

Standard Operating Procedures

#AU 0029 Histology

Histology will be performed on fathead minnows to determine which class of olfactory sensory neurons in the olfactory epithelium respond to carbohydrates.

The procedure is as follows:

- 1) Fish will be anesthetized with MS-222 and placed into a small holding chamber
- 2) A tube delivering aerated water containing MS-222 is delivered into the mouth and over the gills of the fish to ensure the fish remains anesthetized for the duration of the measurement
- 3) A microscope is placed over top of the olfactory chamber, and a flap of tissue that covers the olfactory epithelium is carefully cut away
- 4) A combination of the cue of interest as well as a dye will be added to the olfactory chamber and allowed to sit for a minimum of 30 minutes. Receptor-mediated endocytosis will bring droplets of the dye into the olfactory sensory neurons, and because the receptors that will be involved are recognizing the cue, only olfactory sensory neurons containing receptors to the cue will be stained (this procedure is from Doving et al., 2011)
- 5) The animals will be euthanized via anesthetic overdose and the olfactory epithelium and surrounding tissues will be dissected for histological analysis.

Based on the literature it is believed 5 fish per exposure will be needed.