Guidelines for reporting the results of experiments on fish

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Summary

A detailed account of experimental design, including an accurate description of the animals used, is an essential part of good research practice. Without these details, the reader will be unable not only to form an opinion on the significance of the findings but also to repeat the experiment in another laboratory. This paper presents suggested guidelines for reporting experimental studies using fish.

Keywords Fish; experiment; study; report; refinement

Previous studies have exposed an alarming lack of detail in scientific papers reporting animal experiments (Smith *et al*. 1997). This makes it difficult, if not impossible, to repeat or build upon previous studies, and it contributes to greater scepticism among the general public to science in general. The need to repeat experiments because of insufficient information is not only expensive and time-consuming, but is also unethical.

Fish are now widely used in experimental research, in a large range of environments. In Norway, fish constitute over 90% of all experimental animals used. There is an urgent need to improve the standards on how these studies are reported in the scientific literature.

Guidelines for the specification of animals and husbandry methods have been previously published (Ellery 1985). These guidelines are, however, designed primarily for use in studies involving terrestrial mammals. Aquatic species live in a totally different physical environment and are affected by a range of external influences which are rarely considered when planning experiments on traditional laboratory animals.

The present paper presents the factors which we consider are of importance when reporting the results of studies on fish. These suggestions are based upon the results of Ellery (1985) but have been greatly modified to take into account the special needs of aquatic species. Ellery (1985) noted the following reasons for good reporting, all of which improve the quality of scientific papers:

(1) It enables and encourages the researcher to identify the factors that influence an experiment.
(2) It helps the reader understand more fully the conditions that prevailed during the experiment, making it easier to interpret the results.
(3) It makes it easier for the authorities to accept experimental studies designed to document the safety of drugs and chemicals.
(4) It aids animal breeders in recognizing those factors which influence experiments, and encourages them to take these into account.
(5) It provides a basis for data banks on research animals.

Important factors to be reported

Water is of far greater importance to fish as an environmental medium than air is to ter-

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Accepted 24 November 1999

restrial animals. Not only does it support the fish physically, but it is also a source of oxygen, electrolytes and nutrients. It acts as a medium for osmoregulation and for the dilution of toxic metabolic wastes. Water quality therefore plays a critical role in all fish experiments and must be reported adequately, both to allow critical review of the paper and to enable the experiment to be reproduced elsewhere. Water chemistry varies dramatically between geographical locations. If space is limited, an analysis of the water used in an experiment should be kept by the author and distributed on request. This principle may be applied to other, less important information (Ellery 1985).

Other factors affecting fish used in research include husbandry conditions (Klontz 1995), feed composition (Lovell 1991) and their genetic background (Fevolden *et al*. 1993). Fish react to stress and other environmental challenges in much the same way as traditional research animals (Wedemeyer 1997, Wendelaar Bonga 1997). The effect of these external influences, not only on the intact organism but also on individual organs and even tissue cultures, has been well established (Fabacher 1981, Laitinen *et al*. 1981).

The European organization, Federation of European Laboratory Animal Science Associations (FELASA), has already produced a range of guidelines for the health monitoring of terrestrial laboratory mammals. Attention is now being focussed upon health monitoring of fish used in research.

Recommended minimal information

On this basis, we propose that information about the parameters listed below should either be reported in scientific papers, or at the very least recorded and made available upon request.

Animals

- Species
  - Strain
  - Stock
  - Hybrid
  - Mutant
  - Genetic modifications (e.g. transgenic)
- Age and/or weight and length at start of experiment
- Stage of development
- Sex (if possible)
- Microbiological status
  - Conventional
  - Specified pathogen free (SPF)
  - Gnotobiotic
- Method of transport
- Sedation during transport
- Transport survival (%)
- Quarantine period and/or acclimation period to holding conditions
- Acclimation period to experimental conditions

Water quality

- Water source
- Pre-treatment of inlet water
- Dissolved solids or turbidity
- Temperature (it should be mentioned whether the parameter is regulated or not)
- Dissolved oxygen (at stated temperature, and measured in outlet water)
- pH (at temperature)
- Alkalinity (at temperature)
- Ion strength, conductivity and/or hardness (at temperature)
- Salinity (at temperature)
- Ammonia (NH_3) and ammonium (NH_4^+) ions (at temperature)
- Recirculation of water
  - Treatment of recirculated water

Husbandry during experiment

- Offshore husbandry
  - Net design
    - Type
    - Material
    - Dimensions
- Onshore husbandry
  - Tank design
  - Type
  - Material
  - Colour
  - Dimensions
  - Height of water in tank
- Measures to maintain a given microbiological status

  - Open system (no special protection)
  - Closed system
  - Isolated system (treatment of inlet water)
- Number of fish per tank
- Cleaning procedures and frequency
- Water temperature (°C ± range)
- Lighting
  - Natural
  - Artificial (maximum intensity at water surface)
- Water changes per hour with proportions of fresh and recirculated water
- Water velocity in tank
- Procedures and equipment used during the experiment

Feeding

- Feed
  - Type and composition (Food composition should be related to species, according to the recommendations given by Lovell (1991). It is often sufficient to describe the feed brand name, but in some experiments, such as growth studies, a full description of the feed should be given.)
- Pre-treatment
- Administration method
- Feeding schedule

Experimental procedure

Depending upon the purpose of the experiment the following information should be reported:

- Numbers of fish used for each experimental phase
- Time schedule of the experiment
- Test substances used
  - Manufacturer
  - Supplier
  - Purity
  - Batch number
- Numbers of any fish dying during the experiment
- Statistical treatments

If the fish are anaesthetized the following should be reported:

- Dose
- Induction time
- Exposure time
- Recovery time
- Chemical anaesthesia
  - Agent
  - Manufacturer
  - Supplier
  - Batch number
  - Solute
- Inhalation anaesthesia
  - Concentration
  - Water source
  - pH of anaesthetic solution
  - Temperature
  - Aeration or oxygenation
  - Stage of anaesthesia
- Physical anaesthesia (stunning)
- Electrical
  - Method
  - Equipment
  - Electrical current used
- Injection techniques
  - Site of injection
  - Adjuvant

If sedated before injection, describe the method of sedation.

Examples

Atlantic salmon (Salmo salar)

These were held at ⟨The Laboratory Animal Unit⟩, *B* months old, weight *C* g and length *D* cm, were used. The fish were of conventional, unspecified microbiological status.

The animals were acclimatized to experimental conditions 2 weeks prior to the start of the experiment. Each experimental group of *H* individuals was held outdoors, exposed to natural light in green fibreglass tanks (3 m in diameter and 1.5 m high, supplier) with a water depth of 1 m. The water was from ⟨River⟩ and was untreated. The water had the following characteristics: temperature 6–8°C; oxygen in outlet water 7.2–9.2 mg/l; pH 6.4 ± 0.3; conductivity 150 mS/m; salinity 0.29; ammonia 0.005 mg/l; water velocity 1.5 m/s. The entire water volume was changed every *x* minutes. The tank was covered with a net to prevent attack by birds or the escape of fish. The fish were fed dry

pellets (1 mm diameter) *ad libitum*. This was a commercial standard diet from Fast-food Ltd and was given with an automatic feeder, (Auto-Feed Ltd). The study was carried out from 19 May to 10 July 1999 using five experimental groups (a total of *J* yearlings). *K* individuals died during the experiment (specify the numbers in each group). To measure stress parameters, blood samples were taken at 10:00 h GMT daily using the techniques described below.

A more detailed list of water chemistry and experimental design can be supplied on request.

Zebra fish (Brachydanio reiro)

The study used Zebra fish from Lab Fish-Animals Ltd, Anotherplace, Norway. The *X* adult zebra fish, *B* months old, weight *C* g and length *D* cm were SPF animals (details may be supplied on request). The fish were netted and transported to the research unit by air with 20 individuals in each of *E* plastic bags of 10 l filled with 7 l water and 3 l atmospheric air. Flight time was 3 h, and the total time from netting to placement of the animals in the research tanks was 6 h. The fish were sedated with a dose of *F* mg/l anaesthetic (reference) and the plastic bags placed in an insulated container (supplier) to ensure a stable temperature. On arrival *G* fish were dead. The animals were kept in a separate quarantine tank for 4 weeks for health monitoring. No signs of pathogens were found. The fishes were acclimatized to experimental conditions 2 weeks prior to the start of experiment. Each experimental group, consisting of *H* males and *I* females was held in one of five separated compartments in a fibreglass tank of 150 l (supplier).

Water source: recipient water was treated in several steps, first to remove particles (filter type), then to remove chloride (filter type) and finally UV irradiated to kill micro-organisms (equipment description) before entering a 2000 l holding tank for temperation. The tank was aerated using a pump (description) and temperation was carried out with (description of equipment). The following water parameters were measured and controlled: temperature in water (and room) $28 \pm 1^{\circ}$C; oxygen in outlet water 5.5 mg/l; pH 6.8 ± 0.5; conductivity 93 mS/m; salinity 0.003; ammonia 0.005 mg/l; water velocity 0.1 m/s. The tank volume of water was changed every *x* minutes. The fish were fed *ad libitum* on artemia each morning, from Live-food Ltd (address). The experiment was performed from 23 February to 19 April 1999 using five identical experimental groups (a total of *I* zebra fish). *J* individuals died during the experiment (specify the numbers in each group).

A more detailed list of experimental water chemistry and experimental design can be supplied on request.

Conclusion

We hope that these suggestions may contribute to an improved standardization of experimental work on fish, which will also facilitate the replication of published studies in other laboratories. This should also make it simpler to critically review research work, thereby reducing the danger of perpetuating poor experimental design. In the long term this should also contribute to a better understanding of the current need for animal experimentation, whilst ensuring high ethical standards.

These suggestions do not exclude the need to report other information in some studies.

Acknowledgments The authors wish to acknowledge the findings of the Working committee for the biological characterization of laboratory animals/GV-SOLAS (Ellery 1985). The present paper is based upon the principles embodied in the Committee's report.

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