**Steptozotocin fish injection protocol**

This protocol is for use in creating diabetic fish using intraperitoneal injection of the drug streptozotocin (STZ).

**Required Equipment and Supplies**
- Streptozocin
- Sterile normal saline (pH 7.4-7.6)
- Fish water
- 2-phenoxynethanol
- Plastic containers for anesthetic and recovery water
- Paper towels
- Fish net
- ½ cc syringe with 28.5 gauge needle.
- Petri dish lid
- Scale
- Plastic spoon

1. Prepare anesthetic water: Add 500uL of 2-phenoxynethanol to 500mL of fish water in a plastic “tupperware” container.
2. Prepare recovery water: Use 2 “clear plastic containers – fill containers with fish water ¾ of the way full
3. Prepare a 0.3% solution of STZ. This can be accomplished by adding 6mg of STZ to 2mL of normal saline. This will provide enough injection solution for the injection of approximately 20 fish in 20 minutes.
   - Note: STZ should be kept at -20C at all times when not in use
   - Prepared STZ solution is only good for a maximum of 30 minutes and should not be used for injections after that time
   - STZ is harmful to humans and should be handled carefully and measured in a fume hood.
4. Fill a ½ cc syringe with STZ solution ensuring that there are no air bubbles.
5. Anesthetize a fish by placing the fish in anesthetic water for 1-2 minutes.
6. Briefly place the fish on a paper towel to absorb the extra water.
7. Measure the mass of the fish.
8. Place the fish on a Petri dish lid for injection
9. Inject the fish by inserting the needle into the posterior aspect of the ventral peritoneum
   - Insert the needle only so deep that that the bevel of the needle is fully inside the fish
   - 350 mg/kg STZ is to be injected into each fish. so the following equations apply:
     - $350 \times (\text{mass of fish in g}) = \text{ug STZ}$
     - $0.00067(\text{ug STZ}) = \text{mL to inject}$
• A sheet for volumes to inject per fish mass should be used for quick reference

10. Place the fish in recovery water tank. A recovery water tank is used to limit the number of fish recovering together and to allow for easy observation of fish until they have fully recovered from anesthesia. Following recovery fish are to be transferred to their normal living tank and kept at 22-24°C.
   • 10 fish is the maximum that should be in a recovery tank at a time.

11. The injection series is as follows:
   • Week 1: 3 injections (Monday, Wednesday, Friday is ideal)
   • Week 2: 1 injection (Friday)
   • Week 3: 1 injection (Friday)
   • Week 4: desired experiment is often conducted