Anaesthesia, Recovery, and Euthanasia

Reagents and Equipment

- MS-222 (Sigma #E10521; Ethyl 3-aminobenzoate methanesulfonate, Tricaine) is the most common anaesthetic used for fish and other cold-blooded animals.
- Sodium bicarbonate (Acros Organics #AC217120010)

Protocol for the fighting fish *Betta splendens* (adults)

1. Dissolve MS-222 in RO water and buffer the solution using sodium bicarbonate in a 1:2 (weight:weight) ratio. For example, in 150 mL of RO water prepare:

Stage of Anaesthesia	Exp. procedure	Concentration/Dose for 150 mL
Light anaesthesia (no reaction to painful stimuli)	• blood • measurements	**Cold 400mg/L** (1:2,500 (r) / 0.04% MS-222) 60 mg MS-222 + 120 mg sodium bicarbonate
Deep anaesthesia (absence of reaction to massive stimulation)	• blood • measurements • surgery	**600 mg/L** (1:1,667 (r) / 0.06% MS-222) 90 mg MS-222 + 180 mg sodium bicarbonate
Overdose/euthanasia	• brain dissection	**1 g/L** (1:1,000 (r) / 0.1% MS-222) 150 mg MS-222 + 300 mg sodium bicarbonate

2. Immerse the fish in the anaesthetic bath and use a cover to prevent the fish from coming to the surface and breathing (Fig. 1). Wait for loss of equilibrium and decrease (i.e., for anaesthesia) or cessation of opercular movements (i.e., for euthanasia) before further manipulations (induction time approximately 5-7 min).

Note: If the fish begins to recover prior to completion of the procedure, use a Pasteur pipette to drop some MS-222 solution directly to the skin and opercula, or place the animal back into the anaesthetic bath.

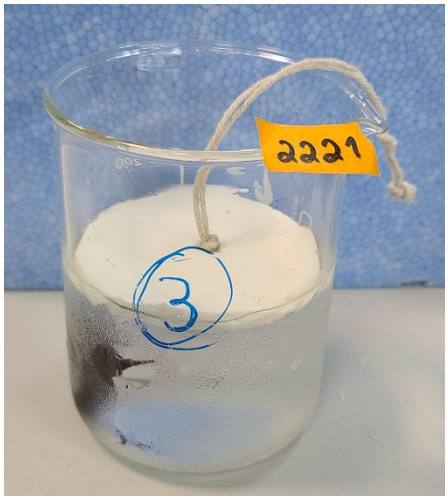

Fig. 1 – Cover for the anaesthetic prevents the *Betta splendens* from coming to the surface to breathe, hence decreasing the anaesthesia time.

3. Prepare the recovery bath (e.g., in the fish housing tank with shelter) with RO water at 250 ppm salinity or water from the system with appropriate aeration flow and leave the fish for a few hours or overnight.

Note: For fish subject to blood collection, prepare the tank with a small water column height (around 4cm) and leave the fish recovering for two days, after which the remaining RO water should be added to the tank. This allows the fish to come easily to the surface and breath and eat.

4. Dispose of MS-222 solution in the sink drain and flush the solution with abundant water.

Protocol for the marine medaka *Oryzias melastigma* (adults)

1. Prepare a stock solution of buffered MS-222 at 1% in miliQ water and store it in the fridge at 4ºC for up to 4 weeks.

   Stock 1% MS-222: In 30 mL miliQ water dissolve 0.3g of MS-222 and 0.6g of sodium bicarbonate.
2. Dissolve anaesthetic from stock solution in RO water prepared at **30 ppt** salinity according to:

Stage of Anaesthesia	Exp. procedure	Concentration/Dose for 200 mL
Light anaesthesia	• electrophysiology • measurements	**0.03% MS-222** 6 mL 1% MS-222 + 194 mL RO at 30ppt

Deep anaesthesia/euthanasia	• blood • measurements • brain dissection	**0.06% MS-222** 12 mL 1% MS-222 + 188 mL RO at 30ppt

3. Immerse the fish in the anaesthetic bath and wait for loss of equilibrium and decrease (i.e., for anaesthesia) or cessation of opercular movements (i.e., for euthanasia) before further manipulations (induction time approximately 2-5 min; Fig. 2).
4. Prepare the recovery bath with RO water at 30 ppt salinity or water from the system with appropriate aeration flow and leave the fish for a few hours or overnight.
5. Dispose of MS-222 solution in the sink drain and flush the solution with abundant water.

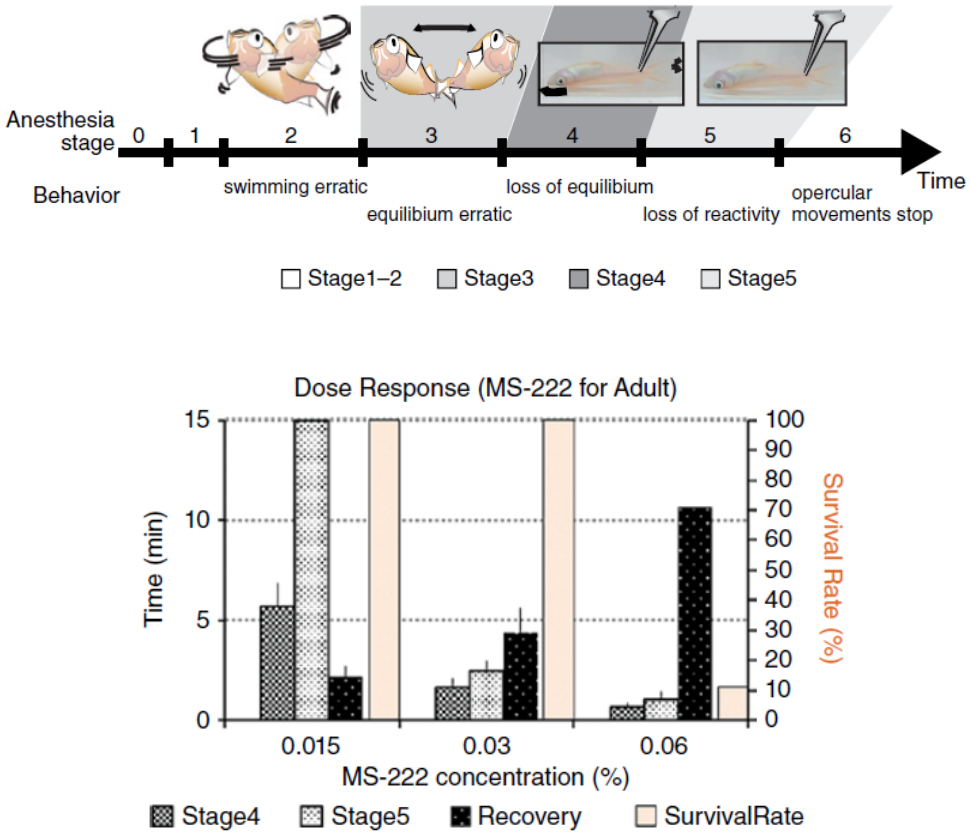

Fig. 2 – Description of anaesthesia stages and a summary of the induction time at three different anaesthetic concentrations on the freshwater medaka *O. latipes* (Murata et al., 2019).

Notes:

- MS-222 powder is stored in the fridge at 4ºC. Alternatively, can be stored on the shelf at temperatures < 25ºC.

- If possible and when appropriate, fish should be starved 12-24 hours prior to anaesthesia as they may regurgitate food.
- Ideally, MS-222 stock solutions are utilized the same day as preparation. When necessary, stock solutions of MS-222 may be kept up to 30 days refrigerated and protected from light.
- MS-222 as it is hazardous to humans, wear gloves and utilize the flow hood.

For more information on anaesthesia in fish see:

Martins, T., Valentim, A. M., Pereira, N., & Antunes, L. M. (2016). Anaesthesia and analgesia in laboratory adult zebrafish: a question of refinement. *Laboratory Animals*, *50*(6), 476–488. https://doi.org/10.1177/0023677216670686

Murata, K., Kinoshita, M., Naruse, K., Tanaka, M., & Kamei, Y. (2019). *Medaka: Biology, Management, and Experimental Protocols, Volume 2*. Wiley-Blackwell.

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