National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

Welfare webinar: Improving the lives of laboratory zebrafish

Question and answer session with Professor Robert Gerlai

This Question and Answer (Q&A) document accompanies the recording of an NC3Rs welfare webinar 'Improving the lives of laboratory zebrafish' held in May 2025. In the webinar our speaker Professor Robert Gerlai explored an ethological approach, grounded in an understanding of zebrafish behaviour and ecology, to improve care of zebrafish in the laboratory and scientific outcomes. Robert provides written answers to the questions submitted during the webinar below, including both those answered live and those he did not have time to answer during the session.

Please watch the webinar recording for full context and further information.

1. When you mentioned pouring the fish is preferred over netting, what would be the best practice for this? Directly from tank to tank?

Yes. We first gently lower the water level (siphoning) in the home tank while this tank is in its usual location, then we move the home tank to the destination tank's location and pour the fish into the destination tank. But, I note this was just a proof-of-concept study (preliminary idea to demonstrate differences in how handling procedures may affect the fish). From a practical standpoint, pouring is often not feasible (for example, when you want to select a particular single fish to test). My recommendation at this point is to use the beaker method. It's simple, almost as minimally invasive as the pouring method, and fewer things can go wrong with it than with the pouring method. The beaker method is when you gently catch the fish with a net the usual way, and then put the fish (while in the net) into a beaker, carry the fish to its intended location (test tank or new holding tank), and lift the fish out of the beaker and put it into the destination tank. This method minimizes the length of air exposure, which, in my opinion, is one of the main reasons for the invasive (fear and stress inducing) nature of human handling for zebrafish.

2. Have the refined handling methods been implemented in your own lab and are you aware of any other labs that have adopted these methods? Has there been any pushback towards the techniques and what barriers have you faced?

Whenever we can, we use the pouring method. But often it's not practical, so instead we use the beaker method. I do not have a systematic survey of current handling practices employed in zebrafish facilities, so I can only answer your question based upon limited and anecdotical evidence: most facilities I know of do not use any of the less invasive handling methods (pouring or the beaker methods). My impression (again, based

upon limited feedback and anecdotes) is that most facilities ignore the potential issue of human handling induced stress. But I also note that our papers on these issues were published quite recently, so they haven't had the time required to make an impact. Also, I noticed the issues I raised in my webinar have started to be discussed more frequently at conferences, workshops and also in peer reviewed literature. So, I think we are moving in the right direction.

3. Do you have any SOPs or guidance you can share on refined methods of handling zebrafish?

We have not developed an SOP. But our peer reviewed publications may be of help here. I'm pasting the bibliographic details of the publications I mentioned in the webinar below in the order they appeared on my slides. The last paper on this list (in bold) is the one directly relevant to your question. The rest are tangential but may be used to answer some other questions posed by others listed after your question.

- Tsang B *et al.* (2020). Maintenance and breeding of zebrafish, with some ethological and ecological considerations in mind. In: Behavioral and Neural Genetics of Zebrafish (Ed. Gerlai R), Elsevier Academic Press.
- Tsang B and Gerlai R (2024). Nature versus laboratory: How to optimize housing conditions for zebrafish neuroscience research. *Trends Neurosci* 47: 985-993. doi: 10.1016/j.tins.2024.08.013.
- Gerlai R (2025). The importance of understanding the ethology and ecology of the zebrafish, and of other fish species, in experimental research. *Biologia Futura* (invited review). doi: 10.1007/s42977-025-00257-3.
- Parichy DM and Postlethwait JH (2020). The biotic and abiotic environment of zebrafish. In: Behavioral and Neural Genetics of Zebrafish (Ed. Gerlai R), Elsevier Academic Press.
- Abozaid A *et al*. (2021). Behavioural effects of acute ethanol in larval zebrafish (*Danio rerio*) depend on genotype and volume of experimental well. *Prog Neuropsychopharm Biol Psych* 112. doi: 10.1016/j.pnpbp.2021.110411.
- Shishis S *et al*. (2022). The effects of housing density and tank size on the behaviour of zebrafish: Towards the optimization of maintenance of zebrafish. *Frontiers in Behav Neurosci* 16:934809. doi: 10.3389/fnbeh.2022.934809.
- Abozaid A and Gerlai R (2024). A search for effective reinforcers in appetitive conditioning for adult zebrafish: Ecologically relevant unconditioned stimuli. *Prog Neuropsychopharmacol Biol Psych* 131(110946). doi: 10.1016/j.pnpbp.2024.110946.
- Mahabir S and Gerlai R (2017). The importance of holding water: Salinity and chemosensory cues affect zebrafish behavior. *Zebrafish* 4: 444-458. doi: 10.1089/zeb.2017.1472.
- **Shishis S *et al*. (2023). Effects of Different Handling Methods on the Behavior of Adult Zebrafish. *Physiol Behav* 262: 114106. doi: 10.1016/j.physbeh.2023.114106**.

4. Do you use aragonite sand for stable pH as well as slightly increase conductivity? if yes, can you advise the volume used to meet your required set points and any other benefits of aragonite sand?

Yes, aragonite will increase pH and will buffer against downward fluctuations in pH (acidity, most often the main issue with pH in zebrafish facilities). The benefit of aragonite is that once put into the system water container (this is what we do) or in the sump or other external filter, it will provide a maintenance free, ”automated”, solution. We have not conducted any systematic experimental studies on how much aragonite would be appropriate and thus, unfortunately, I cannot give specific concrete answers to the question of what weight of aragonite would be needed for a given volume of water to bring the pH and salinity to the appropriate level. In our facility, we put 40 kg of aragonite (in mesh-bags) into our system water container, 4,000 litre volume of water. This weight to volume ratio is not enough to substantially increase salinity, so we do use Instant Ocean (mainly NaCl) to achieve conductivity of around 150-200 micro-Siemens/cm. We decided to continue with NaCl-based adjustment of conductivity simply to make our conditions comparable to those used in most zebrafish facilities. We, however, hope that as others and us are learning more about salinity and salt constitution of the natural waters of zebrafish, the so called “industry standard” of NaCl use will diminish.

Another important point I want to emphasize here concerns your question about pH and whether and how to adjust it. We do not worry about pH itself. What I mean by this is that we use pH only as an indicator of underlying issues not as our main target parameter. Low pH (acidity) is most often the problem in zebrafish facilities (and also in freshwater aquarium hobby). It is the result of too much organic waste (which acidifies the water). For zebrafish it is this excess organic waste amount which is the problem, not low pH. Too much organic waste can accumulate as a result of inefficient filtration, not enough water change, too many fish, too high fish density, overfeeding, for example. Addressing these underlying causes is the way, in my opinion, with which we should increase pH. The usual practice of adding sodium bicarbonate, or sodium carbonate, just covers up, but does not address, the underlying problems. Also, quite often, facilities dump sodium carbonate into the sump of zebrafish racks too fast. Rapid changes in pH are much more harmful than an out of range pH value. Only slow modification of pH with these salts is appropriate.

5. In addition to changing the way the zebrafish respond to conspecifics, are you aware of any other examples where differences in water conductivity or pH (ranging in reasonable values) could result in different phenotypic responses?

There are only sporadic examples. Systematic analyses to address this question are lacking. It is possible that many behaviours, many physiological or biological functions are well buffered against out-of-range environmental parameters. But see the theoretical example I discussed on why requiring this “buffering” can come at a cost, i.e., can result in increased error variation and reduced replicability (for further details and supporting empirical evidence please see Tsang & Gerlai (2024) and Gerlai (2025) from the above list of papers).

6. To my knowledge higher salinity helps with keeping pathogen growth in check and lower salinity could have an impact on the general health of the adult fish. Are you aware of any studies that studied this connection, and what have your own experiences been regarding this when keeping your fish at your facility?

This was exactly one of the questions that the 2017 NIH/ORIP workshop I referred to in my webinar brought up. At that meeting, facility managers advocated high salinity due to its bacteriostatic effect. They observed cleaner fish tanks, less algal growth etc. In our hands, however, salinity levels mimicking what is found in the natural habitats of zebrafish did not have any deleterious effects in terms of diseases or any other practical aspects of zebrafish maintenance. I would argue that if zebrafish live in nature under these low salinity conditions, they must have been adapted to these conditions, and deviating from these conditions may stress the fish (tax their homeostatic processes). Thus, although you may minimize pathogenic load, stressed fish may become more vulnerable to diseases and other problems. Again, this is just my intuition. Due to the absence of conclusive systematic parametric analyses in which salinity levels would be controlled and numerous salinity levels would be examined in terms of their effects on a variety of phenotypes (biology, physiology, diseases, behaviours, etc.) of zebrafish, it is too early to decide what is optimal. But it is time we start asking questions like yours, and start conducting such analyses.

7. What approach(es) could I take back to my facility to start improving zebrafish welfare today?

With some hesitation, and acknowledging that my answer is based upon personal biases, here is my list: increase tank volume; decrease fish density; feed more frequently and less amount of food at a time; increase illumination level; put a photo underneath the tank mimicking a solid bottom surface like gravel or silt; add aragonite; do not adjust pH with salts unless absolutely necessary, instead address underlying causes leading to acidity; if it is a must, adjust pH only slowly (over multiple days) to reach desired value; reduce salinity; handle the fish as gently and as humanely as possible using the least invasive and practically feasible handling method (beaker). Make any changes like the above suggested ones only gradually. Remain flexible. Keep in mind that many of the above suggestions are not based upon systematic empirical research. Remain willing to compromise: that is, consider other factors important for the facility. For example, take into local, practical and financial considerations specific to your facility. Listen to PIs, vets and regulatory personnel, and keep an open discussion about where a healthy compromise for your facility may lie.

8. Are plastic plants in small holding tanks still beneficial even though zebrafish prefer open water and might be restricted in their swimming activity that way?

Likely not. Movement of adult zebrafish in a small tank is already compromised. Adding any structures (plants in this case) to a small tank would further hinder the fish's ability to swim freely. But, as I mentioned above, we do not yet know how much space would be optimal for zebrafish and how much adding plants would help and under what conditions. It (adding plants or hiding places) is just an idea, a working hypothesis to be confirmed/rejected/optimized by future systematic studies.

9. Have you observed increased aggression between zebrafish that have environmental enrichment, such as plastic plants?

Not really. But again, it is just a personal observation that needs to be tested/analysed empirically. For example, plants or other forms of enrichment may enhance spawning activity in the morning, and thus elevated aggression during this period may occur. However, unlike several other fish species, like cichlids or anabantids, cyprinids and other shoaling species are not particularly territorial, they also do not maintain rigid dominance hierarchies. Instead fish of these species form shoals in which individual members tend to be quite peaceful towards each other. Thus, aggression tends to be less of a problem in the maintenance of such shoaling species (like zebrafish, for example). However, again, these are quantitative questions, like most other scientific questions, that is, the answer is not black and white.

10. Have you ever tested for a preference between live and plastic plants? What would the benefit of live plants be compared to plastic plants?

We have not. But it is an interesting question. Brief list of pros and cons. Live plants, pros: can improve water parameters (removal of organic waste/toxins); can grow and be propagated and thus it may be less costly to employ them on the long run. Live plants cons: not many species would be well suited for the zebrafish facility tank (e.g., dim light, lack of nutrient rich substrate), but some anubias species (e.g. *Anubias nana*) would be perfect; non-standard size of these plants may induce error variation; if not kept well, decomposing plants can negatively affect water quality; potential issues with pathogen introduction (but plants can be easily decontaminated). Plastic plants pros: easy and cheap to buy them, there are many options; standard size and shape across tanks and facilities; easy maintenance and cleaning of the plant. Plastic plants cons: toxins, water soluble chemicals, may leach out; sharp or hard edges of the plant may cause physical damage to fish. Cons with both live and plastic plants: may obstruct view of the inside of the tank and thus may limit detection of sick fish; may complicate tank maintenance.

For more details, see: Tsang B and Gerlai R (2022). Common aquarium plants as an enrichment strategy in zebrafish facilities. *Zebrafish* 19: 218-223. doi: 10.1089/zeb.2022.0036.

11. Are we sure that the parameters found in nature are the best to be applied to fish in captivity? For instance, in nature fish density could be less the 1/L because of the environment, but in tank without this environment fish might feel happier at greater density?

True. Rigidly adhering to values of environmental parameters typical of the natural habitat of zebrafish is not a good idea. Deviating from these parameters in an extreme manner, i.e., maintaining zebrafish under conditions that are extremely different from how they live in nature, is also not the best idea. Where the healthy compromise may be must be established empirically. However, there are too many different environmental parameters and too many values for these parameters one would have to consider in said empirical studies. So, where should we start? What I am advocating is to start systematic empirical studies with nature in mind. That is, we should consider the midpoint-value of the given environmental parameter, the

average of values found across habitats in the geographic areas where zebrafish are found in nature, and from this average value extend the range, i.e., analyse the effects of the given environmental parameter when we increase and decrease its values from this starting mid-point. Regarding the specific question of tank size and fish density in the facility vs natural conditions: the fish/volume of water ratio in nature is clearly impossible to match in the lab. We will never be able to have such huge volumes of water per fish as the fish experience in nature. Regarding micro-locality fish density, we already know quite a lot what ideal shoaling distance (inter-individual distance or nearest neighbour distance) is for zebrafish (please see Miller and Gerlai papers referenced below). Based on the latter, we can guestimate what fish density should be idea from a psychological/behavioural standpoint. But I emphasize this is only one aspect. Other questions regarding fish density concern physiological/biological processes and also water quality parameters associated with fish density and tank volume. All of these must be systematically analysed before we can answer what will be ideal for zebrafish in the facility. Nevertheless, from the limited empirical data we and others have collected so far, it appears the crowding the fish in tiny tanks as most of us usually keep our zebrafish, is stressful for them, and is thus suboptimal.

- Miller N and Gerlai R (2012) From Schooling to Shoaling: Patterns of Collective Motion in Zebrafish (*Danio rerio*). *PLOS ONE* 7(11): e48865. doi: 10.1371/journal.pone.0048865.
- Miller N and Gerlai R (2011). Redefining membership in animal groups. *Behavior Research Methods* 43: 364-371. doi: 10.3758/s13428-011-0090-z.
- Miller N and Gerlai R (2011). Shoaling in Zebrafish: What We Don't Know. *Rev Neurosci* 22: 17-25. doi: 10.1515/rns.2011.004.
- Miller N and Gerlai R (2008). Oscillations in Shoal Cohesion in Zebrafish (*Danio rerio*). *Behav. Brain Res* 193: 148-151. doi: 10.1016/j.bbr.2008.05.004.
- Miller N and Gerlai R (2007). Quantification of Shoaling Behaviour in Zebrafish (*Danio rerio*). *Behav. Brain Res* 184:157-166. doi: 10.1016/j.bbr.2007.07.007.

12. Is there any evidence that the light-dark cycle of 12 hour on:12 hours off is optimal compared to natural conditions?

We keep our fish on a cycle of 14h light 10h dark. Others at 12:12. I do not know if this difference affects the behaviour or any other phenotype of zebrafish if maintained consistently. From the wide geographic distribution of zebrafish in nature, I can speculate that likely the species is well adapted to this circadian light variation (fish that live nearer the equator, e.g. Southern parts of India, will experience the 12:12 cycle, whereas fish further North, e.g. in Nepal, will experience an increasing light phase during summer reaching 14:10h light:dark, and an increasing dark phase during winter).

13. Have you studied or are you aware of any studies looking at the effect of salinity on behaviour in both larvae and adult fish?

No. This question needs to be addressed empirically.

14. Do you think rotifer polyculture is ok for larval zebrafish, particularly in regards to the salinity required?

We have been using such cultures, but you are right, increased salinity may be a problem due to salts entering the zebrafish water with the food, if feeding/food preparation is done incorrectly.

15. What do you think about the amount of swimming our zebrafish do in tanks? Also, do you think they are more stationary than they would be in the wild, and would they benefit from encouraged swimming?

Difficult to answer. In small, densely stocked zebrafish tanks, I believe the fish are forced to interact with each other too much. This increases physical activity. In large tanks, zebrafish shoals do not move much after the fish get habituated to their new tank (there is very little synchronized, unidirectional, movement among shoal members), i.e., fish slow down. In nature, I assume shoals keep moving and searching for food and will also respond to predators by moving away from them. What is the “normal”, “healthy”, level of activity, I don’t think we know. But I would recommend against forcing the fish to swim more than what they would normally move.

16. Have you observed any differences in the outcome of sex ratios amongst any of your various studies?

There are many aspects of this question. I will try to address each of them, depending on how I interpret the question. 1, we have not systematically manipulated sex ratio during routine housing of the fish and thus we do not know what, if anything, it may affect. 2, we also have not studied how modification of environmental parameters may affect sex ratio in the fish that develop under these modified circumstances. 3, sex differences have been demonstrated in zebrafish in a variety of phenotypes. In our studies, we most often do not detect significant sex differences. These are all exciting questions, and, especially the 2nd type, has been investigated with other fish species.

17. Do you think a more extensive and consistent reporting of husbandry conditions in publications, would contribute to extract meta-data and impact reproducibility?

Absolutely! This is a very important point. I strongly recommend we disclose as much info as possible on maintenance conditions as well as handling procedures. As you wrote, if available from the literature, these pieces of info then could be used for meta-analyses which then could be highly informative right away, but also could be the starting points for possible future systematic analyses I mentioned above.

18. Do you have any statistically significant controlled experiment that can enable you to back up your statement that any of the parameters you have studied do affect experimental variability.

We do. But, this area of research is just started to expand. So, the proof is limited, at least for zebrafish. For example, we have clear evidence that more invasive handling methods not only lead to elevated anxiety and stress in zebrafish, but also to elevated variance in a variety of behavioural responses. There is a rich literature on other species (mostly in the mouse) that show similar findings across a variety of phenotypes, and thus provide strong support for the theoretical/example I discussed. For further details, please see above cited papers (e.g., Tsang & Gerlai, 2024; Gerlai 2025).

19. Are there any standardised commercial pellet diets that you would recommend for maintaining zebrafish? Or should there be more work in this?

Yes, there are standard foods, and yes there should be more work on this. I hesitate to disclose what brand/food type we use in our facility simply because it may be viewed as conflict of interest. Nevertheless, your question has been one of the issues discussed in the 2017 Zebrafish workshop I referred to in my webinar. The question continues to be discussed with no clear consensus emerging yet. Also consider several other questions surrounding it. Briefly, everyone has their own preference, and sometimes it is based on very different considerations (price, vendor reputation/stability, easiness to obtain the food, quality of the food in terms of health effects, nutrients, growth rate, etc., stability of the components in the food, i.e., how consistent the food is over different batches, time periods, and the list goes on). We have our own preference, but this preference is based mainly upon practical considerations rather than systematic scientific comparison of how different food types affect the fish. But see a recent paper that started to tackle this question, at least from the perspective of how we could find out what food types/items zebrafish prefer:

Abozaid A *et al*. (2025). A novel food delivery method for learning studies detects significant differences in food preference in zebrafish. *Zebrafish* 22: 20-29. doi: 10.1089/zeb.2024.0176.