RSPCA ANIMALS IN SCIENCE

Animals in Science Department
Resources for AWERB members
September 2021

# Environmental enrichment for zebrafish

## Aim of this resource

To help AWERBs discuss local practice for providing laboratory zebrafish with environmental enrichment.

## Relevant AWERB task

Advising staff on animal welfare and the application of the 3Rs; supporting named persons and others dealing with animals.

## Recommendation

Regularly review and discuss different forms of environmental enrichment that can be provided to laboratory zebrafish.

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## The issue

Zebrafish used in scientific research tend to be provided with relatively little environmental enrichment. This may be in part due to a belief that zebrafish do not benefit from enrichment. However, there is now plenty of evidence that providing enrichment can reduce stress and improve welfare in zebrafish. This sheet can be used as a resource to stimulate discussion of different forms of enrichment which can be provided to zebrafish.

## Key points:

- Environmental enrichment refers to modifications which are made to the environment of a captive animal to improve welfare.
- The natural habitat of the zebrafish tends to be highly complex and much more varied than a laboratory tank.
- Zebrafish prefer tanks containing physical structures like plastic plants, shelters and substrates.
- Providing physical structures like plastic plants can improve zebrafish welfare.
- An image of gravel affixed to the bottom of the tank is preferred almost as much as real gravel.
- Some studies suggest that physical structures can increase aggression, but evidence on this is mixed.
- Social contact is sometimes referred to as enrichment, but should be considered a basic need for zebrafish.
- Aggression may be more likely in smaller groups of zebrafish (<6 individuals), so caution should be exercised and groups appropriately monitored.
- Live (invertebrate) food is often used as enrichment, and is likely to benefit zebrafish welfare.
- There is some evidence that occupational enrichments (those which give animals something to do), such as providing fish with flowing water to swim against, may improve welfare.
- Some sensory enrichments, including visual enrichment (e.g. images outside the tank) or auditory enrichment (e.g. music), may benefit zebrafish welfare, although the evidence is currently limited.
- More research into the effects of enrichment would be helpful, but there are already several interventions which we know are likely, on the balance of the evidence, to be worth using.
- Adding enrichment to fish tanks may require extra time and investment, so animal care staff should be provided with the support to introduce an enrichment strategy in their facility.
- More information on the evidence in favour of enrichment for zebrafish can be found at: https://doi.org/10.3390/ani11030698.

# Background Information:

- **Environmental enrichment refers to modifications which are made to the environment of a captive animal to improve welfare.** These modifications can take many forms, such as the addition of physical structures, social contact, occupational items (which give the animal something to do), dietary variety or sensory complexity [1]. They may improve welfare because they make the environment more interesting, or resemble wild habitats more closely.
- **The natural habitat of the zebrafish tends to be highly complex and much more varied than a laboratory tank.** Wild zebrafish are found in India and Nepal, and tend to inhabit small, slow-moving streams and rivers [2–5]. These environments may contain plants, substrate and debris, other zebrafish and other species, including predators, water flow, olfactory (smell) and other sensory cues. These environments are also highly variable, changing with the weather or the season.
- **Zebrafish prefer tanks containing physical structures like plastic plants, shelters and substrates.** When housed in tanks which allow zebrafish to choose between chambers containing physical structures, or empty chambers, zebrafish prefer to spend much more time in the chamber containing the physical structures [6–10]
- **Providing physical structures like plastic plants may improve welfare in zebrafish.** Tanks containing physical structures may contribute to lower anxiety levels, reduced stress, normal social behaviour, better learning and memory, larger brain size, improved fertility and fecundity, and improved ability to cope with pain [11–17].
- **An image of gravel affixed to the bottom of the tank is preferred almost as much as real gravel.** When zebrafish were given a choice between a barren tank and a tank with a gravel image affixed to the base, they showed a preference for the gravel image. This preference was almost as strong as when the fish were given a choice between a barren tank and real gravel [7]. Using gravel images in laboratory settings may be more practical than real gravel as it requires less maintenance.
- **Some studies suggest that physical structures can increase aggression, but evidence on this is mixed.** It has been suggested that adding structures to tanks may increase zebrafish aggression as they may become territorial over certain items, and some studies have found evidence of increases in aggression associated with adding structures to tanks [18,19]. However, other studies have found that adding items to tanks may have no effect on or may reduce aggression [20–23]. Animals should always be monitored carefully after any change to housing or husbandry.
- **Social contact is sometimes referred to as enrichment, but should be considered a basic need for zebrafish.** Zebrafish are a social species [24], so should not be singly-housed – this should be considered an impoverished environment. If zebrafish are to be held individually (e.g. for genotyping), this should only be for short periods and should allow visual and, if possible, olfactory contact between individuals. Singly-housed zebrafish should also be provided with other forms of enrichment, such as plastic plants, to help reduce stress [25,26].
- **Aggression may be more likely in smaller groups of zebrafish (<6 individuals), so caution should be exercised and groups appropriately monitored.** The optimum group size or stocking density for zebrafish has not been determined, and is likely to depend on a number of different factors, but larger group sizes are likely to result in lower levels of aggression [27].

- **Live (invertebrate) food is often used as enrichment, and is likely to benefit zebrafish welfare.** Using live food such as *Artemia* or rotifers as part of the zebrafish diet is likely to be enriching as it provides more variety, and may stimulate natural hunting behaviours. Lots of anecdotal reports suggest that live food benefits zebrafish welfare, but this has not yet been empirically supported by research. Other forms of enrichment, such as plastic plants, have more supporting evidence to show they can improve welfare, so live food should ideally be used in addition to other forms of enrichment.
- **There is some evidence that occupational enrichments (those which give animals something to do), such as providing fish with flowing water to swim against, can improve welfare.** Occupational enrichments are those which give an animal something to do. Zebrafish show a preference for environments containing water flow when it is combined with physical structures, although they show an aversion to environments which contain water flow alone [8]. Exercise in flowing water may also lead to better learning and memory, and has a wide range of physiological and psychological benefits in other vertebrates.
- **Some sensory enrichments, including visual enrichment (e.g. images outside the tank) or auditory enrichment (e.g. music), may benefit zebrafish welfare, but the evidence is currently limited.** For example, auditory enrichment in the form of classical music played in the fish facility may contribute to lower anxiety [28,29]. It is also possible that adding dawn and dusk phases to lighting cycles may reduce fish startle responses and improve welfare, but research is needed to investigate this.
- **More research into the effects of enrichment would be helpful, but there are several interventions which are likely, on the balance of the evidence, to be worth using.** These include adding gravel images underneath tanks, providing live food, and ensuring that any temporarily singly-housed fish are provided with visual and olfactory contact with other fish and with physical enrichment.
- **Adding enrichment to fish tanks may require extra time and investment, so animal care staff should be provided with the support to introduce an enrichment strategy in their facility.** New enrichment items may cost money to purchase, take time to introduce, and require animal care staff to spend more time cleaning or maintaining the items. This investment is worthwhile due to the welfare benefits enrichment can have, but animal care staff should be appropriately supported and given enough time to complete this work.
- **More information on the evidence in favour of providing enrichment for zebrafish can be found at: https://doi.org/10.3390/ani11030698**

**For a full list of references, please click here.**

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