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Managing Fish Welfare for Research

Dr Mark Burdass

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Foreword

Fish are increasingly a significant animal for research. In 2018 they made up 17% of all procedures undertaken in the UK, this is compared to 9% in rats. In the UK, fish use in animal research has increased by 5% in the last 10 years compared to a general reduction in mice and rats over the same period (Annual Statistics of Scientific Procedures on Living Animals, Great Britain, 2018).

In New Zealand research involving animals is more agriculturally focussed but still fish are the fourth most common research animal after cattle, sheep and mice (MPI, 2018), with fish accounting for over 7% of all animals used. Considerations around the welfare of these aquatic animals is therefore of significant importance.

This general increase in the use of fish for animal research has seen a growing number of published papers investigating animal welfare and ethical issues related to holding and housing fish for research, angling and aquaculture (Browman *et al*, 2019, Sloman *et al* 2019, Huntingford and Kadri, 2009).

Introduction

Fish welfare is by no means a straightforward concept. As with all animals, the two major challenges here are the meaning or definition of animal welfare and how best to objectively measure it.

The challenge in undertaking this in fish is their diverse nature. In the specific case of fish, these definitions and measurements need to be applied to an incredibly varied group of animals that consists of over 32,000 species (Helfman *et al*, 2009)! The environmental range that these animals can live in is amazing as well as their physiological and anatomical diversity. There are fish species that inhabit the deepest areas of the world's oceans while others live in the highest lakes in the world. They can live in near freezing water to temperatures over 40°C.

There are air breathing fish that can live in water devoid of oxygen but will suffocate if deprived of air. Anatomically there are jawless fish with a skull and no vertebral column which is thought to have been lost through evolution over time (Janvier, 2010).

It should therefore come as no surprise that defining welfare and specific welfare standards may need to be more species-focused, rather than applied to fish as a single group!

When considering the welfare of fish as a group, however, it is important to take into consideration their taxonomy and that the term refers to a very wide range of animal types.

The majority of the welfare research on this subject is based on teleost fish. However, with increasingly effective systems for housing aquatic organisms becoming available, the diversity of fish types involved in research is increasing. This poses questions in regard to effectively managing the welfare of such a diverse taxonomic group.

How should we approach fish welfare?

Considering the welfare of fish for research purposes fits specifically within the refinement part of the three Rs (Russell and Burch, 1959). Improving welfare will potentially provide more reliable research conclusions and therefore ultimately lead to fewer animals being used.

The welfare of any animal is its overall state of being as regards its attempts to cope with its environment (Broom, 1986). Welfare is therefore a trait of an individual animal's perception of how good its life is. This can obviously vary from very good to very poor. Clearly the environmental challenges it faces are integral to its perception of how good it feels. All aspects of coping methods are included in this definition: feelings in the brain, behavioural, physiological, immunological, etc. Welfare is poor if the individual has difficulty in coping or if it fails to cope.

Health is the specific part of welfare which is concerned with the animal trying to cope with pathological attack or injury. Poor welfare occurs if animals feel pain or fear, if they lack control of their environment, or if they are diseased, injured or starved. The terms stress, pain and fear are applicable to all vertebrate animals, including fish. The hypothalamo-pituitary-interrenal axis (HPI) involving adrenaline, noradrenaline and glucocorticoids such as corticosterone is similar in all vertebrates in controlling the stress response. Too much glucocorticoid production leads to immunosuppression in fish as it does in mammals. Increased stress in fish has also been shown to lead to a marked suppression of brain cell proliferation (Sorensen *et al*, 2013).

In refining what is meant by welfare for fish the review by Huntingford *et al* (2006) clearly lays out the three main perspectives on welfare. These are:

1. **Feelings based welfare.** A feelings-based welfare perspective means that the animal should feel well, should be free from pain or fear and have access to positive experiences.
2. **Function based welfare.** A function-based perspective requires that an animal should be in good health and exist in an environment that does not require it to function beyond its physiological capacity.
3. **Nature based welfare.** A nature-based welfare approach requires that an animal can lead a natural life and express natural behaviour.

In practice, there is great deal of overlap among the three approaches, but when including physiological function, feelings and living conditions into the same concept it becomes very complex and difficult to know how to best measure and assess animal welfare.

When considering the welfare of fish from a feelings-based approach it is important to not anthropomorphise in judgements about housing as these in some cases could in fact impair good welfare. It could be argued that the general public's perception of welfare is very much a feelings-based approach. However, our understanding around the consequence of physiological change on the feelings of aquatic organisms is lacking and difficult to evidence. Previous published reviews on fish welfare have adopted either the feelings-based (Huntingford *et al*,2006) or function-based (Arlinghaus *et al*, 2007) approaches.

Assessing welfare for fish is a relatively new approach to fish husbandry. In fish aquaculture, an early attempt and reviews were developed for farmed fish in the UK by the Farm Animal Welfare Council (FAWC, 1996, 2014). The publication applied the 5 freedoms approach to the welfare of the farmed fish. This states that the fish should have:

1. Freedom from Hunger and Thirst
2. Freedom from Discomfort
3. Freedom from Pain, Injury or Disease
4. Freedom to Express Normal Behaviour
5. Freedom from Fear and Distress

Some of these freedoms are intuitive and easy to interpret, such as the avoidance of disease and injury but others, such as freedom from discomfort, or from mental distress, are more difficult to interpret. As these freedoms were also originally derived for farm livestock, some of them become less clear when applied to an aquatic poikilotherm, e.g. freedom from thirst in fish relates more to their specific osmoregulation strategy rather than simple dehydration. Hunger is also more difficult to interpret as fish do not have the same metabolic activity as a land mammal and so are less driven to eat. In aquaculture, periods of food deprivation are quite commonly used prior to harvest and prior to transportation. This is to ensure better welfare for the fish as it reduces poor water quality issues and reduces stress during these activities.

In recent years the importance of welfare in fish aquaculture has increased dramatically and most intensive farming operations operate under welfare standards. Many aquaculture industry bodies have clear guidance around welfare requirements (New Zealand Salmon Farmers Association, 2019, Federation of European Aquaculture Producers, 2000).

How do our perceptions of welfare apply to fish?

Holding aquatic animals in an artificial environment is likely to present welfare challenges which the animals are seldom equipped to deal with: Space restraints, unnatural aggregations, barren environments, handling and other frequent artificial stressors, etc. can all impact on the welfare of captive aquatic organisms.

The drive to improve animal welfare can be in part linked to the public's desire to reduce 'suffering' in captivity. However, the public's perception of what animals can suffer often skews the concern. In the case of fish, public concern over welfare is generally far less than it is for mammals.

As humans, we often intuitively respond to animal welfare in other mammals through the belief that we share many welfare responses. However, with aquatic animals it could be argued that we are less able to empathise with fish and therefore judgements around welfare can be more difficult.

The scientific understanding of perception of pain by fish is a highly debated current area of interest by different scientific groups with very divergent views (Sneddon, 2003, Sneddon *et al*, 2003, Rose, 2002, Braithwaite and Boulcott, 2007, Rose, 2007, Newby *et al* 2008, Braithwaite 2010, Rose *et al* 2013.). In light of these differences in scientific opinion, and the finding that fish possess the physiological and anatomical means to 'detect painful stimuli,' it seems appropriate to give fish the benefit of the scientific doubt, and to frame our deliberations around an assumption that fish are able to detect noxious stimuli or tissue damage, and to experience pain.

There is no single effective measure of welfare and although a wide range of physiological, biochemical and behavioural measures are used to assess welfare, none of these are considered reliable in isolation and multiple measures need to be taken (Broom, 1997, 1999). This is partly due to the need for careful interpretation of the functional context of many of the measures used (Dawkins, 1998). Physiological stress responses such as cortisol release, for example, are autonomic responses that indicate activity or arousal rather than being specific to poor welfare. These measures may, therefore, be affected by several different parameters (Dawkins, 1998, FSBI, 2002). Interpretation of behavioural measures in isolation is also subject to some doubt (Dantzer, 1986, Dantzer, 1991, Barnett and Hemsworth, 1990, Broom, 1991, Mason, 1991, Dawkins, 1998). Behavioural and physiological measures are intrinsically linked and are dependent on one another for correct interpretation with regard to maintaining good welfare (Dawkins, 1998). Appropriate combinations of welfare measures will, therefore, be specific to a given situation, but the greater our understanding of both the behaviour and physiology associated with a given situation the better equipped we are to answer questions of welfare standards.

This is highly relevant for research establishments managing fish, as a key element is usually the Standard Operating Procedure (SOP). In the case of fish and their diverse nature and wide range of environmental tolerances, these SOP's will need to be specific to the species being housed and generic SOP's are less useful.

As scientists, a function-based approach might appear to be the most attractive in having clearly defined physiological and health measurements that can be applied to evaluating the welfare status of the animal. However, research into fish welfare is a fast-developing field, often driven by global developments in aquaculture and ornamental fish keeping. The application of animal welfare concepts to fishes has lagged behind that of mammals, but fish welfare has received a considerable amount of attention in recent years - both in relation to research and to commercial practices such as aquaculture and the ornamental fish trade.

In the last 5 years there have been significant developments in quantifying welfare in aquaculture, specifically the salmon industry in Europe. Similarly, the OIE (OIE 2019) considers both schools of thought in its approach to animal welfare, recommending:

a) The use of fish carries with it an ethical responsibility to ensure the welfare of such animals to the greatest extent practicable.

b) The scientific assessment of fish welfare involves both scientifically derived data and value-based assumptions that need to be considered together. The process of making these assessments should be made as explicit as possible.

Feeding and Nutrition

Feed requirements

Feed requirements for fish are as equally diverse as their species diversity. To ensure good health and welfare their nutritional needs should be met. Regular access to nutritious and healthy food is essential.

For species which are more commonly held in captivity this is relatively easy as formulated diets can be obtained. For less common species or fish with unusual dietary requirements, specific strategies need to be used.

All food used for fish should be stored adequately to ensure the nutritional value isn't degraded. Food should be kept cool and stored in a dry area prior to use. If "wet diets" are to be used it is essential that the vitamin and mineral requirements of a species are met, and that the impact on water quality of feeding these types of diet is managed appropriately.

Food sources for fish which have not had extensive nutritional research on requirements may benefit from varying their diet. Ornamental fish certainly benefit from varying the food sources and occasionally using live feeds to increase dietary range and potentially improve nutritional balance. The use of live feeds should be done with some caution as they are a significant risk factor in introducing parasites into the system.

Feeding regimes

Feeding regime has been identified as one of the most important factors influencing the welfare of fish (Huntingford *et al.*, 2006) and should be managed appropriately for the specific species being held. The feeding of fish should be done in a predictable regular manner, the number of feeding events necessary to ensure good welfare is often related to the fish species or stage of life cycle. Predictable feeding events certainly improve welfare, feeding, and digestion in the fish (Vera *et al.*, 2007, Noble *et al*, 2012).

Feeding fish also runs the risk of increased aggression between individuals if insufficient food is presented in the system. Poor feeding can lead to increased size differences in fish which can increase aggression in captivity and ultimately poor welfare outcomes for some fish. It is therefore important for good welfare standards that feeding effectiveness is continuously reviewed to ensure good welfare.

Feed withdrawal has far less welfare consequences for adult fish compared to mammals. Most adult fish are unlikely to be stressed by a lack of feeding for a few days. However, larval or juvenile fish can be impacted by lack of food as they have lower body reserves. Withdrawal of food for these fish will quickly impact negatively on their welfare, and so frequent regular feeding is essential to ensure good welfare.

Managing waste

It is important to remember that the food is the main source of pollutants in the system so efficient feeding is important. For most systems faecal waste and uneaten food should be removed from the holding system either automatically by the nature of the system design or manually by regular siphoning. Uneaten food presents issues for the fish and can negatively affect their welfare.

Firstly, uneaten food will degrade and pollute the water causing a deterioration in water quality. For example oxidised food can contain toxins or microbial toxins which are harmful to fish health. Secondly, the nutritional value of old food will have declined.

Respiration

Providing an environment for good respiration is obviously essential for housing fish. This includes providing both enough oxygen and sufficient de-gassing to remove CO_2 from the environment.

The inverse relationship between temperature and oxygen saturation in water can be a problematic one, especially as increasing temperatures drive higher levels of oxygen demand in fish! In considering the respiratory environment the fish are housed in, any system used to improve oxygen availability also needs to consider the impact on the fish. For example fish held in a small tank with vigorous aeration delivered with an air stone is likely to cause significant distress as they struggle to maintain station or move around in the tank.

Osmotic Balance

Fish as a group have a wide range of salinity tolerances. To ensure good welfare the fish need to be housed in water within suitable salinity ranges.

Individual fish species often have preferences in terms of the hardness of the water they have adapted to. It is ideal to maintain the water hardness in similar ranges to the species requirements.

Fish may often adapt to ranges outside of their preferred range, but this is likely to lead to minor levels of chronic stress or a failure to thrive, and should therefore be avoided.

Thermal Regulation

Fish have a wide range of thermal tolerance as a group; however, each species of fish will have preferred ranges.

Ensuring that the housing facility provides an environment that is maintained within the preferred range for each species held is important for good welfare.

Possibly a more significant issue, is protecting against sudden temperature changes. Aquatic organisms are particularly impacted by sudden temperature changes as the aquatic environment is generally very stable in terms of temperature. Typically, temperature changes of more than 3 or 4°C in a day is likely to cause welfare issues.

Water Quality

Good water quality is essential for good welfare.

If fish in the wild encounter poor water quality their first response is to move away from the area. Fish in captivity do not have this option so ensuring that good water quality is being maintained is vital.

What constitutes good water quality can often be unique to the fish being housed but common problems include poor oxygenation, carbon dioxide build up, high levels of ammonia or nitrite, high suspended solids and toxic contaminants such as fire retardants and heavy metals.

Adequate filtration must be used and regular monitoring undertaken to ensure the systems are working. In modern aquaculture systems the use of continuous automated water quality monitoring provides assurance that optimal water quality is being maintained.

The key factors are dissolved oxygen, temperature, pH, ammonia, nitrite and water hardness. Information regarding the water quality and the absence of deleterious concentrations of gasses and ions, metabolites, toxins, and particles can be recorded continuously and provide further information to ensure good welfare standards are maintained.

Housing

General Buildings

Systems for holding aquatic life can be constructed both indoors and outside. The requirements for the buildings and infrastructure are to control adverse conditions that can impact on the animals and provide a protective environment which is not subject to sudden environmental changes.

Significant consideration should be given to emergency planning. If the systems water supply is interrupted or power is lost to systems using filtration etc, then there needs to be plans in terms of environmental maintenance. Plans can include automatic systems that create an alert to issues, such as water level indicators that dial predetermined telephone numbers when triggered. The use of backup power supplies is also increasingly common to ensure systems continue to function during power cuts. Reserve supplies of oxygen are also used to maintain stock during emergency events. These all allow stock to be maintained and minimise the welfare impacts of such events. Poor welfare/fish mortality resulting from a lack of emergency planning is not acceptable.

Water flow

Housing systems for aquatic animals are generally quite varied. The main considerations include security of the animals, and visibility of the animals for monitoring. A key area to address is the water maintenance system used to maintain the water quality of the system.

Systems can be separated into two main forms:

1) Open

New water flows through the system and is then discharged to waste after a single pass.

These systems usually have screening and a pumped water supply to minimise potential biofouling and diseases or pest species being introduced to the housing system.

Some form of pre-disinfection of incoming water with ultraviolet systems or ozone treatment can further reduce stock's exposure to problems from the source water supply.

2) Closed

Water is recirculated after filtration to restore water quality, with only minor new water exchanges undertaken.

Filtration

Most systems use some form of filtration to maintain a good environment for the fish as the use of simple water exchange systems can be expensive, and may be restricted by location. For example maintaining marine fish in facilities away from a direct source of good seawater would mean continuously transporting tankers of seawater to the facility.

If external water sources are used in this way, the water quality of the incoming water should be monitored to maintain consistency and therefore good welfare conditions.

For freshwater systems the use of mains water without pre-treatment should be avoided, as it can result in temperature or pH shock, and potentially introduce toxins.

For closed systems filtration is used to maintain the environment and water quality. These filtration systems are available commercially and the requirements are well documented for the housing of fish (Spotte, 1979, Lawson, 1995, Timmons *et al* 2002).

Holding Systems

Holding systems used to house fish can be extremely diverse from glass aquariums to concrete tanks. Whatever the system used there are certain key aspects that need to be considered. These are:

- Easy access to stock
- Stock easily observed without disturbance
- Appropriate levels of space to house the stock and not produce aggression through either too high or too low a stocking density
- Material used is inert and will not leach toxic materials into the water
- System is easy to maintain to ensure high hygiene standards

Consideration should be given to the colour of tanks made of opaque material, as well as the lighting.

For larval and very young fish pale tank colours and high light intensities would seem to be ideal for many fish species (Tamazouzta *et al*, 2000; Downing and Litvak, 1999). This probably is in response to their need to visual feeding and contrast with the food items is critical.

For older fish slightly darker shades such as green would seem to be preferred (Luchiari and Pirhonen,(2008).

For glass or acrylic aquariums consideration should be given to the surrounding environment. Fish can be startled and stressed by movement in their surrounds and also by objects looming over the tanks. Placing opaque barriers between tanks and putting lids on tanks will reduce startling and also prevent fish jumping out!

Lighting

Lighting systems are important for good welfare in fish. The key considerations are light quality and photoperiod. Various commercial lighting systems are now available for fish facilities, but the intensity of the light source and its colour range are important to ensure good welfare.

Modern LED lighting systems give incredible control over photoperiod, light intensity and frequency. These systems are more than capable of identically representing the lit environment that fish might experience in the wild. Photoperiod or the length of day is critical for prolonged good welfare. The day length the fish experience can have significant impacts on their physiology. In many cases fish exposed to 24 hours of light will become stressed, fish also require periods of "sleep" (Zhdanova, 2011) which generally take place at night and so depriving fish of a dark period can impact on their welfare.

External stimuli

When housing fish consideration should be given to control issues around the C start response (Eaton and Emberley, 1991).

This is an involuntary action in fish when exposed to strong stimuli such as sudden noise or light. The stimulus triggers large axons in the spine called Mauthner cells to fire which causes the fish to turn and form a rough c shape in body form then shoot forward. The reaction is involuntary and so fish can easily put themselves at risk in an enclosed tank system.

Sudden banging on housing systems or lights suddenly being turned on in a darkened room can all cause this, so systems should be housed in rooms where the lights go on gradually and the ambient noise is controlled.

Harmful organisms

The holding systems used for fish in research can run the risk of exposing the fish to low concentrations of harmful organisms (e.g. parasites, bacteria and viruses).

A key issue to consider with housing of fish therefore is the biosecurity of the system to maintain health status. New fish not only pose a potential risk of introducing new disease organisms, but their own health status may be compromised by existing pathogens in the system.

It is essential that all facilities should operate some form of independent quarantine system for new stock. This allows potential health issues to be identified early before they develop. The sourcing of new fish for research is a major risk to the health of existing animals, so ensuring new animals are monitored and allowed to recover from transportation is good practice. Regular health testing should also be routine for new and established animals to prevent infectious diseases developing or allow early intervention to improve welfare outcomes.

Once disease organisms become established in a aquatic housing system they are generally very difficult to eliminate.

Environmental Enrichment

Consideration of the environment the animals are being held in is important. The issue of enrichment requirements for fish is often overlooked or absent because of potential water quality maintenance issues. However, some species respond well to the changes in housing systems considered earlier to make their environment more complex.

A wide variety of enrichment techniques can be considered from structures to varying dietary choices. These all have potential benefits and potential problems (Williams *et al*, 2009) but should be considered in the housing strategy for fish.

Research has clearly shown that fish being reared for release into the wild benefit enormously from having complex rearing habitats prior to release. The presence of complex structures reduces maladaptive behaviours that reduce survivability in the wild (Roberts *et al*, 2011) and in burbot the fish reared in systems with no structure showed signs of stress and poor welfare (Wocher *et al*, 2011).

The use of structures and substrate in tanks has both positives and negatives in terms of fish welfare. Structural complexity in an aquatic environment is common in many natural aquatic habitats and therefore fish that occupy these types of environment may benefit from enhanced welfare if this is replicated in the artificial environment. Fish that are more pelagic in nature would benefit far less from this type of environment.

Structures in a tank can be as simple as offcuts of suitable pipes to provide hides or rigid plastic sheets suspended in the tank. These provide a more varied environment and act as enrichment for several fish species. Structures can also act to reduce aggressive social interactions between fish, acting to break eyeline and visibility of sub-dominant fish. Research on zebrafish has certainly shown both reductions in aggression (Carfagnini *et al*, 2009) or delays in developing social hierarchies (Wilkes *et al*, 2012) when provided with more structure in the tank system. Structures in tanks can therefore lift the overall welfare of the animals, particularly in fish species with strong social hierarchies or territorial species.

The use of substrates in holding facilities can also cause issues. The simple use of gravel like substrate has been shown to improve fish growth and therefore welfare (Batzina and Karakatsouli, 2011).The study also highlighted that the substrate colour could be important in producing the welfare benefits. Many holding systems are designed to be self-cleaning in terms of removing particulates and debris from the system. If structures are placed into the system, the flow dynamics in the tank can be disrupted and create dead spots were debris can settle. This can lead to localised deterioration in water quality. Increased structural complexity in rearing systems also creates issues for capturing and handling fish. This can lead to increased risks of the fish damaging themselves while trying to avoid capture. It can also create issues when undertaking observations on fish or simply stocktaking fish numbers in the system.

The use of gravel or sand substrates in toxicity testing exposure tanks has been proposed as environmental enrichment for some species of fish (e.g. flatfish). However, in addition to the type of problems described above, substrates can harbour disease-causing organisms (e.g. White spot cysts [*Icthyophthirius multifiliis* (freshwater) and *Cryptocaryon irritans* (seawater)]). In addition, the 'abrasive' nature of such substrates may cause skin damage and injury to fish in contact with these materials. Such injuries can expose the fish to secondary infections and leave them more susceptible to disease, and includes increased metabolic costs of maintaining homeostasis (e.g. osmotic balance), immune response and necessary tissue repair. The use of substrate also presents a problem for toxicity testing in terms of dosing. Some substrates may bind toxins and therefore adversely impact on the quality of the study. In aquaculture production the use of substrate has also been shown to reduce feeding behaviour, (Wocher *et al*, 2011) though the study was not able to show an impact on specific growth rates.

When making decisions on housing system and enrichment requirements for fish, the natural history of an animal can be used as an initial guide for defining an appropriate captive environment for the species in question (Williams *et al*, 2009). This approach can help identify the key scientific and practical issues involved and show where potential improvements in environmental enrichment can be made in both husbandry and test situations. The review of natural history/ecology need not be exhaustive (e.g. information of the natural history of some species may be limited by comparison with well-studied species such as zebrafish), but can provide a valuable starting point for initiating or improving laboratory culture procedures for captive fish species.

Stocking Density

The number of animals and the biomass being housed in a system is important to manage and control.

The stocking density can be either too high or too low. Low stocking densities can cause increased aggression in stock as they become more territorial (Baras *et al*, 1998, Jørgensen *et al*, 1993. High stocking densities can also lead to aggression and also localised deterioration in water quality (Ellis *et al*, 2002, Jørgensen *et al*, 1993). Monitoring of aggressive behaviours and body condition can be used to pick up these issues and need to be addressed to minimise impacts on overall welfare.

The requirement to maintain adequate stocking densities can also impact on the number of animals used in research. To ensure good welfare conditions, stocking levels should be sufficient to minimise aggression even if relatively low numbers of animals are to actually be sampled.

Fish species that shoal in the wild do this as part of an anti-predator strategy. If these species of fish are held individually or in low densities it seems likely that they may feel more vulnerable and become more stressed (Saxby *et al*, 2010).

Historically, the author has had numerous conversations with members of the general public regarding stocking densities for Clarias catfish. The stocking densities for adult catfish was often well over 400kg per m^3 of rearing space. To put this in context the recommended maximum stocking density for farming salmon is around 25kg per m^3. At first glance this might seem excessive but Clarias are an air-breathing catfish and become very aggressive and cannibalistic if the stocking density dropped below 50kg per m^3. Observation of the fish at these high stocking densities showed that they would actually congregate at the bottom of the tank stacked on top of each other, so being crowded wasn't really a problem for them. So, for Clarias catfish specifically, lots of room in their tanks would have led to really poor welfare outcomes!

Handling

It is almost inevitable in undertaking research studies that aquatic animals will require handling to either sample or to measure specific parameters. For fish specifically this can present a real issue in terms of welfare of the animal.

For any procedure which requires fish to be handled some thought should be given to minimising the impact on their welfare. Training fish is possible; certainly fish will learn to respond to feeding events quickly and so can be trained to feed from the hand; this may allow better observation and handling of the animals. Training can be particularly useful for larger fish which are difficult to handle, and the use of anaesthesia is impractical. Habituation can also allow easy access to large fish to manipulate them and for example exploit the use of tonic immobility (Kessel *et al*., 2015) for procedures. However, the use of training can also entrain abnormal behaviours that may impact on a study and so be impractical.

Key issues include:

Capture

If fish are going to be caught out of the system, the time it takes is critical to minimise stress. Use soft mesh nets and avoid chasing fish around systems. Once caught either keep the fish in the net to manipulate or gently drop the fish out of the net, do not roll fish out of nets as this increases damage to the skin. Once caught covering the eyes with a wet cloth will quieten most fish species for handling. It is also important to ensure that anything that is going to touch the fish including hands, cloths etc all need to be wet before handling. The mucus layer on the skin surface of fish is an integral part of the fish's immune system preventing infection. Dry cloths and hands will remove this layer and increase the risk of disease outbreak.

Anaesthesia

If handling of fish is particularly invasive or prolonged, then using anaesthetics can help minimise the potential to damage fish during handling. The process does need to be strictly managed to avoid welfare issues. The use of anaesthesia can also have potentially longer lasting physiological and behavioural consequences post procedure and should be thoroughly reviewed before using (Cooke *et al*, 2016). Two of the greatest risks for fish under anaesthesia are overdose and hypercapnia. If fish are kept in the anaesthetic too long, they can receive too great a dose. The hypercapnia risk comes from prolonged anaesthesia which reduces breathing rate. A lack of breathing can allow carbon dioxide to build up in tissues and cause an acidosis in the fish. It is therefore good practice to ensure good oxygenation during this time, so oxygen levels remain high.

Frequency

If fish are regularly handled, then there is a risk of cumulative stressful events increasing welfare problems with the fish including increased disease susceptibility and death. Fish that are regularly handled will acclimate to this and so handling impacts less on these fish with time.

Training

The management of fish for research requires reasonably specialist knowledge and experience. The environmental and husbandry requirements for most aquatic animals are radically different to those of reptiles and mammals. To ensure good welfare of any aquatic animal facility needs specific experience and training for aquatic animals. In the UK, researchers wishing to obtain a personal licence to work with fish have to go through a fish specific training programme before they can undertake research (UK Home Office, 2020). A clear priority therefore, of any research facility is to ensure that the staff have sufficient experience and training to guarantee good welfare standards are maintained. All staff working with fish and housing systems should be familiar with basic fish husbandry and water quality management. Modern recirculating systems are often very technical and require specialist knowledge and training to operate and maintain. This therefore requires duty cover for these facilities to ensure their smooth operation. Staff undertaking specific manipulations such as anaesthesia or injections should also have specific training and experience in the relevant SOP for the activity. Ideally, training records and records of the levels of expertise of staff should be maintained. Staff who are undertaking new manipulations or procedures should be signed off as competent and initially supervised by experienced staff to ensure the welfare of the animals isn't compromised during training.

Summary

Managing the welfare of aquatic animals such as fish in research is key not only to minimising suffering of research animals but also to good research outcomes. Fish which are stressed and/or welfare compromised will not generate consistent realistic results as they become physiologically compromised.

As terrestrial mammals, humans are often not innately in tune with what constitutes a good environment to house fish in an aquatic environment. We are therefore reliant on using a combination of behavioural cues, physiological parameters and physical measurements of water quality to determine if the housing environment we are holding fish in will provide for good welfare. To provide fish with a good environment can only lead to less stress and better welfare which ultimately will lead to better research outcomes. It is key to use an evidence-based approach to ensure good welfare.

The data regarding animal usage shows a clear trend for increasing use of fish for research, it is therefore essential to ensure good welfare standards are maintained.

References

Annual Statistics of Scientific Procedures on Living Animals, Great Britain, 2018) ISBN 978-1-5286-1336-1, HC 2475 2019-20

Arlinghaus, R., Cooke, S.J., Schwab, A. and Cowx, I.G. (2007) Fish welfare: a challenge to the feelings-based approach, with implications for recreational fishing. *Fish and Fisheries* 8, 57–71. https://doi.org/10.1111/j.1467-2979.2007.00233.x

Baras, E., Tissier, F., Westerloppe, L., Mélard C., and Philippart J. (1998) Feeding in darkness alleviates density-dependent growth of juvenile vundu catfish *Heterobranchus longifilis* (*Clariidae*) Aquat. *Living Resour*. Volume 11, Number 5, September 1998Page(s) 335 – 340 https://doi.org/10.1016/S0990-7440(98)80004-1

Barnett, J.L. and Hemsworth P.H. (1990) The validity of physiological and behavioral measures of animal welfare *Appl. Anim. Behav. Sci*., 25 pp. 177-187 https://doi.org/10.1016/0168-1591(90)90079-S

Batzina A.and Karakatsouli N.(2011) The presence of substrate as a means of environmental enrichment in intensively reared gilthead seabream Sparus aurata: Growth and behavioral effects. *Aquaculture*, Volumes 370–371, 11 December 2012, Pages 54-60. https://doi.org/10.1016/j.aquaculture.2012.10.005

Braithwaite V.A. (2010) *Do fish feel pain?* Oxford University Press ISBN978-0-19-955120-0

Braithwaite V.A. and Boulcott P. (2007) Pain perception, aversion and fear in fish. *Diseases of Aquatic Organisms* 75(2): 131-138 31 Op cit 23 32

Broom, D.M. (1986) Indicators of poor welfare. *British Veterinary Journal 142*, 524–526.

Broom D.M. (1991) *Assessing welfare and suffering Behav. Process*., 25, pp. 117-123 https://doi.org/10.1016/0376-6357(91)90014-Q

Broom D.M. (1997) Welfare evaluation Appl. Anim. Behav. Sci., 54 (1997), pp. 21-23 https://doi.org/10.1016/S0168-1591(96)01200-2

Broom, D.M. (1999) *In Proceedings of Aquavision 1999, Fish welfare and the public perception of farmed fish 1-6*. Stavanger: Proceedings of Aquavision.

Browman, H. I. Cooke S. J., Cowx I. G.,. Derbyshire S. W. G, Kasumyan A., Key B.,. Rose J. D, Schwab A., Skiftesvik A.B., Stevens E. D., Watson C. A., Arlinghaus R. (2019) Welfare of aquatic animals: where things are, where they are going, and what it means for research, aquaculture, recreational angling, and commercial fishing. *ICES Journal of Marine Science* (2019), 76(1), 82–92. doi:10.1093/icesjms/fsy067

Carfagnini, A.G., Rodd, F.H., Jeffers, K.B. and Bruce, A.E.E. (2009) The effects of habitat complexity on aggression and fecundity in zebrafish (Danio rerio). *Environmental Biology of Fishes* 86, 403–409. https://doi.org/10.1007/s10641-009-9539-7

Cooke, S. J., Wilson, A. D. M., Elvidge, C. K., Lennox, R. J., Jepsen, N., Colotelo, A. H., & Brown, R. S. (2016). 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.

Dantzer R. (1986) *Behavioral, physiological and functional aspects of stereotyped behavior—a review and a reinterpretation* J. Anim. Sci., 62 (1986), pp. 1776-178 DOI: 10.2527/jas1986.6261776x

Dantzer R. (1991 *Stress, stereotypies and welfare Behav. Process*., 25, pp. 95-102 https://doi.org/10.1016/0376-6357(91)90012-O

Dawkins M.S. (1998) Evolution and animal welfare Q. Rev. Biol., 73 pp. 305-328 https://www.journals.uchicago.edu/doi/abs/10.1086/420307

Downing G. and Litvak M.K. (1999) The effect of photoperiod, tank colour and light intensity on growth of larval haddock *Aquaculture International* 7: 369–382, 1999. DOI:10.1023/A:1009204909992

Eaton R.C., Emberley D.S. (1991) How stimulus direction determines the trajectory of the Mauthner-initiated escape response in a teleost fish. J Exp Biol. 1991 Nov;161:469-87.

Ellis T. North B. Scott A. P. Bromage N.R. Porter andM. Gadd D. (2002) *The relationships between stocking density and welfare in farmed rainbow trout Journal of Fish Biology*, Wiley. https://doi.org/10.1111/j.1095-8649.2002.tb00893.x

FAWC (Farmed Animal Welfare Council), (2014) FAWC *Opinion on farmed fish welfare Surbiton*, Surrey.

FAWC (Farmed Animal Welfare Council), (1996). *Report on the Welfare of Farmed Fish*. Surbiton, Surrey.

Federation of European Aquaculture Producers, (2000) *Code of Conduct*.

FSBI, 2002 FSBI (Fisheries Society of the British Isles), 2002. *Fish Welfare. Briefing Report* 2. Granta Information systems.

Helfman G., Collette B.B., Facey D.E., Bowen B.W. (2009) *The diversity of fishes: biology, evolution and ecology*. Wiley, New Jersey

Huntingford F. A. and Kadri S. (2009) Taking account of fish welfare: lessons from aquaculture. *Journal of Fish Biology* (2009) 75, 2862–2867 Wiley.doi:10.1111/j.1095-8649.2009.02465.x,

Huntingford F., Adams A. C., Braithwaite V. A., Kadri S., Pottinger T. G., Sandøe P., Turnbull J. F. (2006) Current issues in fish welfare. *Journal of Fish Biology*, Wiley

https://doi.org/10.1111/j.0022-1112.2006.001046.x

Janvier P. (2010) *microRNAs revive old views about jawless vertebrate divergence and evolution*. Proc Natl Acad Sci U S A. 2010 Nov 9; 107(45): 19137–19138. doi: 10.1073/pnas.1014583107

Jørgensen, E.H. Christiansen, J.S. Jobling M. Effects of stocking density on food intake, growth performance and oxygen consumption in Arctic charr (*Salvelinus alpinus*) *Aquaculture*, 110 (1993), pp. 191-204 https://doi.org/10.1016/0044-8486(93)90272-Z

Kessel, S. T., Hussey, N. E., & MacLatchey, D. (2015). Tonic immobility as an anaesthetic for elasmobranchs during surgical implantation procedures. *Canadian Journal of Fisheries and Aquatic Sciences*, 72,1287–1291.

Lawson T. B., 1995 *Fundamentals of Aquacultural Engineering Springer*, ISBN 978-1-4613-0479-1

Luchiari A. C. Pirhonen J. (2008) Effects of ambient colour on colour preference and growth of juvenile rainbow trout *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Biology*, Wiley. https://doi.org/10.1111/j.1095-8649.2008.01824.x

Mason G.J. (1991), Stereotypies and suffering Behav. Process., 25 pp. 103-115 https://doi.org/10.1016/0376-6357(91)90013-P

MPI 92018) Report on statistics on the use of animals in research, testing, and teaching for 2016

Newby NC and Stevens ED (2008) The effects of acetic acid 'pain test' on feeding, swimming and respiratory responses of rainbow trout (*Oncorhynchus mykiss*) *Applied Animal Behaviour Science* 114: 260- 269 33 https://doi.org/10.1016/j.applanim.2007.12.006

New Zealand Salmon farmers Association, 2019 *Salmon Biosecurity Standards*

Noble C, Cañon Jones, H. A. Damsgård, B., Flood, M.J. Midling, K. Ø. A.Roque, Sæther B. & Yue Cottee S. (2012) Injuries and deformities in fish: their potential impacts upon aquacultural production and welfare *Fish Physiol Biochem* 38, 61–83 https://doi.org/10.1007/s10695-011-9557-1

OIE (2019) Aquatic Animal Health Code (2019) Chapter 7 *Welfare of Farmed Fish* https://www.oie.int/index.php?id=171&L=0&htmfile=titre_1.7.htm

Roberts, L.J., Taylor, J., and Garcia de Leaniz, C. (2011). Environmental enrichment reduces maladaptive risk-taking behavior in salmon reared for conservation. Biol. Conserv. 144(7): 1972– 1979. https://doi.org/10.1016/j.biocon.2011.04.017

Rose J.D.(2002) Anthropomorphism and 'mental welfare' of fishes *Diseases of Aquatic Oorganisms Vol. 75*: 139–154,

Rose J.D.(2002) The Neurobehavioral nature of fishes and the question of awareness and pain Rev. Fish. Sci., 10 (2002), pp. 1-38 https://doi.org/10.1080/20026491051668

Rose JD, Arlinghaus R, Cooke SJ, Diggles BK, Sawynok W, Stevens ED and Wynne CDL (2013) Can fish really feel pain? Fish and Fisheries DOI 10.111/faf.12010 35

Russell, W. M. S., & Burch, R. L. (1959). *The principles of humane experimental technique*. London, UK: Methuen. 117.239.25.194

Saxby A., Adams L., Snellgrove D., Wilson R.W., Sloman K. A. (2010) The effect of group size on the behaviour and welfare of four fish species commonly kept in home aquaria. *Applied Animal Behaviour Science Volume* 125, Issues 3–4, July 2010, Pages 195-205. https://doi.org/10.1016/j.applanim.2010.04.008

Sloman K. A. Bouyoucos I. A. Brooks E. J. Sneddon L. U. Ethical considerations in fish research *Journal of Fish Biology*. Volume 94, Issue 4 April 2019 Pages 556-577 https://doi.org/10.1111/jfb.13946

Sneddon LU (2003) Trigeninal somatosensory innervation of the head of the rainbow trout with particular reference to nociception. *Brain Research* 972: 44-52 29 DOI:10.1016/s0006-8993(03)02483-1

Sneddon L.U., Braithwaite VA and Gentle MJ (2003) Do fish have nociceptors: Evidence for the evolution of a vertebrate sensory system. *Proceedings of the Royal Society: Biological Sciences*, 270 (1520) 30 https://doi.org/10.1098/rspb.2003.2349

Sørensen C. Johansen I B Øverli Ø (2013) Neural plasticity and stress coping in teleost fishes *General and Comparative Endocrinology* Volume 181, 15 January 2013, Pages 25-34 https://doi.org/10.1016/j.ygcen.2012.12.003

Spotte S. (1979) Seawater Aquariums: The Captive Environment. New York/Chichester/Brisbane/Toronto: John Wiley & Sons, 1979. xxii, 413 pp. DOI: https://doi.org/10.1017/S0025315400040509

Tamazouzt L., Chatain B. and Fontaine P., (2000) Tank wall colour and light level affect growth and survival of Eurasian perch larvae (Perca fluviatilis L.) Aquaculture; 182(1-2) : 85-90 http://dx.doi.org/10.1016/S0044-8486(99)00244-6

Timmons M. B. , Ebeling J. M., Wheaton F. W., Summerfelt S. T. , Vinci B. J. *Recirculating Aquaculture Systems Cayuga Aqua Ventures Llc*; 2 edition ISBN 0971264619

United Kingdom Home Office (2020) *Guidance Personal licence: standard conditions*.

Vera, L.M. De Pedro, N. Gómez-Milán, E. Delgado, M.J. Sánchez-Muros, M.J. Madrid, J.A. Sánchez-Vázquez F.J. (2007) Feeding entrainment of locomotor activity rhythms, digestive enzymes and neuroendocrine factors in goldfish *Physiology & Behavior*, 90 (2007), pp. 518-524 https://doi.org/10.1016/j.physbeh.2006.10.017

Wilkes, L., Owen, S.F., Readman, G.D., Sloman, K.A. and Wilson, R.W. (2012) Does structural enrichment for toxicology studies improve zebrafish welfare? *Applied Animal Behaviour Science* 139, 143–150. https://doi.org/10.1016/j.applanim.2012.03.011

Williams, T.D. Readman, G.D. Owen S.F. (2009) Key issues concerning environmental enrichment for laboratory-held fish species. Laboratory Animals Volume: 43 issue: 2, page(s): 107-120 https://doi.org/10.1258/la.2007.007023

Wocher H., Harsányi A., Schwarz F. J.(2011) Husbandry conditions in burbot (Lota lota L.): Impact of shelter availability and stocking density on growth and behaviour. *Aquaculture* Volume 315, Issues 3–4, 21 May 2011, Pages 340-347 https://doi.org/10.1016/j.aquaculture.2011.01.051

Zhdanova I. V (2011) *Sleep and its regulation in zebrafish* Rev Neurosci. 2011;22(1):27-36. doi: 10.1515/RNS.2011.005.

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