pain and the use of an additional analgesic has been recommended (Schroeder & Sneddon, 2017). The importance of the caudal fin for fish locomotion is well documented (Lauder, 2000) and caudal amputation has significant consequences for swimming efficiency (Fu <i>et al.</i>, 2013). While there is evidence in some species that fin clipping does not affect behaviour (e.g. Champagne <i>et al.</i>, 2008), it has been suggested that fin clipping can compromise scientific results through secondary infections and changes in behaviours involving fins (Breacker <i>et al.</i>, 2017). A technique of skin swabbing to obtain a DNA sample has been used in larger fishes (Campanella & Smalley, 2006; Taslima <i>et al.</i>, 2016) and recently validated as a non-invasive alternative to obtain DNA in zebrafish and stickleback (Breacker <i>et al.</i>, 2017) with significant ethical implications. The removal of the adipose fin in salmonids is a common procedure used particularly for distinguishing between hatchery-reared and wild fishes. The adipose fin lacks musculature and skeletal structures; however, its assumed vestigial role has recently been challenged with evidence of extensive nervous tissue and mechanosensory function (Buckland-Nicks <i>et al.</i> 2012; Aiello <i>et al.</i> 2016; Buckland-Nicks, 2016).</p>

Surgical Procedures	<p>Lengthy and invasive surgeries almost always require anaesthesia, while the use of anaesthesia for more minor surgeries is debated (Cooke <i>et al.</i>, 2016a). Major surgery usually refers to the penetration of the body cavity, or surgery that will cause significant physiological or physical disturbance to the fish (AFS, 2014). In particular, surgery methods for the implantation of electronic tags have been reviewed (Wagner <i>et al.</i>, 2011) with surgeon training and experience playing an important role in influencing surgical success (Deters <i>et al.</i>, 2010). Post-operative care is also a crucial part of the surgical procedure (Cooke <i>et al.</i>, 2016a). Individuals should be carefully monitored to ensure there are no unintended effects of surgery, which would lead to the need for euthanasia.</p>
Toxicology and Infection Studies	<p>There are obvious ethical considerations with studies where the intent is to expose fishes to potentially deleterious compounds or organisms. Such studies may include lethal endpoints, however, in some countries legislation requires full justification to explain why lethal endpoints are necessary and clear evidence that the objectives cannot be achieved by any other means. Nevertheless, the fish acute median lethality test (LC50) is still widely used for testing the toxicity of novel compounds (Schaeck <i>et al.</i>, 2013). The application of deleterious conditions that may result in suffering or mortality need to be monitored frequently (ASAB, 2012, 2018). Within toxicity testing, there has been a clear move away from the use of adult fishes towards the use of embryos as a form of partial replacement (Schaeck <i>et al.</i>, 2013), however, some caution is required in relation to ethical interpretation with emerging evidence that larval fishes respond to noxious stimuli (Lopez-Luna <i>et al.</i>, 2017a).</p>

Blood sampling	Non-terminal blood samples are usually taken from the caudal vein running beneath the vertebrae (Bennett <i>et al.</i> , 2016) using a needle diameter and syringe size suitable for the size of fish. Needles should be sharp to ensure clean entrance to the vein and anti-coagulants are often used to stop clotting in the collection vessel. For small fishes, non-terminal blood sampling is not possible; caudal severance to allow collection of blood by capillary action can be combined with euthanasia. Other methods of blood sampling such as cardiac puncture are more invasive and can affect survival.
Intra or Interspecific Aggression	Competition studies involving the formation of dominance hierarchies or escalated fighting may elicit physiological consequences (Sloman & Armstrong, 2002). Injury can be minimised by the provision of shelters and continuous monitoring so that opponents can be removed as soon as contests are complete. Mirror image tests have been used as an alternative to dyadic encounters (Sloman & Tamilselvan, 2017) and while there are circumstances when they may not provide all the information required for the experimental objectives there are many instances where they are an acceptable alternative (Balzarini <i>et al.</i> , 2014; Elwood <i>et al.</i> , 2014). The main advantage of the mirror image test is that it removes the potential for physical injury.
Predation Studies (where fishes are prey)	In their natural environment, fishes usually eat other fishes and of course they will eat them alive. However, when feeding live fishes to live fishes in a captive environment, we need to consider the welfare of both the predator and the prey (Metcalf & Craig, 2011). Often the predator is the focus of the study and so welfare is adequately considered but the welfare of the prey fish is ignored. In the wild a prey fish is likely to have a much greater chance of

escape, compared to predation in a bare tank without refuge. In a barren environment where prey is confined in close contact with the predator, the environmental relevance of the situation must be considered and consequences for results (Lind & Cresswell, 2005). For example, a toxicant may be found have an impact on a fish's ability to catch another fish in a tank environment, but such an experiment tells us next to nothing about the ability of an exposed fish to catch another fish in a natural situation. Close confinement with a predator inevitably will induce a stress response in the prey prior to and during the predation event (Hart, 1997). Mesocosm experiments may reduce stress and may overcome some of the problems associated with the interpretation of simple laboratory studies. Many fishes swallow their prey whole (Gill, 2003), and it could be argued that a predation event by an efficient predator is quick with minimal suffering. Additionally, some fishes will not eat anything other than live food thus the welfare of the predator becomes a necessary consideration. Where predation of live fishes is deemed the only method, and the scientific advancement is justified, continuous observation with intervention to cull any injured prey during failed predation attempts is recommended (ASAB, 2012, 2018).

Genetic Modification

Studies which alter the genetic material of a fish are prevalent in the zebrafish and medaka literature. There is also a significant literature surrounding the genetic modification of salmonids (Sundström & Devlin, 2016). Ethical considerations include whether the modified phenotype is detrimental to the welfare of the individual (ASAB, 2012, 2018), and the risk to humans and the wider environment if they escape captivity (Smith *et al.*, 2010).