K. Species-specific provisions for fish

1. Introduction

The use of fish in scientific experiments 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 with immediate physiological consequences that can be relatively long-lasting and such changes, as well as having obvious welfare implications, will also 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

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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 keeping 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 that 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

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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 food 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 stocking density or temperature, or biofiltration.

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Susceptibility to the effects of 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 into 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.

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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 species of fish 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 and vibration

Fish can be acutely sensitive to sounds and vibrations, 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)

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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, or separate equipment should be used. 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.

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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

Consideration should always be given to enriching the environment of fish. 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

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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 usually 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 food.

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 food. Artificial diet is preferable, providing it meets the nutritional requirements of the species, and is acceptable to the fish. However, some fish species or life stages will not consume artificial diets. Artificial diets tend to have less impact on water quality.

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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. The feeding regime, palatability and the presentation of food should be designed to 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 food. 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

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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

(See paragraph 4.11 of the General section)

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 injection techniques are considered the least invasive method of marking. Careful consideration is needed before more invasive methods such as fin clipping or tagging are used. Mechanical tagging should not be used unless no other method is suitable.

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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.

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