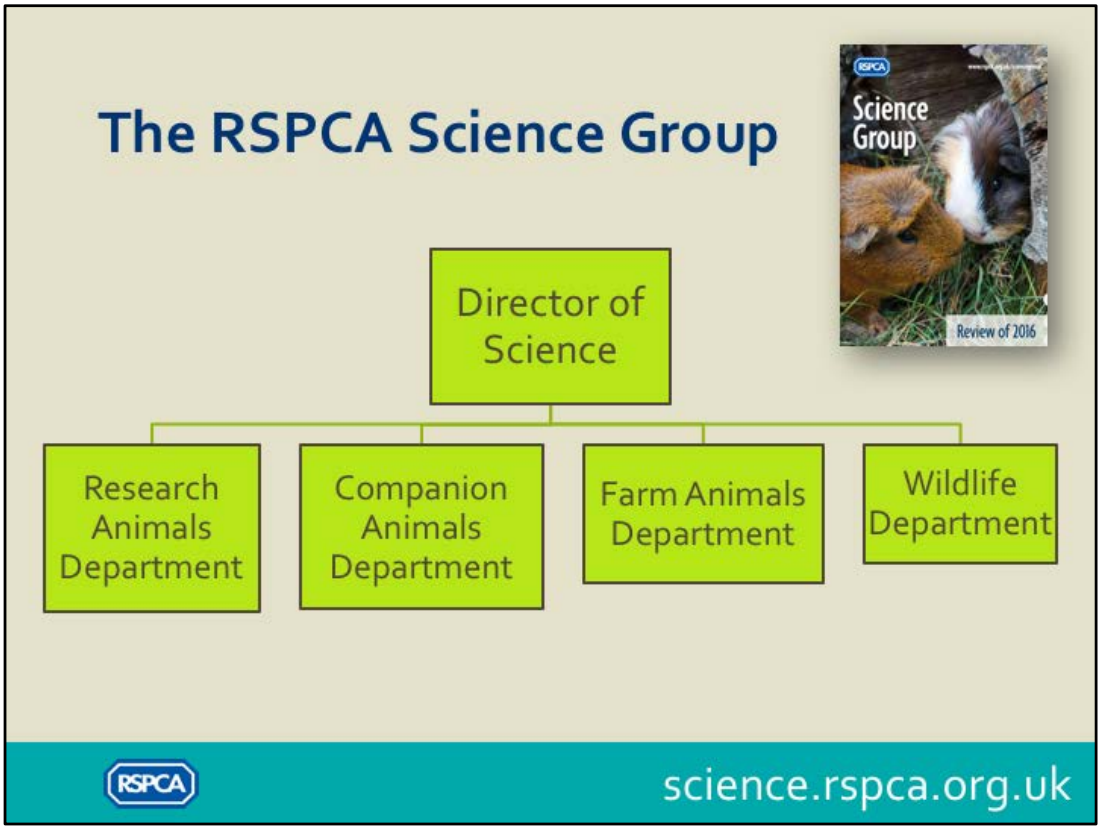
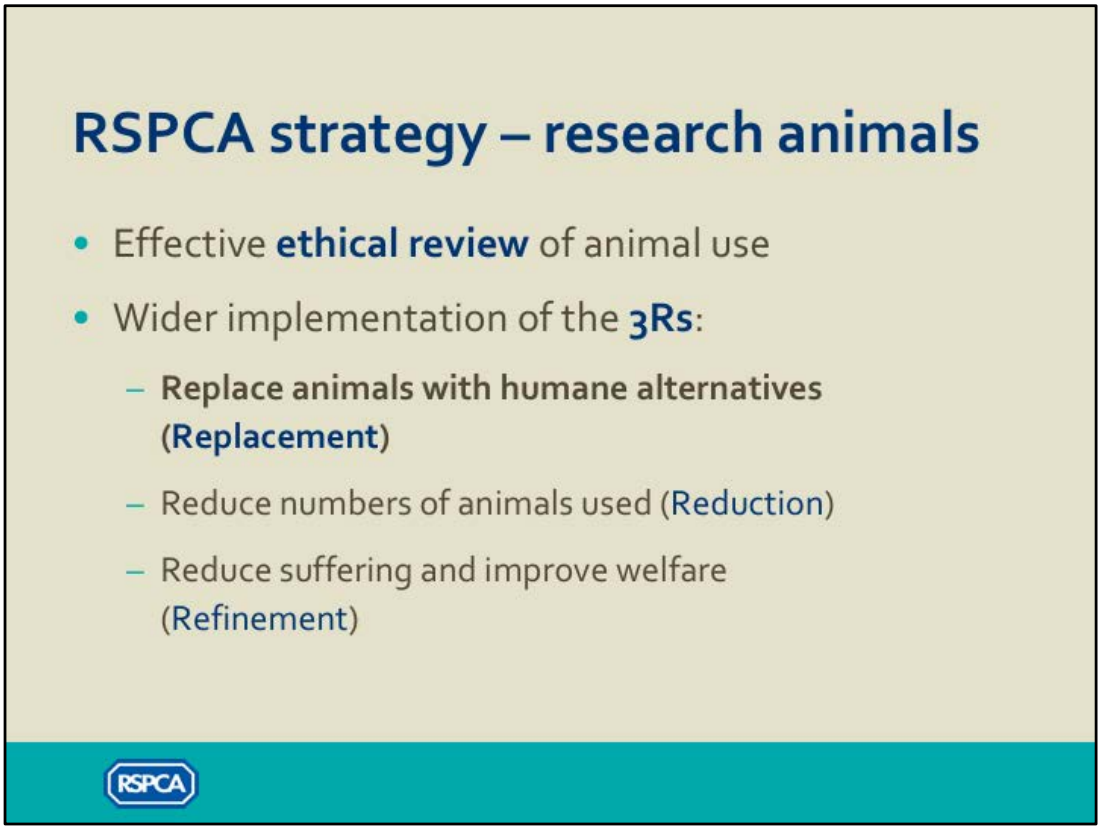
European legislation on environmental enrichment: what this means for fish

PENNY HAWKINS BSC PHD
RESEARCH ANIMALS DEPARTMENT, RSPCA

The RSPCA is the leading scientific animal welfare organisation in England and Wales. It was founded 193 years ago and is best known for its work to rescue and rehabilitate companion and wild animals. It has a network of local Branches, animal hospitals and clinics, and uniformed Inspectors who advise the public on animal care and investigate cases of cruelty or neglect. The RSPCA also has an Education team that produces resources and trains speakers for schools, and its own ethical food label, RSPCA Assured, which promotes better farmed animal welfare. All of the RSPCA's policies, campaigns and advocacy work are evidence-based and informed by the Society's Science Group.



The Science Group comprises four departments; Companion Animals, Farm Animals, Wildlife and Research Animals. All provide the scientific basis for RSPCA policy and strategy, and implement Society strategy in their respective area. The four departments cover pretty much all aspects of human-animal interaction between them, and there is a lot of useful cross over between us. For example, species are used in research and testing that are 'covered' by all three of the other departments, and the problem with bovine tuberculosis in the UK cuts across Farm Animals, Wildlife and Research Animals. ENRICH Fish is also an example of an initiative that is important for my Department and Farm Animals. You can find out more about the Science Group by going to the URL at the bottom right.

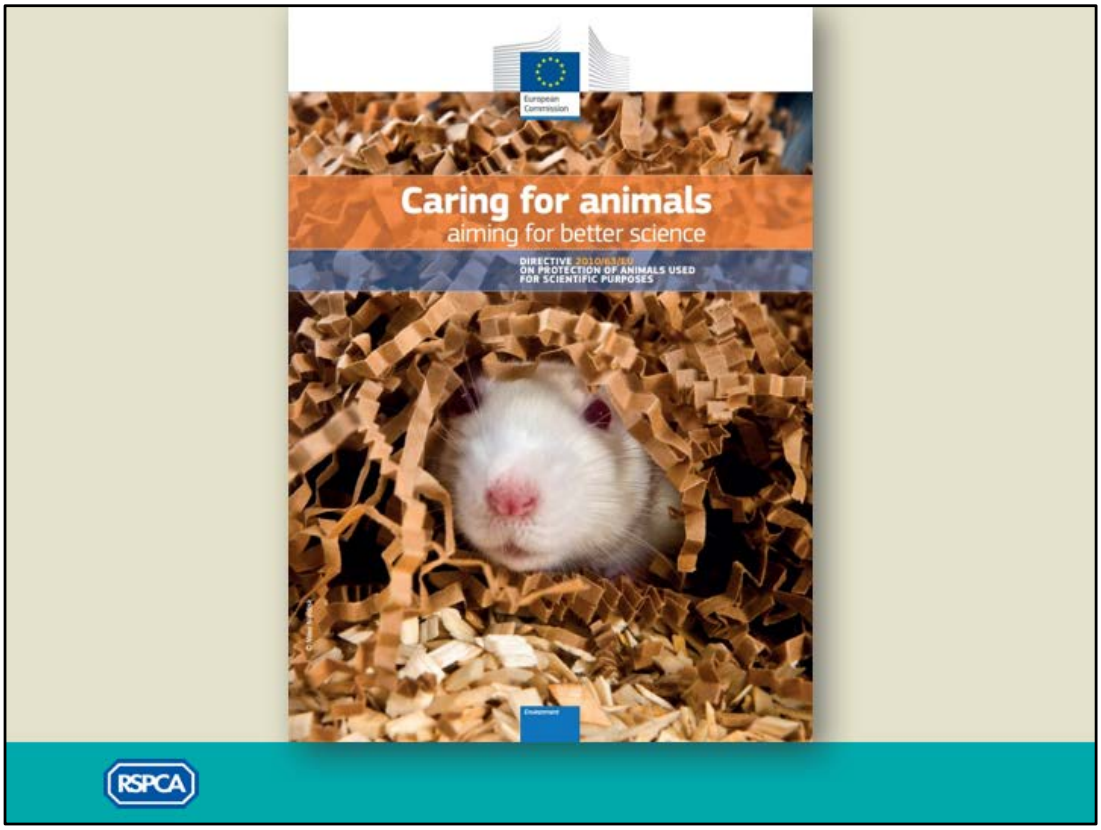


The Research Animals Department implements the RSPCA's strategy with respect to laboratory animals, which has two main strands.

First, we aim to achieve more effective ethical review of animal use, in which the harms and benefits, and whether and how animals should be used, are given careful scrutiny that involves a range of expertise and perspectives. A major area of work for us is promoting and supporting Ethical Review Bodies, such as the Animal Welfare Bodies required by the European Directive regulating animal care and use, of which more later.

Second, we believe (along with many others) that the Three Rs of replacement, reduction and refinement are essential for humane science. Replacement is our ultimate objective, but we also want to see numbers reduced to the minimum necessary to answer the scientific question, suffering minimised and welfare improved for as long as animal use continues. We were keen to become involved in the ENRICH Fish project, as large numbers of fish are used in research and testing and their refinement is often neglected – in fact, they are sometimes described as 'alternatives' even though they are clearly nothing of the sort! As the law recognises, fish are sentient and capable of suffering like all other species whose use is regulated (and probably some others besides).

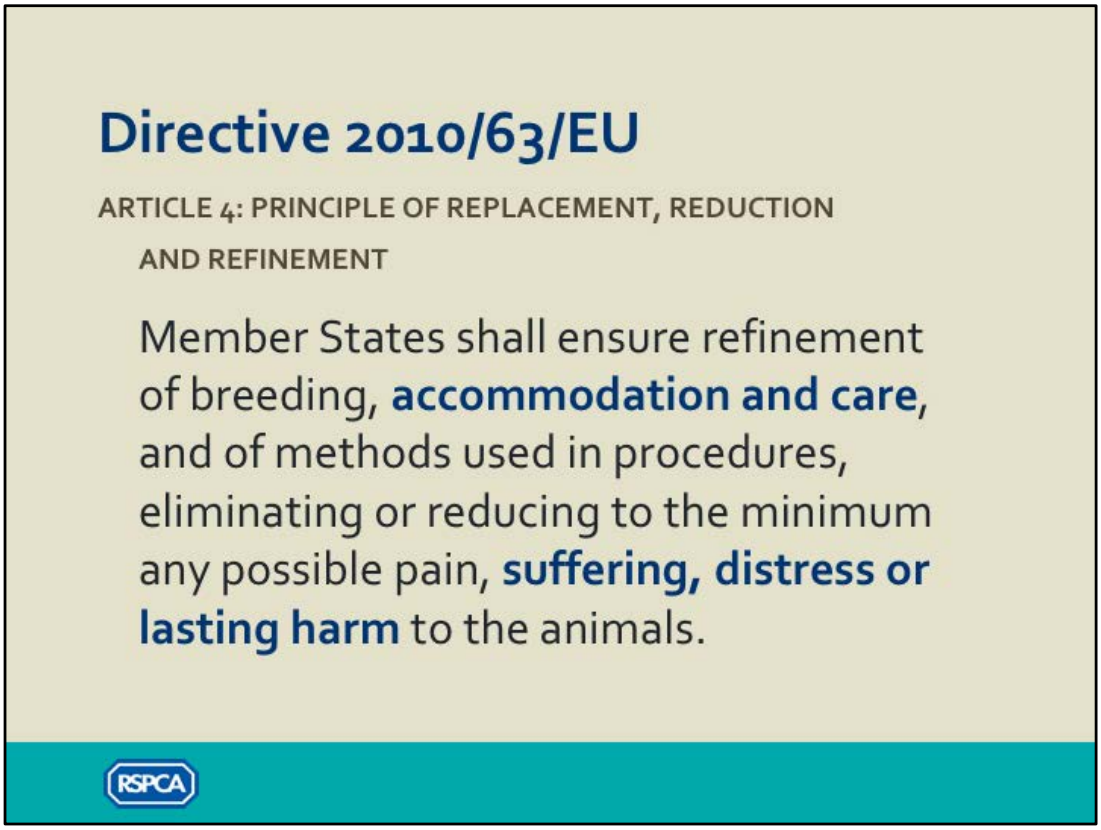
I have also liaised with the RSPCA Farm Animals Department when inputting into the ENRICH Fish project, and we are keen to see how we might apply the outcomes to the RSPCA Assured standards for Atlantic salmon. Our other standards for fish are currently rainbow trout, just in the process of being updated, and standards for cleaner fish are still in progress. The next standards are likely to be for sea bream and sea bass.



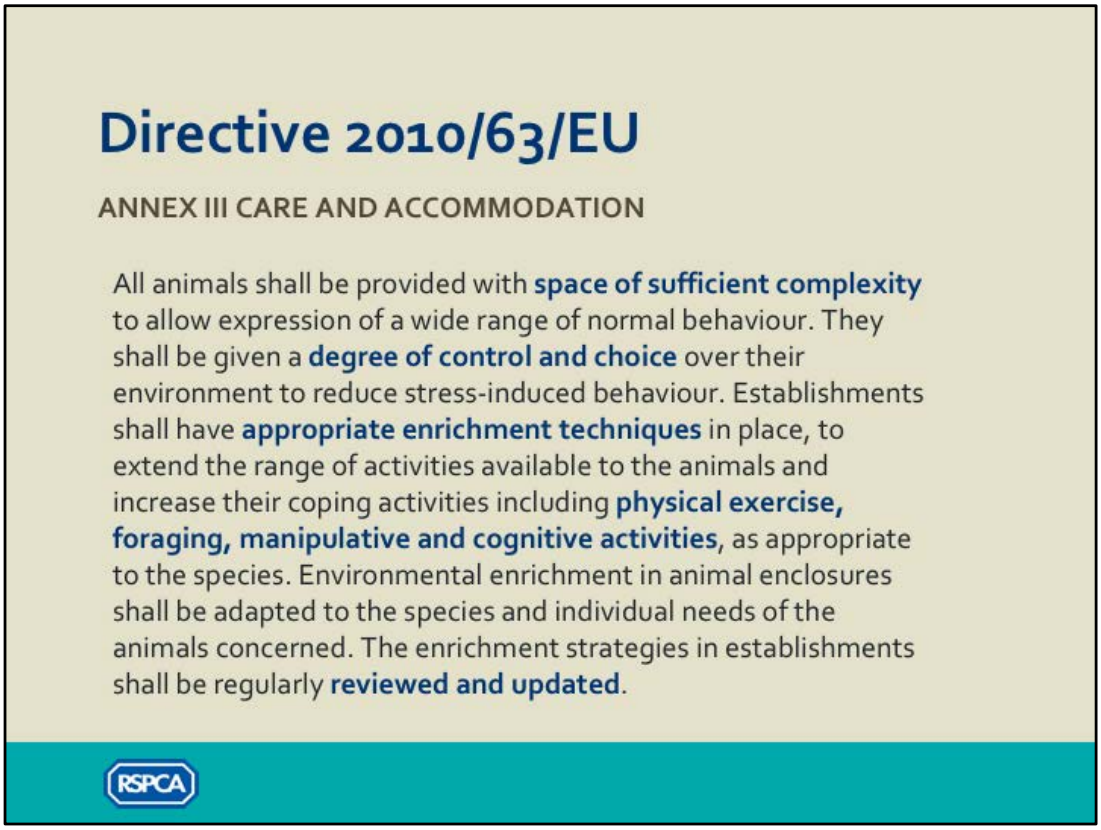
So, moving on to look at the European legislation on the housing, husbandry and care of animals used for scientific purposes, and what this means for fish. This is Directive 2010/63/EU, which sets out requirements for regulating research and testing, and also includes an Annex on animal accommodation and care.

But it begins with some Recitals, which come before the legally binding Articles of the Directive and explain what drove the revision of the Directive, which took years of hard work in the late nineties and early noughties. These are informative because they set the context for the Directive and its Annexes and associated working documents.

The Recitals explain how the revision of the previous Directive, which dated back to 1986, was prompted by new knowledge about animal welfare and capacity of animals to sense and express pain, suffering, distress and lasting harm. This was coupled with changes in attitudes towards animals, and demands by some Member States for more extensive 'animal welfare rules'. Importantly, the Recitals spell out that animals have intrinsic value and should be treated as sentient – they also refer to ethical concerns of the public about animal use. And of course, as for all EU Directives, harmonisation of legislation was a key objective.

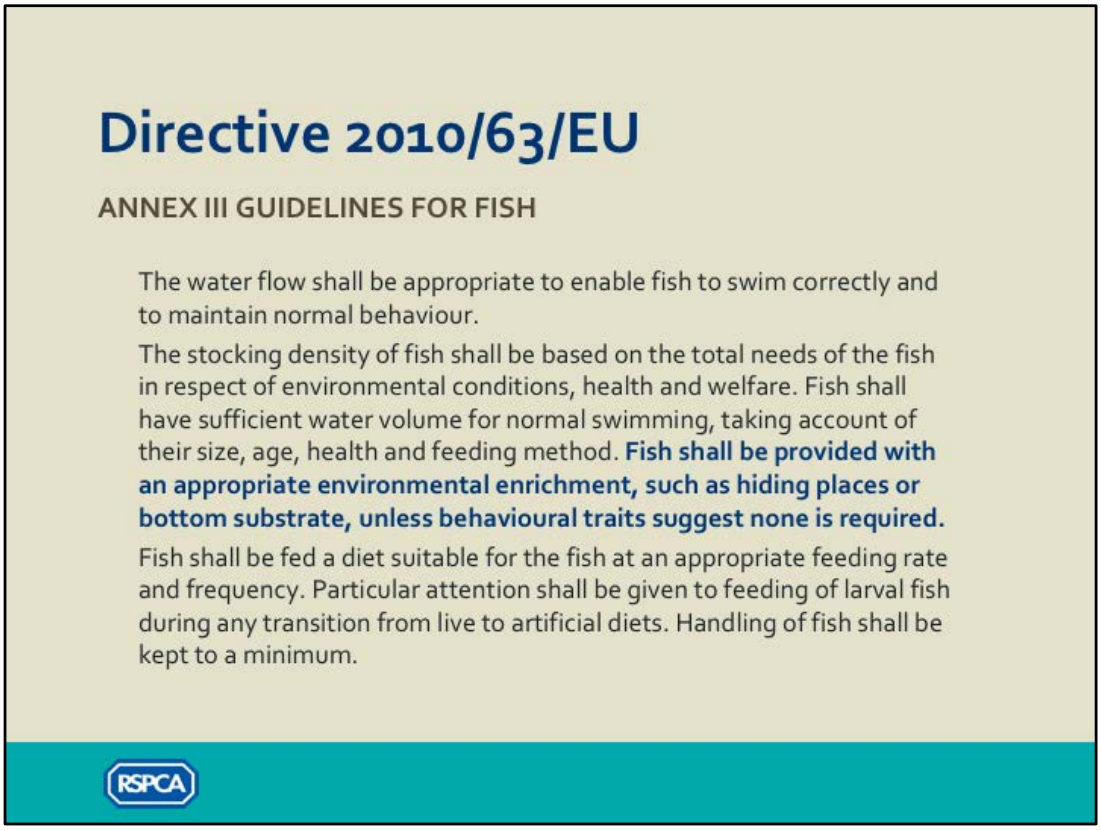


The relevant Article in the Directive is this one, number four, which requires that animal accommodation and care shall eliminate, or reduce to the minimum, any possible suffering, distress or lasting harm to the animals. Guidelines for animal accommodation and care are set out in Directive Annex III.



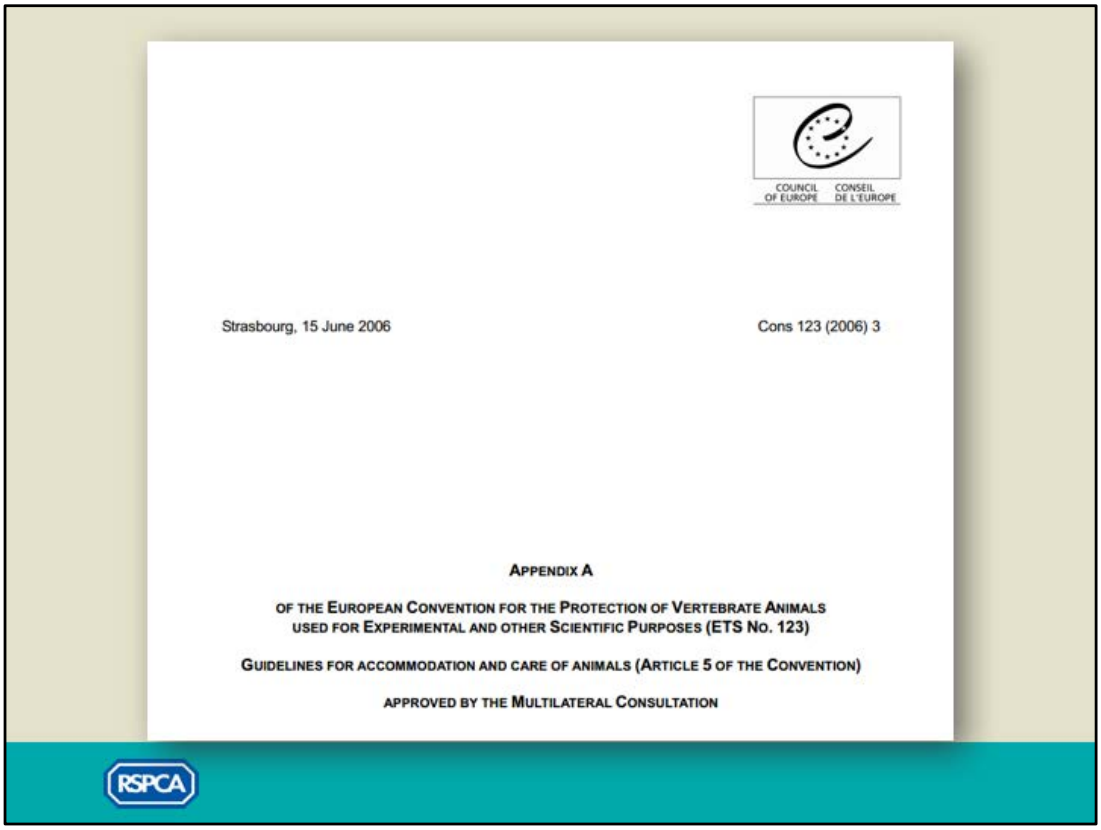
And this is what the Annex has to say about environmental enrichment, for all species in general. There are lots of essential principles with reference to complex space, degrees of control and choice over the environment; appropriate enrichment techniques; opportunities to exercise, forage, manipulate things and undertake cognitive activities; with a requirement for establishments to review and update their enrichment strategies.

All of this sounds very good, but what does the Directive advise for enriching the lives of fish in practice?

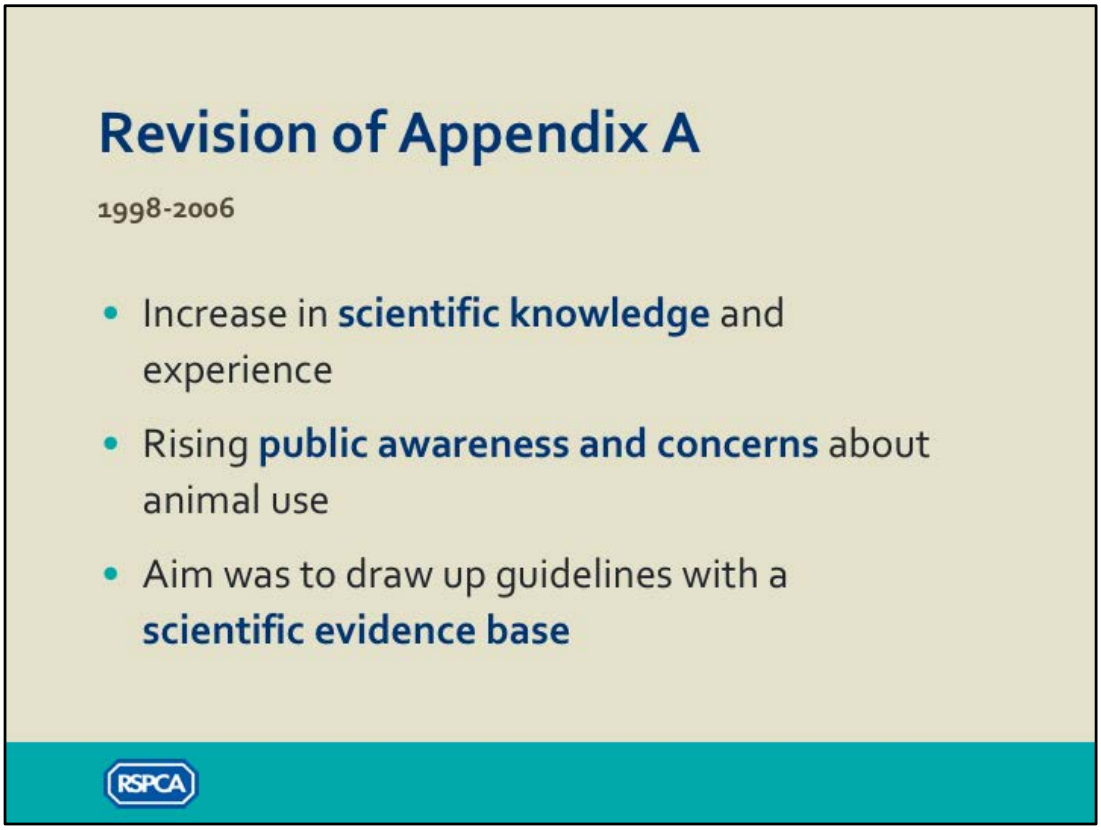


Here are the 'guidelines for fish'. The first thing to note is that these refer to 'fish', although there are over 25,000 species of fish. The second is that they are rather sparse and do not provide much guidance at all – appropriate enrichment, like hiding places or substrate, unless they don't need anything!

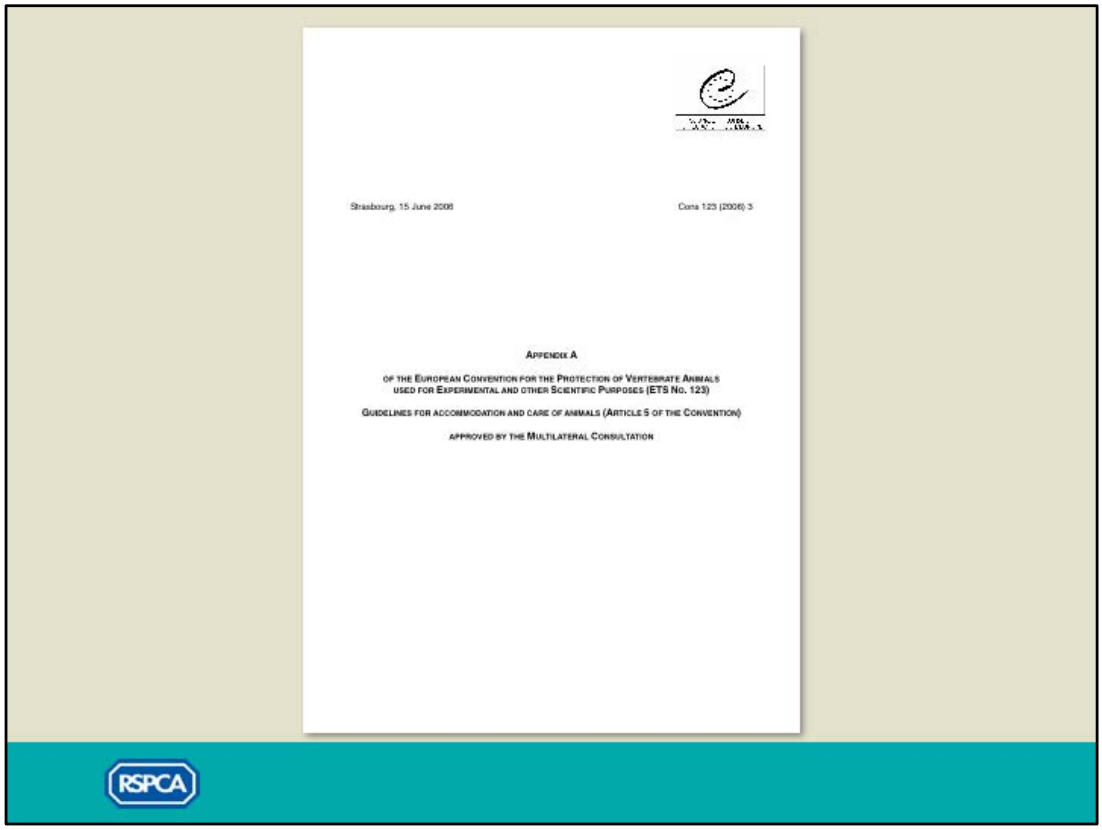
Annex III to the Directive was taken from this European Commission Recommendation, which goes into slightly more, species-group specific detail, but not very much. This recommendation 2007/526 was, in turn, taken from an Appendix to the Council of Europe Convention on the 'protection' of animals used for scientific purposes ...



... which was revised in 2006. This is not a legally binding regulation, but the revision of its Appendix A on accommodation and care was the basis for the equivalent Annex of the Directive.



The revision of the Appendix, which took place via a series of Council of Europe working groups between 1998 and 2006, was driven by very similar factors to the revision of the Directive – largely, increased knowledge and changing attitudes. Plus, there was a desire to incorporate new knowledge about animal behaviour, physiology and welfare into the guidelines.



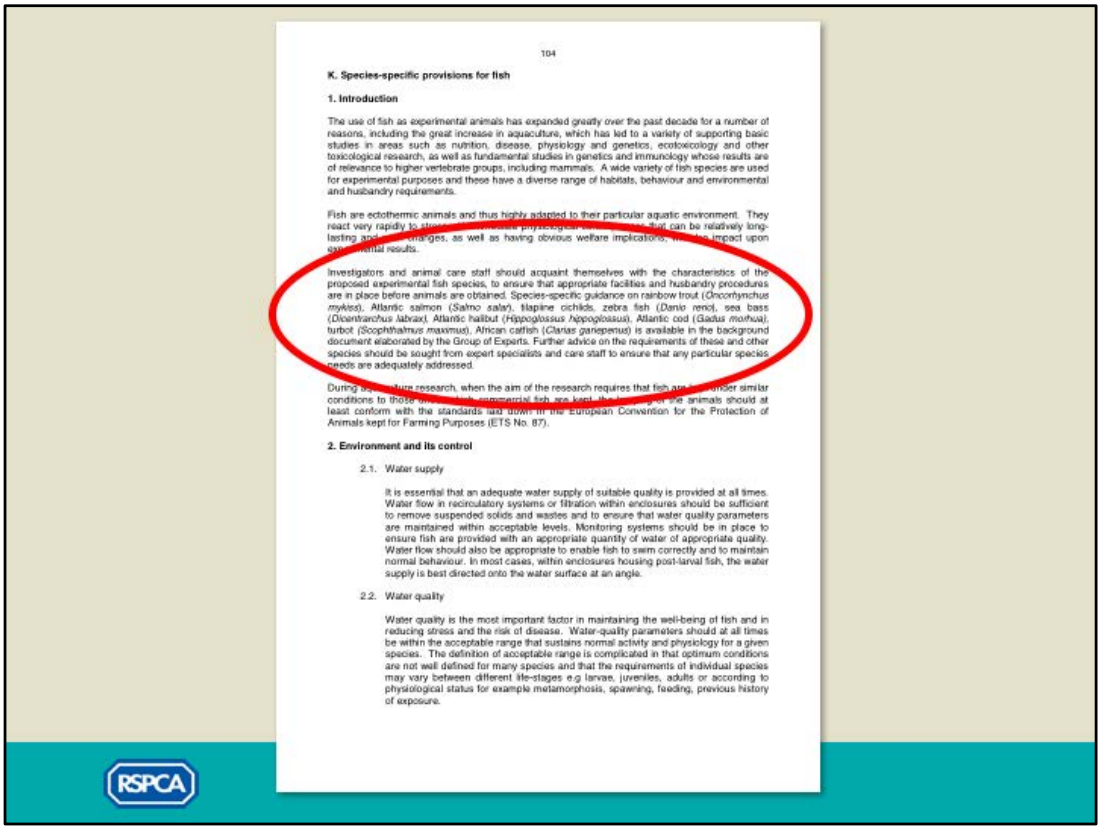
Strasbourg, 15 June 2006 Cons 123 (2006) 3

APPENDIX A

OF THE EUROPEAN CONVENTION FOR THE PROTECTION OF VERTEBRATE ANIMALS USED FOR EXPERIMENTAL AND OTHER SCIENTIFIC PURPOSES (ETS No. 123)

GUIDELINES FOR ACCOMMODATION AND CARE OF ANIMALS (ARTICLE 5 OF THE CONVENTION)

APPROVED BY THE MULTILATERAL CONSULTATION



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K. Species-specific provisions for fish

1. Introduction

The use of fish as experimental animals has expanded greatly over the past decade for a number of reasons, including the great increase in aquaculture, which has led to a variety of supporting basic studies in areas such as nutrition, disease, physiology and genetics, ecotoxicology and other toxicological research, as well as fundamental studies in genetics and immunology whose results are of relevance to higher vertebrate groups, including mammals. A wide variety of fish species are used for experimental purposes and these have a diverse range of habitats, behaviour and environmental and husbandry requirements.

Fish are ectothermic animals and thus highly adapted to their particular aquatic environment. They react very rapidly to stress [illegible] that can be relatively long-lasting and [illegible] changes, as well as having obvious welfare implications, [illegible] impact upon experimental results.

Investigators and animal care staff should acquaint themselves with the characteristics of the proposed experimental fish species, to ensure that appropriate facilities and husbandry procedures are in place before animals are obtained. Species-specific guidance on rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), tilapiine cichlids, zebra fish (*Danio rerio*), sea bass (*Dicentrarchus labrax*), Atlantic halibut (*Hippoglossus hippoglossus*), Atlantic cod (*Gadus morhua*), turbot (*Scophthalmus maximus*), African catfish (*Clarias gariepenus*) is available in the background document elaborated by the Group of Experts. Further advice on the requirements of these and other species should be sought from expert specialists and care staff to ensure that any particular species needs are adequately addressed.

During aquaculture research, when the aim of the research requires that fish are kept under similar conditions to those under which commercial fish are kept, the housing of the animals should at least conform with the standards laid down in the European Convention for the Protection of Animals kept for Farming Purposes (ETS No. 87).

2. Environment and its control

2.1. Water supply

It is essential that an adequate water supply of suitable quality is provided at all times. Water flow in recirculatory systems or filtration within enclosures should be sufficient to remove suspended solids and wastes and to ensure that water quality parameters are maintained within acceptable levels. Monitoring systems should be in place to ensure fish are provided with an appropriate quantity of water of appropriate quality. Water flow should also be appropriate to enable fish to swim correctly and to maintain normal behaviour. In most cases, within enclosures housing post-larval fish, the water supply is best directed onto the water surface at an angle.

2.2. Water quality

Water quality is the most important factor in maintaining the well-being of fish and in reducing stress and the risk of disease. Water-quality parameters should at all times be within the acceptable range that sustains normal activity and physiology for a given species. The definition of acceptable range is complicated in that optimum conditions are not well defined for many species and that the requirements of individual species may vary between different life-stages e.g larvae, juveniles, adults or according to physiological status for example metamorphosis, spawning, feeding, previous history of exposure.

Fish show varying degrees of adaptability to changing water-quality conditions. Some degree of acclimatisation may be necessary and this should be carried out for a period appropriate for the fish species in question.

As most fish species cannot function well in water containing a high level of suspended solids, these should be maintained within an acceptable range. Where necessary water supply to facilities should be appropriately filtered to remove substances harmful to fish and to maintain suitable water physico-chemical parameters.

2.2.1. Oxygen

Oxygen concentration should be appropriate to the species and the context in which they are held. Required oxygen concentration will vary according to temperature, carbon dioxide concentration, salinity, feeding level and amount of handling. Where necessary supplementary aeration of water should be provided.

2.2.2. Nitrogen compounds

Ammonia is the main excretory product of fish. Dissolved urea, as well as feed and faeces, are converted to inorganic compounds such as ammonia and phosphate. Ammonia will be further converted into nitrite and nitrate. Ammonia and nitrite are very toxic to fish and their accumulation should be avoided by increasing flow rate, reducing density or temperature, or biofiltration.

Susceptibility to ammonia varies between fish species and in general marine and younger fish are more susceptible. The toxic form of ammonia is unionised ammonia, the amount of which depends not only on total ammonia concentration, but also on pH, salinity and temperature.

2.2.3. Carbon dioxide (CO_2)

Carbon dioxide is produced by fish during respiration and dissolves in water to form carbonic acid, thus lowering the pH. Accumulation of carbon dioxide can be a problem at a high stocking density if pure oxygen is used instead of air to maintain the oxygen content in the water. Although high concentrations of free carbon dioxide can be fatal to fish this is most unlikely to be a problem under normal housing conditions. However, care should be taken that water supply systems, particularly in the case of groundwater-based systems, do not introduce harmful quantities of carbon dioxide in the enclosures.

2.2.4. pH

Acceptable pH levels depend on many water quality factors, for example, carbon dioxide and calcium. As far as possible pH should be kept stable as any changes in pH will influence other water quality parameters. In general pH may be lower in freshwater than in salt water. If necessary supply water should be buffered.

2.2.5. Salinity

Salinity requirements of fish will vary according to whether they are marine or freshwater in origin or adapted. Some species are able to tolerate a wide range of salinity. In others salinity tolerance may vary according to life stage. Changes in salinity should be introduced gradually.

2.3. Temperature

Temperature should be maintained within the optimal range of the fish species involved and any changes should take place gradually. At high temperatures it may be necessary to provide supplementary aeration of enclosure water.

2.4. Lighting

Many fish require light for feeding and other behavioural activities. Fish should be maintained on an appropriate photoperiod as far as possible since the day/night cycle influences the physiology and the behaviour of fish.

Many fish species should not normally be kept in bright light, although some tropical species naturally encounter very bright light. As appropriate for the species, lighting should be subdued or tanks should be covered and suitable hiding places provided. Abrupt changes in light should be avoided as far as possible.

2.5. Noise

Fish can be acutely sensitive to sounds, even at very low levels. Noise levels within experimental facilities should be kept to a minimum. Where possible equipment causing noise or vibration, such as power generators or filtration systems, should be separated from fish-holding facilities. Fish reared in a particular environment will adapt to the stimuli presented there and may become stressed if moved to unfamiliar surroundings.

2.6 Alarm systems

(See Paragraph 2.6 of the General Section)

3. Health

3.1. General

Appropriate attention should be paid to hygiene within experimental facilities. The health of fish is intimately bound up with their environmental and husbandry conditions. Most diseases are associated with stress arising from deficiencies in these conditions and any attempt to control disease should address these areas if problems are to be successfully eradicated. Fish health management is almost always concerned with populations rather than single individuals, and control measures should be designed accordingly.

3.2. Hygiene and disinfection

Fish-holding facilities, including associated pipework, should be cleaned and disinfected when appropriate. In closed systems cleaning and disinfection should be compatible with maintenance of optimal microbiological conditions. Equipment, for example nets, should be disinfected between use. Staff should take precautions to prevent cross-contamination between fish enclosures.

3.3. Quarantine

Newly introduced stocks, both from farmed and wild fish, should be given an appropriate quarantine period, as far as possible separate from existing stocks. During quarantine they should be closely monitored and any disease problem which arises should be treated or the stock destroyed. Farmed fish should be procured from reputable suppliers and as far as possible have a verified health status.

4. Housing, enrichment and care

4.1. Housing

Fish behaviour will influence stocking density and schooling or territorial behaviour should be considered. The stocking density of fish should be based on the total needs of the fish in respect of environmental conditions, health and welfare. Fish should have sufficient water volume for normal swimming. Measures should be taken to avoid or minimise conspecific aggression without otherwise compromising animal welfare. Acceptable stocking density for a given species will vary depending on water flow and current, water quality, fish size, age, health and feeding method. In principle, groups should consist of fish of the same size to minimise the risk of injuries or cannibalism.

4.2. Enrichment

For some species, environmental enrichment may be necessary to take account of their behavioural traits, for example, in reproduction or predation. Examples of such needs include provision of hiding places for wrasse, or substrate such as sand for some flatfish. Care is needed to ensure that environmental enrichment does not adversely affect water quality, but this should not impede the development of suitable measures to enhance the welfare of fish.

4.3. Enclosures

4.3.1. Fish holding facilities

Fish can be maintained in land-based enclosures in dedicated buildings or in external areas, or in enclosures in open-water systems. Where practical, these should have controlled access and be arranged to minimise disturbance of the fish, and to facilitate maintenance of suitable environmental conditions.

4.3.2. Land-based enclosures

The materials used to construct the enclosures should be non-toxic, durable and with a smooth internal surface to prevent abrasions to the fish. Enclosures should be of an appropriate size to accommodate the required stocking density of fish and should be able to receive the necessary water flow. Enclosures should be of an appropriate shape to accommodate the behavioural needs and preferences of the particular experimental fish species; for example, circular enclosures are most appropriate for salmonids. Enclosures should be designed to prevent escape. Enclosures should where appropriate be self-cleaning to aid removal of waste products and surplus feed.

4.3.3. Open-water enclosures

Fish, especially marine species, may be kept in large floating enclosures. The enclosure dimensions, including depth, should permit active swimming and shoaling of the fish. Mesh size should permit good water exchange while preventing escape of fish. Enclosures should be designed to minimise the risk of attack by predators. Enclosures should be rigged so as to prevent their shape distorting in tidal flows or running water and thus trapping fish.

4.4. Feeding

Fish may be fed either on artificial diet or fresh/frozen natural feed. Artificial diet is preferable, providing it meets the nutritional requirements of the species, and is acceptable to the fish. Some fish species or life stages will not take artificial diets. Artificial diets also tend to have less impact on water quality.

It is important that fish are fed at an appropriate feeding rate and frequency, and this will depend on a number of factors including temperature, size and maturity. As high temperature increases the metabolic rate, feeding level should also be increased. It may not always be necessary to feed fish daily. Presentation of diet is also very important to ensure adequate feeding. Consideration should be given to the number of meals per day, the age of the fish, the water temperature and the size of the pellet or food fragment offered. Feeding regime, palatability and the presentation of food should ensure that all fish obtain sufficient food. Particular attention should be paid to feeding of larval fish, especially where feeding is switched from live to artificial diets.

4.5. Cleaning of enclosures

All enclosures should be kept free of fish waste products or uneaten feed. If these are allowed to accumulate, water quality and thus fish health will be adversely affected. Enclosures should be regularly treated and cleaned to prevent fouling and reduced water exchange. There should be no risk of back-flushing and consequent fouling of enclosure water and the risk of infection. If enclosures are not self-cleaning, waste material should be siphoned off as necessary, generally as soon as possible after feeding. The sides and bottom of enclosures should be cleaned regularly to avoid build up of algae and other detritus. Care should be taken to minimise stress during cleaning.

4.6. Handling

Fish may be severely stressed by handling which should therefore be kept to the minimum possible. Fish should normally be netted out from the normal enclosure and anaesthetised in a smaller container before handling. Fish should be kept under anaesthetic for as short a time as possible and be placed in clean aerated water for recovery. An effective concentration of anaesthetic should be maintained throughout the procedure.

When catching fish, nets with an appropriate frame and mesh size should be used. Knotted net mesh should be avoided. Nets should be disinfected and rinsed in clean water before use.

Out of water fish should be handled with wet gloves or wet hands and on a moist surface to avoid scale and mucus loss. Particular attention should be paid to handling practices to avoid desiccation, suffocation and other injury.

4.7. Humane killing

Most fish should be killed by either:

- an overdose of anaesthetic using appropriate route and anaesthetic agent for the size and species. When killed by immersion, fish should be left in the anaesthetic solution for at least five minutes following the cessation of opercular movement and/or vestibulo-ocular reflex (VOR), or

- concussion of the brain by striking of the cranium

Death should be confirmed, for example, by physical destruction of the brain or exsanguination.

4.8. Records

Records should be maintained on appropriate water quality parameters.

4.9. Identification

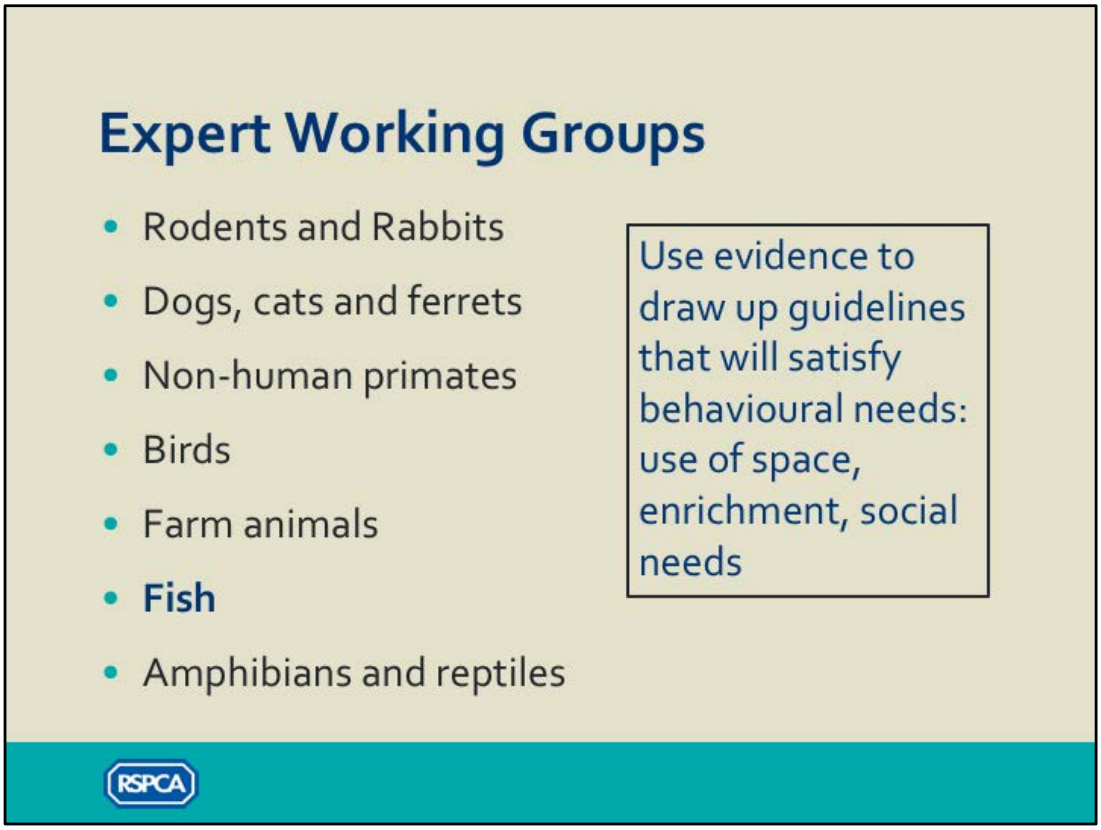
It is not always necessary or feasible to individually identify all fish within a facility.

If it is necessary to mark fish for identification purposes, subcutaneous dye injection is considered the least invasive method of marking. Careful consideration is needed before more invasive methods such as fin clipping or PIT tagging are used. Mechanical tagging should not be used unless no other method is suitable.

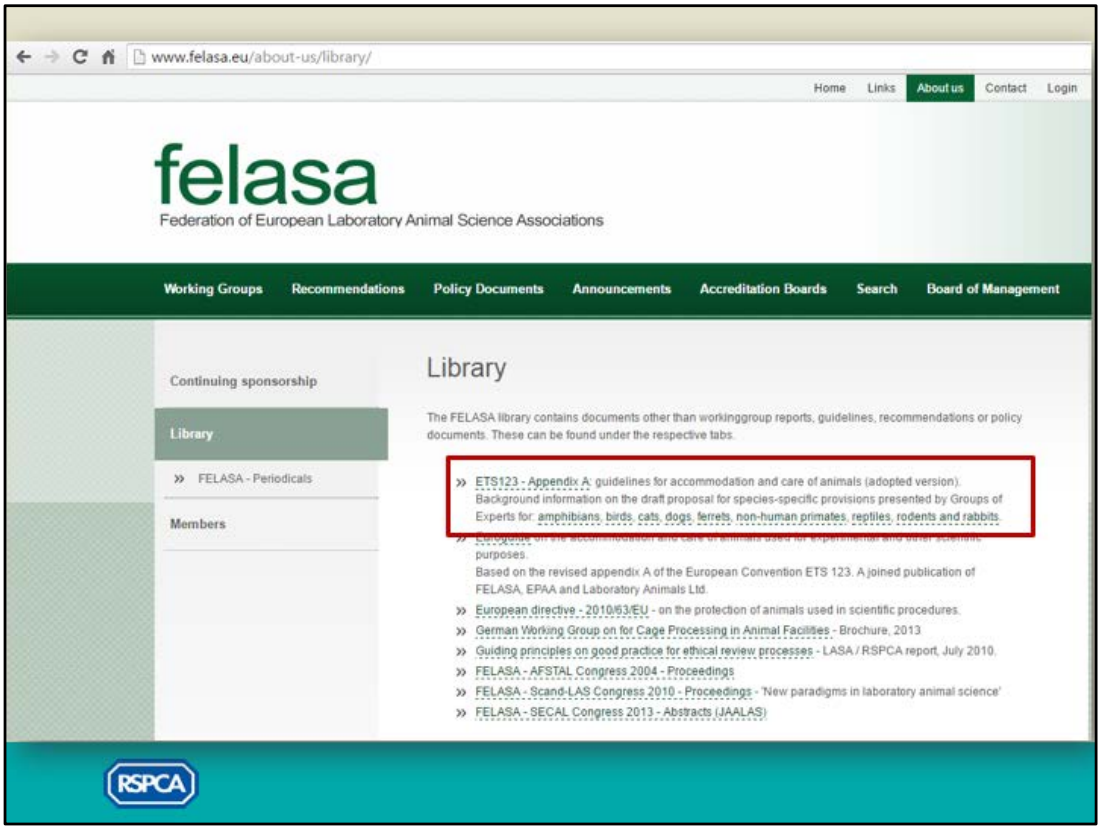
Marking should generally be carried out under anaesthesia in order to ease handling and minimise the risk of injury, morbidity and stress.

5. Transport

Fish should be deprived of food prior to transportation for a period sufficient to allow the gut to clear and reduce faecal contamination of the transport system. Care should be taken to prevent injury and stress to fish during capture, loading, transportation and unloading. Abrupt temperature changes, periods of hypoxia and any deterioration in water quality due to excretory products should be avoided.

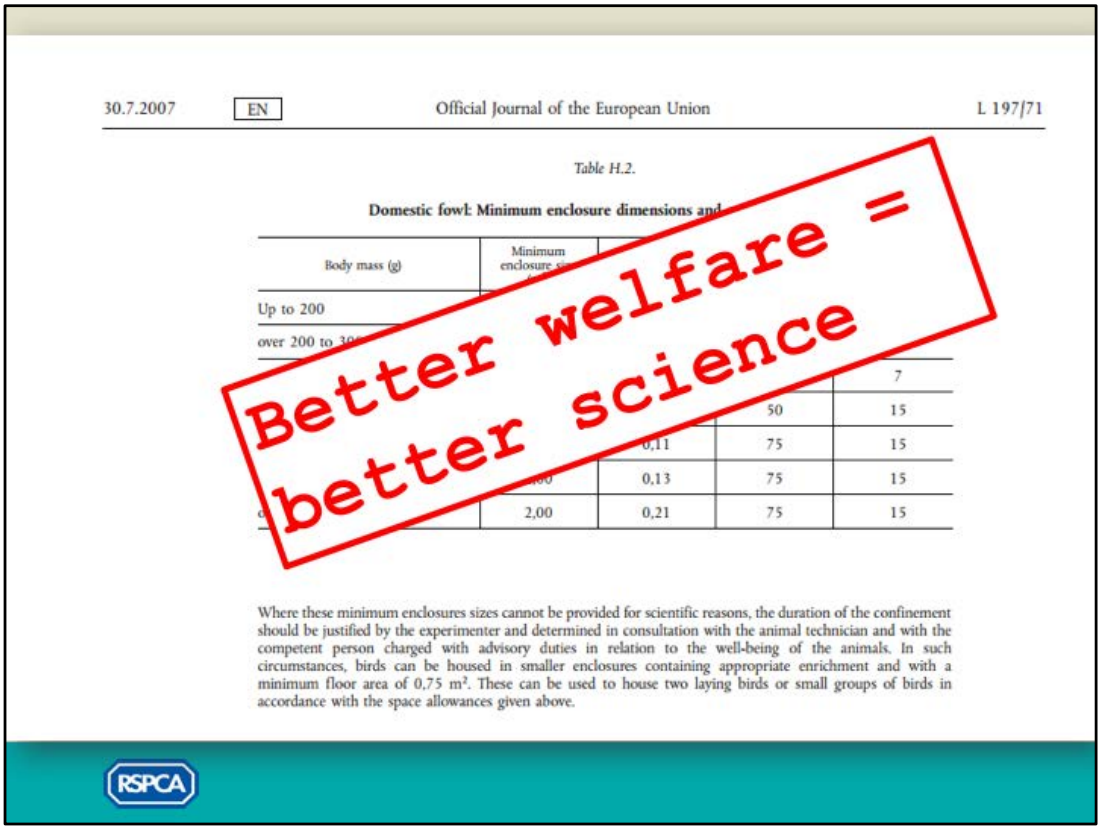


These were the Expert Working Groups set up by the Council of Europe, with representation from a range of stakeholder organisations including scientists, animal technologists, breeders, animal welfare organisations, regulators and Member States. I was involved in these, and we were given the brief of using evidence – both from the scientific literature and examples of current good practice – to draw up guidelines that would satisfy behavioural needs, including a good quality and quantity of space and satisfying social needs (for social animals). As you can see, one of these groups was tasked with achieving this for 'fish'.

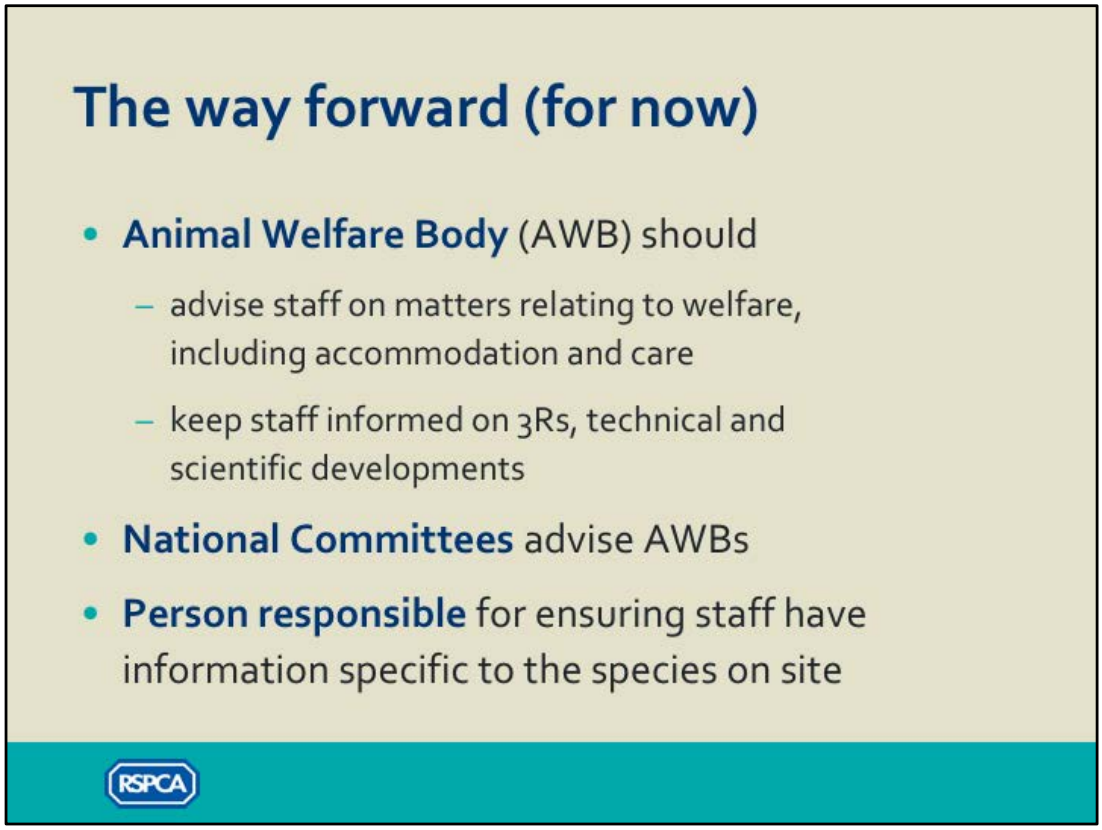


We all had to produce a background review document, including recommendations for the Appendix guidelines document itself plus all of the evidence that we had gathered to substantiate our recommendations, with other useful and up-to-date information about good practice refinements for accommodation and care. Following publication of the Appendix, all of the so-called 'part B' documents were uploaded onto the Federation of European Laboratory Animal Science Association's website ... apart from the one for fish. This has never been made publicly available and seems to have sunk without trace.

So in my view fish have had a raw deal throughout the revision of the Convention and Directive and their Appendix and Annex. The thousands of different species are lumped together as 'fish' – when it would have been possible to account for many (or the majority) of fish used in the lab by focusing on a few widely used species, as we did for birds. Without a part B, there was no evidence that people could use to try to define species-specific standards that would improve the welfare of laboratory fish.



And of course this is not just about welfare – it is widely recognised that providing better quality and quantity of space, as in these larger enclosures for domestic fowl, also means better science. Given the current serious concerns amongst the scientific community with respect to the design, conduct, analysis and reporting of research, this has never been more important.



So what can be done, in the absence of defined and substantiated guidelines for fish species used in the laboratory, like the Atlantic salmon?

For now, there is one requirement in the Directive that can help to refine fish husbandry and care, including environmental enrichment. The local Animal Welfare Body has a number of important tasks including advising staff on matters relating to welfare, including accommodation and care, and keeping staff informed on 3Rs, technical and scientific developments. The AWBs are supposed to receive advice from their respective National Committees; this is not yet the norm, but the UK National Committee has begun formalising its communication with AWBs and encouraging them to network more widely. Other Member States, such as the Netherlands and Belgium, are developing networks for AWBs.

There is also a requirement in the Directive for establishments to have a person on site who is responsible for ensuring that staff have access to species-specific knowledge about the animals who they use and care for. These people, like the UK Named Information Officer, can be instrumental in bringing new scientific developments and good practice to the attention of research institutions.

All of these provisions, if properly implemented, could provide important channels for ensuring that new knowledge about fish behaviour, welfare and needs, and about empirically evaluated refinements, can reach establishments using fish. The ENRICH Fish project is playing an important role in helping to inform refinement for both laboratory and farmed Atlantic salmon, and I hope that projects like this will help lead to fish welfare being afforded the same priority as so-called 'higher' species.

Thank you