Chapter 1

Keeping and raising zebrafish

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1 Introduction

Over the past decade, the zebrafish has become an important model organism for biological research. In recent years, more than 1800 zebrafish mutants have been identified, providing a rich resource for the study of embryonic development in vertebrates. An increasing number of laboratories are now starting to use this unique collection of zebrafish mutants for their research.

Raising and maintaining zebrafish is more demanding than keeping stocks of invertebrates, but less so than keeping stocks of mammals. This chapter is aimed at giving a background on how to raise mutant and laboratory wild-type stocks and how to maintain them in good breeding conditions. We describe aquarium systems and water conditions as well as procedures to raise and breed zebrafish. While this chapter is aimed primarily at investigators entering the zebrafish field, the recipes and protocols may also provide useful information for experienced zebrafish researchers.

2 Aquaria systems and water conditions

2.1 Aquaria systems

The major demands to aquaria systems are posed by the problem of keeping the water in good condition. If the water is not constantly kept clean, the fish will suffer from the toxicity of their own excretions and derivatives thereof. The simplest way to keep the water clean is to keep fish at very low densities and to replace the water at regular intervals. A more elaborate method is to equip the aquaria with biological filters through which the water is circulated and purified. The filters contain material with a large surface area on which bacteria can grow, which degrade toxic ammonium to less toxic nitrates. In larger systems, water from several (often more than 1000) tanks may be recycled through one large common filter (recirculating systems).

The exact type of facility required depends very much on the actual work to be done. For experiments involving only wild-type embryos and one or two mutant strains, a few large tanks are sufficient. However, genetic experiments with zebrafish often need facilities with up to several thousand tanks. When setting up such a system, the space requirements have to be considered, as well as the costs of maintenance and the overall safety of the system. In several laboratories, large-scale aquaria systems that are tailored to a variety of experimental needs have been built. The principles of such systems are described below.

2.1.1 Systems without filtering

On the one hand, non-circulating systems avoid all costs of establishing and servicing filters and are therefore relatively cheap to set up. On the other hand, they are very space-demanding, because fish have to be kept at low densities. Moreover, they require high maintenance and, in practice, they work only in areas where water of very high quality can be produced at a low cost.

Adult fish can be maintained without feeding and water exchange for up to 10 days at low densities (no more than two fish per litre). Keeping fish for longer periods requires feeding and regular water exchange. This can be done on a daily basis or continuously. In flow-through systems the tanks are typically large (20–200 litres) and each has its own adjustable inlet and an overflow (for example, a hole, equipped with a sieve, drilled just below the upper rim of the tank). The rate of freshwater supply should be about 0.3 tank volumes per day, allowing fish densities of one fish in 1–3 litres, depending on the feeding regimen. The tap water should be charcoal filtered and run through ultraviolet (UV) lamps before entering the aquaria. As the charcoal removes oxygen from the water, it is necessary to aerate the tanks using bubble stones. Care has to be taken to avoid overfeeding of fish, as surplus food quickly rots and thus decreases the water quality. The tanks have to be cleaned daily to remove faeces and surplus food after the last feeding. To avoid spread of infections between tanks, it is routine to handle fish only with sterilized or disposable items. However, it is not easy to avoid spreading infections in genetic experiments, where it is often necessary to mix fish from different tanks and different rooms.

2.1.2 Recirculation systems

With biological filters, the water is purified, cleaned, and reused. A principal advantage of recirculation systems is that they provide high-quality water and a high water exchange rate. For small-scale experiments, fully equipped tanks with an appropriate filter, illumination, heater, and aeration can be purchased at local pet stores. Gravel and plants are not required. The tanks should be placed on a dark surface (because the fish like those better than light surfaces) and not in the vicinity of windows, in order to avoid growth of algae. With an appropriate filter, which is serviced regularly, and bi-weekly exchange of one-third of the water, fish can be kept at densities of about one fish per litre with little maintenance.

In large-scale recirculating systems, the water of many tanks is cleaned and regenerated through a common filter unit. Such systems therefore permit the raising and maintaining of very large numbers of fish in a comparatively small space. As hundreds, or more, tanks can be serviced by one filter, very high-quality water is provided with little maintenance. On the other hand, diseases can spread between the interconnected tanks in recirculation systems. Sterilizing the water by UV irradiation before redistribution can reduce this problem.

A large-scale recirculation system suited for genetic screening purposes was developed for the 1993 Tübingen mutagenesis screen. This system has proved to be very reliable and effective, and is therefore described here in detail (*Figure 1*). Smaller-scale versions of this system, with minor modifications, are now in use in many zebrafish laboratories throughout the world, including the authors' laboratories. The system was built by an experienced company (Schwarz, Germany), but similar systems are now also available from other companies (e.g. Müller & Pfleger, Germany or Marine Biotech Inc., USA).