***Animal***

**Review: Assessing fish welfare in research and aquaculture, with a focus on European directives**

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**Supplementary Material**

**Supplementary Table S1** *The impact on fish welfare, risk assessment and mitigation procedures of environmental parameters in aquaculture (A) and laboratory research (LR) contexts*

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| Water supply and quality |
| Impact on fish welfare | Intoxication due to the presence of toxic substances such as pollutants, metals, chlorine and ammonia that can influence the physiology, behaviour, and development of fish. Overly intense water flow can impair swimming ability of fish. |
| Risk assessment | A: water quality strongly depends on the source of water used (natural or artificial), and this must be carefully considered in the design phase of the farm. Sources of pollution in close proximity to the aquaculture facility can result in deterioration of water quality.LR: the source of water is usually tap water purified by reverse osmosis and purification systems that guarantee good water quality. Potential risks are represented by the unexpected deterioration of tap water.  |
| Mitigation | A: using filtration techniques such as mechanical, biological and chemical filtration and sedimentation.LR: using circulating systems equipped with mechanical and biological filters and periodic water changes. |
| Oxygen |
| Impact on fish welfare | Variation in the concentration of O2 affects the metabolism, growth and survival rates of fish and induces behavioural changes (swimming, escape response and schooling behaviour). |
| Risk assessment | The increase of temperature and eutrophication phenomena reduce the amount of oxygen in the water. |
| Mitigation | Reducing the consumption of oxygen by regulating fish density, by keeping the ambient area cleaned from rotting materials (faeces and food residues) and increasing the amount of oxygen in the water. A: oxygenation of the water can be ensured by gravity, surface diffusers and turbine aerators such as jumps of water, perforated trays, trickling filters, injection aerators, aerator pumps with a fountain spray pattern, paddlewheel aerators, ceramic plate diffusers for micro bubbles, and oxygen generator systems for intensive aquaculture, depending on the cases. LR: the amount of oxygen in the water can be increased by a wide area of ventilation, providing an efficient ventilation system with air stones and, if compatible with the experimental design, by using plants that produce oxygen through photosynthesis. |
| pH |
| Impact on fish welfare | pH affects metabolism and cell homeostasis, and variation can affect animal health by damaging the outer surface of gills, eyes and skin. |
| Risk assessment | Oscillation of pH values in the water source. |
| Mitigation | A: providing centralized buffering systems for pH control. To replace lost alkalinity and sustain the buffering capacity of water, carbonate (CO32-), in the form of limestone, bicarbonate of soda, or other common sources can be added. LR: using water with a proper pH and effective filtration systems with biological filters that help to maintain a constant pH. Water changes can help restore the pH level. Other options include use of rocks or substrates, increasing the aeration, adding bicarbonate of soda or using commercial buffers.  |
| Nitrogenous compounds |
| Impact on fish welfare | Intoxication due to the presence of urea and ammonia that compromise animal growth, cause generalized stress effects and induce histopathological changes in gill structure. |
| Risk assessment | Eutrophication due to a high density of fish or excessive food release and inefficient depuration systems. A: variation in nitrogenous compound amounts in the water source. LR: inefficient biological filters of the re-circulating system. |
| Mitigation | Using appropriate ratios between the amount of administered food and the weight of the animals and removing excess food and faeces.A: using centralized systems for the removal of food remains and faeces. LR: removing excess food and faeces after feeding and using efficient biological filters. |
| Environmental salinity |
| Impact on fish welfare | Variations in environmental salinity increase sensitivity to other stressors, lead to disease development and can affect neurochemical parameters. |
| Risk assessment | The evaporation of water causes an increase in the concentration of salt in the tank. A: salinity variation at the level of the water available for the farms. LR: proper salt concentration is usually obtained by mixing purified water with commercial artificial sea salt. Salinity variation between experimental tanks could be caused by mistakes in water preparation or differences between commercial salt composition. |
| Mitigation | A: preventing oscillations of salinity at the water source. LR: checking the salinity values using a refractometer and correcting the salinity in the tank by means of water changes. |
| Temperature |
| Impact on fish welfare | Variations in ambient temperature strongly affect fish biology and influence growth rate, food consumption, feed conversion, neurochemical parameters, physiology, and behaviour along with other body functions. |
| Risk assessment | A: change in temperature of the water source during the year. If the temperature of the water source does not correspond to the optimal temperature for the farmed species, the control and the change in temperature requires a very high expenditure of energy and is in most cases sustainable in closed systems or limited to specific phases of animal growth. LR: small differences in water temperature between tanks that may influence the experimental results. |
| Mitigation | A: using heating systems to regulate temperature at the water source or in the tanks.LR: maintaining a constant temperature in the room and using digital thermostats connected to heaters or coolers to ensure proper water temperature. |
| Light |
| Impact on fish welfare | Light affects life cycle, locomotion, and behaviour. |
| Risk assessment | Variation in light intensity and light-dark alternation. A: depending on the type of aquaculture system, animals can be exposed to artificial or natural light with consequent variations in light intensity and light-dark alternation during the year and between the tanks.LR: variation of light intensity and wavelength of the light spectrum between laboratories and tanks. |
| Mitigation | A: preventing bright light in the tanks, using shelters and artificial structures that allow the fish to be exposed to the optimal light level. When possible, light and photo period regulation for each rearing tank should be used. LR: using light sources with the correct wavelength and photo period regulation for each experimental tank, or for the laboratory room. |
| Noise (including vibration) |
| Impact on fish welfare | Noise can affect swimming modality and speed, group cohesion, reproductive success and behaviour. |
| Risk assessment | Excessive ambient noise and variation in its intensity. A: presence of intense low frequency sources of noise in the farm, or near the farm. LR: presence of intense low frequency sources of noise in the experimental room or due to noisy filtration systems. |
| Mitigation | Reducing environmental noise by using quieter equipment that produces less vibration and by isolating the working environment or the tanks with sound absorbing material. Vibrations can be kept at a minimum by placing the tank on top of a thick layer of acoustically insulating material (such as rock wool) that has proven to be effective in minimizing the conduction of external noise. This mitigation procedure is the same used by fish bio-acousticians to collect high quality sound production from their experimental fish. |
| Stocking density |
| Impact on fish welfare | Density affects growth rate and behaviour and impacts the quality of water. |
| Risk assessment | A: too high density used to maximize production. Limited knowledge of the social and predominance systems of the reared fish.LR: limited space in the experimental tank with consequences for territorial behaviour and inter-individual spacing. |
| Mitigation | A: reducing fish density in the rearing tanks, assessing the social and predominance systems of the target species. LR: shelters and compartments can be provided in the experimental tanks to regulate territorial behaviour. Experimental groups should be formed based on the social and dominance behaviour of the experimental species. |
| Environmental complexity |
| Impact on fish welfare | Environmental complexity influences coping activity and behaviour. |
| Risk assessment | The use of bare tanks with no opportunities for the fish to express sheltering or exploring behaviour and to develop a proper behavioural repertoire. The lack of environmental complexity can cause stress in animals.  |
| Mitigation | A: using artificial structures to offer shelters. LR: if compatible with the experimental design, environmental enrichment may be introduced in the tank. |
| Feeding |
| Impact on fish welfare | Feeding affects food conversion and growth rate. The amount of food impacts the production of waste and metabolites. |
| Risk assessment | A: inefficient food distribution within the rearing tanks due to high density and suboptimal regulation of territorial and social behaviour. LR: food quantity not calibrated in relation to density and the social system of the experimental species, with the risk of unequal distribution of food among individuals. |
| Mitigation | A: ensuring an equal distribution of food in the tank, by means of proper systems of food distribution. Calibration of food quantity in relation to stocking density. LR: assessing and calibrating food quantity and distribution in relation to the density and composition of the experimental groups and the social system of the experimental species. |
| Handling |
| Impact on fish welfare | Handling procedures increase animal stress and affect behaviour. |
| Risk assessment | A: frequent handling for transport and grading practice.LR: frequent hand-netting due to experimental work and transport. |
| Mitigation | A: ensuring recovery and acclimation periods and the use of appropriate anaesthesia. LR: experimental design with longer acclimation and recovery periods. |

**Supplementary Material S1**

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