

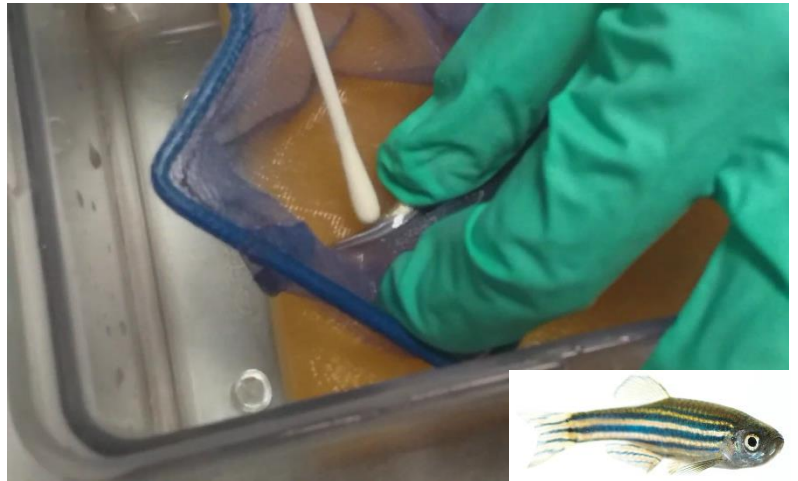
Refined methods of DNA collection in fishes

Aim of this resource

To help AWERBs discuss good practice for collecting DNA samples for genotyping from small laboratory fishes.

Relevant AWERB tasks

Advising staff on animal welfare and the application of the 3Rs.



Screenshot from <https://youtu.be/DTxIEGCCZXA>

Recommendation

Review current practices and discuss DNA collection techniques, equipment and training, as well as issues around different sampling methods to ensure the most refined method is being used.

The issue

DNA samples for genotyping small laboratory fishes such as zebrafish are frequently collected by fin clipping – removing a small portion of the caudal (tail) fin. However, this process is invasive and painful, and also requires the fish to be handled, which can be stressful. There are a number of alternative sampling techniques available which should be reviewed by the AWERB, to ensure the most refined method is used.

Key points:

- Fin clipping is an invasive and painful procedure.
- Fin clipping and the associated handling and anaesthesia can cause adverse effects which may affect experimental results.
- Skin swabbing has been shown to be an equally reliable method of DNA collection for small laboratory fishes, and may be used for individuals as small as 20mm in length.
- Skin swabbing has less impact on behavioural and physiological indicators of welfare than fin clipping – it is therefore a refined method and should be used instead of fin clipping.
- Skin swabbing may also be a more practical choice in many facilities, as it is not considered a regulated procedure in the UK.
- In larger fish species, buccal swabbing may be considered as an alternative to fin clipping, but is unlikely to be preferable to skin swabbing in terms of animal welfare.
- Methods have been developed which involve collection of DNA samples from embryos; these may also be considered refinements over fin clipping.
- If fin clipping is still proposed, staff carrying out the procedure must be appropriately trained. Specific and compelling scientific or veterinary justification should be given for the use of fin clipping instead of skin swabbing.
- As well as anaesthesia, fishes should be provided with appropriate pain relief when fin clipped.

Background information:

- **Fin clipping is an invasive and painful procedure.** Fin clipping involves anaesthetising the fish, restraining them out of water and removing a section of fin, usually the caudal (tail) fin, with a scalpel. This procedure has been shown to cause a range of behavioural and physiological responses consistent with pain and distress in fishes, including reduced activity, increased ventilation, decreased feeding, more time spent at the bottom of the tank and increased cortisol levels^[1-3].
- **Fin clipping and the associated handling and anaesthesia can cause side effects which may affect experimental results.** These may include an increase in cortisol release, an elevated non-specific immune response, and a risk of secondary infections^[3,4]. Fin clipping may also have short and long-term effects on fish behaviour, either as a direct result of pain or distress, or because fins are used by some species in behavioural interactions which may be affected^[4]. Fin clipping also requires the use of anaesthesia, which may have further impacts on fish welfare as some commonly-used anaesthetics cause aversive responses in fishes^[5].
- **Skin swabbing has been shown to be an equally reliable method of DNA collection for small laboratory fishes.** DNA samples can be collected by restraining a fish in a net, placing them on a wet sponge and gently swabbing the side of the fish several times with a sterile swab. This method can be used to obtain DNA concentrations and purities which are comparable to those obtained from fin clips^[4,6]. Videos demonstrating the technique are available^[7,8], and more detailed guidance for researchers can be found on the [NC3Rs website](#).
- **Skin swabbing can even be used for very small fishes.** Comparable amounts of DNA were collected from fish that were 20 mm and 30 mm long to larger fish (50 mm long)^[4]. This makes this technique appropriate for a wide range of small laboratory species, including zebrafish and sticklebacks.
- **Skin swabbing has less impact on behavioural and physiological indicators of welfare than fin clipping – it is therefore a refined method and should be used instead of fin clipping.** Skin swabbing has been shown to cause a smaller change in cortisol levels than fin clipping, as well as fewer changes in stress-related behaviours and the expression of genes associated with stress compared with fin clipping^[3]. Skin swabbing can also be performed without the use of anaesthesia – this may be important for fish welfare as some anaesthetics cause aversive responses in fishes^[5].
- **Skin swabbing may be a more practical choice in many facilities, as it is not considered a regulated procedure in the UK^[3].** This means it does not need to be carried out by a personal licence holder. However, those carrying out the procedure should be fully trained, competent and empathetic to ensure that the impact on the animals is minimised.
- **In larger fish species, buccal swabbing may be considered as an alternative to fin clipping, but is unlikely to be preferable to skin swabbing in terms of animal welfare.** Buccal swabbing involves swabbing the inside of a fish's mouth with a sterile swab. Like skin-swabbing, it can be carried out without anaesthesia, is a reliable method of DNA collection and is less invasive than fin-clipping^[9-11]. However, buccal swabbing may be

more difficult to carry out than skin-swabbing, is unlikely to have any welfare benefits over skin-swabbing, and is not considered suitable for smaller species like zebrafish.

- **Methods have been developed which involve collection of DNA samples from embryos; these may also be considered refinements over fin clipping.** These methods include the Zebrafish Embryonic Genotyping (ZEG) system, which works by oscillation of live zebrafish embryos on a roughened glass surface to collect genetic material^[12]. This system can be used with embryos that are 72 hours post-fertilisation, which is before the onset of protection in the UK, when the animals are not believed to be capable of experiencing pain, suffering or distress. The use of this system has not been found to have negative effects on survival, development or behaviour^[12]; however, it has been shown to cause an acute low-level stress response^[13].
- **If fin clipping is still proposed, staff carrying out the procedure must be appropriately trained. Specific and compelling scientific or veterinary justification should be given for the use of fin clipping instead of skin swabbing.** Fin clipping is a regulated procedure, so must be carried out by a licensed individual. The fact that the fish must be handled and held out of air, both of which are stressful, should be taken into account and every effort made to minimise suffering, e.g. by refining capture and handling and minimising air exposure. The operator should also take care to not remove too much tail fin - only a small amount of fin tissue is needed for DNA extraction, and it is important to leave a healthy portion of tail.
- **As well as anaesthesia, fishes should be provided with appropriate pain relief when fin clipped.** Fin clipping must be carried out under anaesthesia in the UK, and surveys suggest that the use of anaesthesia for fin-clipping is widespread in other countries as well^[14]. However, as fin clipping is a painful procedure, fishes should also be provided with analgesia. Administration of lidocaine has been found to reduce behaviours associated with pain, as well as cortisol levels, after fin clipping^[2].

For the list of references, please [click here](#).