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The Care and Management of Zebrafish (*Danio rerio*) in Research Laboratories

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The zebrafish (*Danio rerio*) has grown to be one of the most significant vertebrate model organisms in biomedical research because of its extraordinary genetic similarities to humans, rapid development, external fertilization, high reproductive capacity and transparent embryos. Zebrafish are widely employed in genetics, toxicology, pharmacology, neuroscience, cancer biology, regenerative medicine, environmental monitoring and aquaculture research in addition to their importance in developmental biology (Lawrence, 2011; Avdesh et al., 2012). The growing use of zebrafish in scientific research has brought attention to how crucial it is to uphold strict guidelines for animal management and welfare. Fish can sense pain, stress and unfavorable environmental conditions, according to scientific research, therefore providing them with compassionate care is both morally and scientifically required. In addition to improving animal wellbeing, appropriate husbandry techniques lower experimental variability and increase the validity and repeatability of study findings (CCAC, 2005; OECD, 2013).

The globally recognized 3Rs—Replacement, Reduction and Refinement—should be followed when managing laboratory zebrafish.he least number of fish needed to produce statistically significant results should be employed wherever feasible and every technique should be improved to reduce discomfort, agony and stress. The Institutional Animal Ethics Committee (IAEC) or the appropriate regulatory body must first approve any experimental protocols involving zebrafish and all personnel involved must be properly trained in fish husbandry, handling, breeding, health monitoring and experimental procedures (CCSEA, 2023). Only accredited laboratory animal suppliers or authorized breeding facilities with verified health and genetic status should be used to obtain healthy zebrafish. To reduce stress and stop the spread of disease, proper handling, transportation and acclimatization techniques are crucial. Before being introduced into established colonies, newly obtained fish should be kept in quarantine for a minimum of 14 days. Fish obtained from different sources should never be mixed until their health status has been confirmed. During quarantine, fish should be checked every day for odd behavior, mortality, parasite infections, or clinical symptoms of disease (Lawrence, 2011).

Housing and Environmental Management

Only accredited laboratory animal suppliers or authorized breeding facilities with verified health and genetic status should be used to obtain healthy zebrafish. To reduce stress and stop the spread of disease, proper handling, transportation and acclimatization techniques are crucial. Before being introduced into established colonies, newly obtained fish should be kept in quarantine for a minimum of 14 days. Fish obtained from different sources should never be mixed until their health status has been confirmed. During quarantine, fish should be checked every day for odd behavior, mortality, parasite infections, or clinical symptoms of disease (Lawrence, 2011). Recirculating Aquaculture Systems (RAS), which continually filter, disinfect and recirculate water to preserve superior water quality while lowering water usage, are frequently used in modern zebrafish facilities. Mechanical filtration (120-µm filter pad and 50-µm cartridge filter), biological filtration, activated carbon filtration, ultraviolet (UV) sterilization, aeration and temperature control make up a typical RAS. Sterilization efficiency generally decreases with extended usage, therefore routine filter cleaning and planned UV bulb replacement are crucial. To stop the spread of pathogens, fish nets and equipment should be properly cleaned with clean water, dried fully and disinfected with 70% ethanol or other approved disinfectants before being used again (Lawrence, 2011; Avdesh et al., 2012).

Controlling the environment properly is similarly crucial. The ideal photoperiod for zebrafish is 14 hours of light and 10 hours of darkness, with progressive dawn and twilight transitions lasting roughly 20 to 30 minutes to minimize stress. Continuous light exposure should be avoided as it inhibits normal spawning behavior. Fish behavior and reproductive success can be adversely affected by excessive vibration,

noise and needless human disturbance, all of which should be avoided while placing tanks. Zebrafish are mostly surface-oriented swimmers, despite the fact that they naturally inhabit the whole water column. As a result, rather than offering too much depth, tanks should offer sufficient surface area. While breeding tanks typically function best at a depth of around 10 cm, which facilitates successful spawning and egg collecting, a water depth of about 25 cm is appropriate for ordinary maintenance. Maintaining a healthy aquatic environment requires regular cleanliness. Every day, around one-third of the tank water in static systems should be replenished while collected organic waste is being removed. A weekly replacement of roughly 50% of the system water is usually adequate in recirculating systems. Fish should not be disturbed during cleaning methods and before tanks are put back into service, any disinfectants should be completely eliminated.

Stocking Density and Daily Husbandry

One of the most crucial elements affecting zebrafish welfare, growth, breeding effectiveness and water quality is appropriate stocking density. While excessively low stocking densities may have a detrimental effect on social behavior, overstocking

increases aggressive interactions, degrades water quality, stunts growth and increases susceptibility to disease. Larvae are supplied at around 20 larvae per 400 mL of water, whereas embryos are typically maintained at about 20 eggs per 100 mL of water. In well-maintained recirculating systems with effective filtration and feeding control, juveniles and adults can be kept at roughly 5 fish/L. Breeding tanks should have lower stocking densities of 2-3 fish/L, while static systems without biological filtration shouldn't have more than 1-2 fish/L. Fish size and stocking density should also be correlated with tank capacity. For isolation or nursery reasons, one or two adult fish should be kept in small tanks (0.8–1.5 L). Larger tanks (6–11 L) may hold 60–110 adult zebrafish under ideal filtration conditions, while medium tanks (1.8–3.5 L) can hold roughly 18–35 adults (Lawrence, 2011). Careful observation of swimming behavior, hunger, mortality, body condition and clinical symptoms of sickness should be part of everyday husbandry. To reduce the spread of disease within the colony, sick or damaged fish should be isolated right away for diagnosis and treatment.

Water Quality Management

The most significant environmental element affecting zebrafish health, reproduction, development and survival is water quality. Therefore, it is crucial to continuously monitor physical and chemical factors in order to maintain stable ambient conditions and guarantee repeatable study results. The ideal water temperature for growth and embryonic development is 28.5°C, while 26–28.5°C is advised. Conductivity should be between 300 and 1,500 µS/cm, dissolved oxygen should be above 6 mg/Land water pH should be kept between 6.8 and 7.5. Alkalinity should be between 50 and 150 mg/L CaCO3and total hardness should be kept between 50 and 100 mg/L CaCO3. Unionized ammonia below 0.02 mg/L, nitrite below 0.1 mg/Land nitrate below 50 mg/L are the minimum quantities of toxic nitrogenous chemicals. For laboratory zebrafish, salinity is typically kept between 0.5 and 1.0 g/L (Avdesh et al., 2012).Timely remedial actions are made possible by routine monitoring. While dissolved oxygen, hardness, alkalinity, ammonia, nitrite and nitrate should be tested at least once a week, or more frequently if anomalies arise, temperature, pH and conductivity should be checked every day.

Feeding and Nutritional Management

Normal growth, reproduction, immunological function and consistency in experiments all depend on a balanced diet. Particle size, feeding frequency, storage conditions and feed quality should all be properly controlled. Commercial diets of superior quality should be kept dry and refrigerated to avoid microbial contamination and nutrient deterioration. At five days post-fertilization (5 dpf), larvae start feeding. Initially, they should be fed live foods like rotifers, Paramecium, or micro-diets that are the right size (about 100 µm). As fish mature, the size of feed particles should progressively increase from 100 µm to 200–400 µm. While adults should get a combination of dry prepared feed and live or frozen feeds twice daily to maintain physical condition and reproductive

performance, juveniles are typically fed formulated diets supplemented with freshly hatched Artemia (Lawrence, 2011; Avdesh et al., 2012). Because uneaten feed quickly degrades water quality by raising ammonia concentrations and biological oxygen demand, overfeeding should be avoided. Therefore, after feeding,

residual feed should be removed as soon as possible.

Broodstock Management and Breeding

Maintaining healthy broodstock under ideal environmental conditions is essential to successful breeding. Strict biosecurity, regular health monitoring and good genetic management reduce the spread of disease and guarantee the generation of healthy progeny. While optimum reproductive function happens between 6 and 18 months of age, zebrafish usually reach sexual maturity at about 3 months of age. In a photoperiod of 14 hours of light and 10 hours of darkness, spawning is triggered soon after light begins. Typically, females lay between 70 to 300 eggs during a spawning and fertilization rates of more than 80% are regarded as acceptable. In order to preserve the health of their reproductive systems, females should be given at least one week to recuperate between spawning sessions. To prevent adults from eating eggs, breeding tanks should have spawning grids, marbles, or mesh inserts. Usually hatching in 72 hours, fertilized eggs are cultured in embryo medium at 28.5°C. Feeding begins at 5 dpf with live feeds or properly proportioned manufactured diets as development proceeds through the embryonic, larval, juvenile and adult stages (Reed & Jennings, 2010; Lawrence, 2011; Avdesh et al., 2012).

Conclusion

Reliable study results and animal welfare depend on the proper handling of laboratory fish, especially zebrafish (Danio rerio). Healthy fish populations are maintained by appropriate housing, ideal water quality, balanced feeding, appropriate stocking density and efficient breeding management. Stress and illness risks are further decreased by routine health monitoring, sanitation and biosecurity. The humane use of fish in research is encouraged by adherence to ethical principles and the 3Rs (Replacement, Reduction and Refinement). The reliability and scientific validity of experimental research are enhanced by standardized husbandry techniques. In general, superior biomedical and aquaculture research is built upon efficient fish management.

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