



APPENDIX F

ACOUSTIC TAGGING SOP - VAMP 2011

Equipment Set up

- Fill disinfection trays for surgical instruments with Novalsan
- Fill rinse tray with de-ionized or distilled water.
- Set up scale, measuring board, and surgery tray.
- Fill fresh carboy with water from source tank and fill MS-222 carboy to the line with water from the same source tank. Add 2ml MS-222 and 2ml bicarb to the water in the MS-222 carboy.
- Fill anesthesia bucket to line with water from source tank. Add 7ml MS-222 and 7ml bicarb. Cover with a lid.
- Place a study fish recovery bucket in a sleeve and fill with water from source tank. Check to be sure that the bucket is labeled on the handle and that the label on the lid matches.
- Place a dummy fish recovery bucket in a sleeve and fill with water from source tank. Check to be sure that the bucket is labeled on the handle and that the label on the lid matches. Dummy buckets should have red lids and will begin with "CX"
- Check that a reject bucket has been filled with water from the source tank and is available nearby.
- Check that a lethal bucket has been filled with water from the source tank and a lethal dose of MS-222, and ensure this lethal bucket is available nearby.
- Start a data sheet.
- Obtain tags and place the first tag and its vial in disinfectant solution. Record the Tube ID on the datasheet. Move the tag and vial to the rinse water before implanting the tag.
- Prepare the flume by inserting the standpipe, and adjusting water sources to achieve the desired temperature based on temperature of the source tank and temperature at Durham Ferry. Direct the flow to circulate around the tank and use a cinder block between the standpipe and the incoming water to assist circulation. Cover the flume with lids to shade the buckets and to minimize heating of the water.

Surgery

- Anesthetize fish
 - o Net one fish from source tank and place directly into the anesthesia bucket. Place a lid on the bucket. Start your stopwatch immediately to track how long the fish is in the anesthesia bucket.
 - o Remove the lid at about 1 minute to observe the fish for loss of equilibrium. Keep the fish in the water for an additional 30-60 seconds after it has lost equilibrium. Time of sedation should normally be 2-4 minutes, with an average of about 3 minutes. If loss of equilibrium takes less than 1 minute or if a fish is in the anesthesia bucket for more than 5 minutes, reject that fish. If after sedating a few fish they are consistently losing equilibrium in more or less time than typical, the anesthesia concentration may need to be adjusted. This should only be done after consultation with the coordinator.
 - o If a fish is unacceptable for tagging, place the fish in the "Reject" bucket and inform the data recorder.
- Recording fish length, weight, and condition
 - o Transfer the fish to the scale and weigh to the nearest 0.1g.
 - o Transfer the fish to the measuring board and determine forklength (FL) to the nearest mm.
 - o Check for any abnormalities and descaling.
 - o Data must be vocally relayed to the recorder and the recorder should repeat the information back to the tagger to avoid miscommunication.
 - o Any fish dropped on the floor must be rejected.
- Tag implantation
 - o Place the fish into the surgical tray ventral side up. Immediately start a stopwatch to track surgery time.
 - o Anesthesia should be administered through the gravity feed tube as soon as the fish is on the surgery table. Using the in-line valve, adjust the flow as needed so that the gilling rate of the fish is steady.

- o Using a scalpel, make an incision approximately 5 mm in length beginning a few mm in front of the pelvic girdle. The incision should be just deep enough to penetrate the peritoneum, avoiding the internal organs. The spleen is generally near the incision point so pay close attention to the depth of the incision.
- o Use forceps to open the incision to check that you did not damage any internal organs or cause excessive bleeding. If you observe damage or think you damaged an organ, do not implant the tag – reject that fish.
- o One scalpel blade can be used on about 5 fish. If the scalpel is pulling rough or making jagged incisions, the blade needs to be changed prior to tagging the next fish.
- o Gently push the tag into the body cavity and position it so that it lies directly beneath the incision and the ceramic head is facing forward. This positioning will provide a barrier between the suture needle and internal organs.
- o Suture the incision with two to three interrupted stitches.
- o Transfer the fish from the surgical table to the appropriate recovery bucket.
- o Three fish will be placed in each recovery bucket. Call out the count of fish in the recovery bucket to the recorder for confirmation. Put the lid back on the bucket. Once 3 fish are in a bucket, place the datasheet on top of the lid and signal to the tag validating crew for the bucket to be removed.
- o Confirm the tube ID with the recorder and place the empty vial into the lid of the tray which holds the tags.
- o Between surgeries the tagger should move the tools just used into the disinfectant bath. Each tagger will have 3 sets of surgical instruments to rotate through to ensure that tools get a thorough soaking in disinfectant between uses. Once disinfected, tools should be rinsed in distilled or de-ionized water. Organic debris in the disinfectant bath reduced effectiveness so be sure to change the bath regularly.

Tag Validation

- Obtain bucket and datasheet from tagging crew and gently place hydrophone in bucket.
- Set display to the first tag periods on the datasheet and confirm the signals. Record the time of confirmation on the datasheet.
- Once all tags in a bucket have been heard, remove the hydrophone, securely fasten the lid, and transfer the bucket to the flume.
- If a tag is not heard, isolate the individual carrying the non-functioning tag, euthanize the fish, and retrieve the tag. Indicate on data sheet that tag was not heard and that fish was removed. Note on the bucket lid using a grease pencil the number of fish remaining in the bucket. Place the tag back into the vial from which it came.
- Return the datasheet to the tagging crew.

Holding Between Tagging and Transport

- After tags are validated, transfer buckets to the flume. After placing a bucket in the flume cover the tank with a trash can lid to provide shade.
- Check temperature in the flume periodically during each tagging session and adjust as needed to maintain desired water temperature.

Loading

- Begin fish loading/ transport/ release data sheets.
- Fill hauling tank. Do not use water from the chiller – it will not be able to keep up. Allow water to sit in the tank for at least 15 minutes before purging.
- Re-fill tank with water at the same temperature, or 1-2 degrees cooler than the source tank depending on temperature at Durham Ferry. Add non-chlorinated ice if needed to reach/maintain desired temperatures.
- Turn on oxygen.
- Bring buckets to the truck and check each for morts before placing into the tank. If a mort is found, the recovery bucket containing the mort must be returned to the tagging area. The tag must be removed and identified by the validation crew. The tag will then be implanted into a new fish with a new entry on a datasheet and comment should read re-tagged from mort. The original entry should be crossed out on the data sheet with a comment of mort at loading.
- Call out the number of the bucket to the recorder and the number of fish in the bucket.
- Once all buckets have been loaded, confirm that the number of buckets matches the number that should be loaded and that there are no buckets remaining in the flume or in the tagging area.
- Record water temperature and DO in each tank and record the departure time.

- Secure the tank.
- Send datasheets with transport crew.

Cleanup

- Return tag tray with empty vials and datasheets to coordinator at the end of each tagging session.
- Wipe down or spray all surfaces with ETOH to disinfect
- Soak surgical instruments in Novalsan for at least 15 minutes. Scrub with small brush. Rinse with water and dry thoroughly to prevent rusting. Leave on a dry towel.
- Rinse buckets with hose and place upside down to dry.

Important things to remember:

- Anesthesia and fresh carboys and buckets should be filled just prior to tagging to avoid temperature changes and should be changed often. Check levels of carboys before each surgery to be certain that you will not run out of water during a surgery.
- Keep a lid on any bucket that contains fish.
- Any fish dropped on the floor must be rejected. If a fish is dropped on the floor after it has been tagged, euthanize the fish, remove the tag, and place it into another fish.
- Carefully handle buckets. Try not to bang them around, slam the handles, or otherwise handle in a rough manner as this can stress fish.