

Standard Operating Procedures

#AU 0028 Inducing Anosmia in Fish

This procedure can be used to apply a variety of treatments to the olfactory epithelium of fish to induce anosmia. That being said, the intended anosmia-inducing agent is tissue glue (e.g., VetBond). Tissue glue is ideal as it is commonly used for closing wounds in animals and has been demonstrated to degrade and eventually fall off over time.

Anesthetizing fish

- 1) Prepare a solution of buffered MS-222 for anesthesia. The concentration of the solution is species-specific, and should induce a very light anesthesia. Use the lowest concentration possible. If the concentration is unknown for the species you are using, start with 50 mg/L buffered MS-222 and slowly increase until fish lose their balance in the water column. Commonly used species and their concentrations are 80 mg/L for goldfish, 120-180 mg/L for fathead minnows, and 50 mg/L for rainbow trout. To prepare the solution:
 - a. Place 750 mL of water matched to the holding tank (both chemically and thermally) into a clean 1L beaker or something comparable
 - b. Add MS-222 and stir with a stirring rod
 - c. Adjust solution to a pH of 7.4 using sodium bicarbonate (NaHCO_3). NOTE: you can usually achieve a pH of 7.4 by adding in twice the mass of MS-222. For example, if you use 50 mg of MS-222, add 100 mg of sodium bicarbonate
 - d. Bring the final volume up to 1L
- 2) Set up a recovery tank (minimum volume of 1 L) that contains by volume 70% water from the holding tank from where the fish originates and 30% fresh room temperature dechlorinated water (this is so the recovery tank will contain water familiar to the fish and water that has high oxygen and low ammonia levels)
- 3) Provide supplemental aeration to the recovery tank to ensure there is ample dissolved oxygen for the fish
- 4) Collect a fish from its tank and place it in a small dark appropriately sized transport container (or a 1 L beaker) wrapped with paper towel and filled with water from the tank that the fish originated from (the familiarity of the water and the darkness is meant to calm the fish)
- 5) Using a small net collect the fish from the transport container and place it in the MS-222 bath
- 6) Anaesthesia occurs when the fish has lost equilibrium (i.e. it has turned on its side) and is no longer resisting loss of equilibrium, as well the fish will have slowed regular opercular movement
- 7) It can take 20 seconds to 5 minutes for a fish to succumb to the anaesthesia
- 8) Remove the fish from the MS-222 bath and conduct the necessary procedure (see anosmia procedure below)
- 9) If the recovery tank is located in the same room as where the procedure is performed return the fish directly to the recovery tank. If it is not, place the fish into an appropriately sized transport container and transport to and place the fish into the recovery tank
- 10) Closely monitor the recovery for 24 hours after anaesthesia to make sure there is no prolonged stress
- 11) After 24 hours the fish can be moved to a holding tank or used for experimental procedures.

Inducing Anosmia

- 1) Perform steps 1) – 7) in the anesthetizing fish procedure above
- 2) Remove the fish from the MS-222 bath and place on a moist paper towel
- 3) Dry nares off with paper towel, use the corner of a folded Kim wipe to remove excess water from within the olfactory chamber
- 4) Using a 1-10 μ L pipettor, fill both olfactory chambers with tissue glue. NOTE: you must dispense the minimum possible amount, as any additional tissue glue will spread over the surface of the fish and may affect their eyes or mouth. The exact amount used depends on the size of the olfactory chamber
 - a. For sham-anosmic fish, pass a small amount of water into the olfactory chamber. This will mimic the procedure but exclude the tissue glue
- 5) After application the tissue glue will quickly dry (<60 seconds). Once dry, return the fish to a recovery tank and monitor as per the anesthetizing fish procedure
- 6) Once recovered for a minimum of 24 hours, the anosmic and sham-anosmic fish are ready to be used in behavioural trials
- 7) After trials, anosmic fish should be euthanized as per SOP AU #0007