**Checklist of additional questions on the use of zebrafish overseas**

The expectations of the major UK public funding bodies for the use animals in bioscience research are set out in the document ‘[Responsibility in the Use of Animals in Bioscience Research’](https://www.nc3rs.org.uk/responsibility-use-animals-bioscience-research). Compliance with the principles in this document is a condition of receiving funds for animal research. Welfare standards consistent with the principles of UK legislation must be applied and maintained,whereverthe work is conducted. For further information, see [Use of animals overseas](https://www.nc3rs.org.uk/3rs-resources/peer-review-and-advice-service).

Please confirm the following for the list below: **Yes/No**. Where ‘No’ is given, please justify this in the [Exceptions box](#Text12) at the end of this form. Please note that the below items refer to both stock and experimental fish.

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| 1. The zebrafish have been bred and reared in captivity for scientific purposes.
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| 1. Adult and larval fish are housed separately. Fish are housed in mixed-sex groups at age-appropriate densities (e.g. 10-30 larvae/litre for ~5-30 dpf; 5-10 fish/l for >30-90 dpf; 4-6 fish/l >90 dpf). If individual housing is required temporarily (e.g. for genotyping), other fish are visible in immediately adjacent tanks and fish are returned to group housing as soon as possible.
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| 1. Larval fish up to ~6-7 dpf are kept in shallow water (<20cm) until their swim bladders are fully inflated and normal swimming is observed. Physical disturbances, such as handling, are minimised or avoided during the early life stage.
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| 1. For all life stages, water changes/exchange and cleaning protocols are appropriate for the tank system in use and the quality of water entering the system. Solid waste is routinely removed from the tanks of adult fish. All tanks are covered with lids.
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| 1. Water quality parameters fall within the appropriate bounds of the following ranges, taking into account that the potential toxicity of ammonia increases with pH: total ammonia (NH3/NH4) <0.1 mg/l; nitrite (NO2) <0.1 mg/l; nitrate (NO3) <25 mg/l; pH 6.5-8; conductivity between 150-1700 µS/cm; general water hardness (dGH) between 3-8; dissolved O2 >4 mg/l and CO2 <20 mg/l. Chlorine is removed from tap water before use.
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| 1. Water temperature is maintained within a range of 24-29oC for adult and larval fish. Incoming replacement water is the same temperature as the water it is replacing.
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| 1. Stable water quality and temperature are provided at all times, including during experimental procedures; this is verified at least once per day. Contingency plans are in place for maintaining stable environmental conditions in case of system breakdown or loss of power. When fish are held in static tanks, the holding, breeding, and/or experimental tanks are monitored at an increased frequency to ensure that water quality and temperature do not deteriorate.
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| 1. Handling is kept to a minimum and care is taken not to damage the mucus layer. Soft nets are used and gloves are kept moist. Small fish and those at the delicate larval stage (~<40 dpf) are lowered into the water and allowed to swim from the net, rather than the net being inverted by hand.
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| 1. Where transport of adult zebrafish is justified, the density of fish within the transport bag does not exceed five fish per litre of water; the transport bag contains approximately two thirds of air/oxygen; food has been withheld 24-hours prior to bagging to minimise ammonia build up and an ammonia binder is added to the water. Upon arrival, fish are acclimatised to the temperature of the receiving water (e.g. by floating the unopened transport bag until temperature equilibrium is achieved). Fish are then released from the transport bag immediately upon opening and the water from the transport bag is discarded.
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| 1. The risk of introducing pathogens or contaminates from an outside source is minimised. A quarantine system, including a health screening policy, is in place and only bleached embryos are introduced to the main facility. Where use of bleach is not possible for experimental reasons, please provide details of the biosecurity measures to prevent cross-contamination in the Exceptions box below.
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| 1. The risk of spreading pathogens between tanks and fish is minimised. Appropriate biosecurity plans and training are in place for staff and users. Hand nets are designated to specific tanks/racks and sanitised between uses. No equipment used for handling/housing fish is shared between rooms unless it has been appropriately disinfected. The filtration systems supplying the water to the fish rooms includes a UV steriliser which is routinely serviced.
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| 1. Larval zebrafish are routinely fed 2-3 times a day on a diet that is appropriately sized for their gape. Adult zebrafish are routinely fed 1-3 times per day.
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| 1. Stock fish are provided with environmental enrichment, including at least one of the following: feeding of live prey, refuges within the tank (e.g., synthetic plants or shelters); gravel substrate or images of gravel on the bottom of the tank.
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| 1. DNA for genotyping is obtained using a non-invasive method (e.g. the Zebrafish Embryo Genotyper or [skin swabbing](https://www.nc3rs.org.uk/skin-swabbing-dna-sampling-zebrafish)) rather than tissue biopsy (e.g. fin clip).
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| 1. When fin clipping is justified, this is carried out under anaesthesia, the minimum amount of tissue required is taken (e.g., the tip of one tail lobe ~1-2mm2), fish are provided with analgesia before and after the procedure and recover in visual contact with other fish.
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| 1. When any procedure may cause pain, appropriate contemporary anaesthesia and analgesia is provided. Agents with the least aversive properties are prioritised following advice of the veterinarian.
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| 1. Surgery is performed using methods that minimise the risk of post-operative infection. The least invasive surgical approaches are used, and appropriate perioperative care (pre-operative medications and maintenance of body temperature) is provided. Assisted recovery/resuscitation is provided if necessary (e.g., through use of an aerated recovery tank).
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| 1. Fish are monitored with a frequency appropriate to keep pain and distress to a minimum, using appropriate, tailored welfare indicators for the study (e.g. erratic movements, increased respiration, blanching of colour). A dedicated time slot is allocated to complete daily health checks on all stock and experimental animals.
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| 1. Humane endpoints have been established for any experiment with the potential to cause moderate or severe harm following consultation with the veterinarian and animal care staff. Implementation of these is recorded during the experiment.
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| 1. Euthanasia is conducted for all life stages beyond five days post fertilisation using buffered lidocaine (1 g/l lidocaine hydrochloride + 2 g/l sodium bicarbonate (NaHCO3) + 50 ml/l 96% ethanol). If this is not possible (e.g. due to regional licensing restrictions), the method used is appropriate for the life stage and is either permitted in Schedule 1 of the ASPA (overdose of another anaesthetic agent or concussion of the brain by striking the cranium) or recommended by the AVMA (2020).
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**Exceptions box**: Where there are deviations from the above, please explain and justify these in the space provided below; include details of how any potential welfare issues will be mitigated.

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