4.0 Blood Sampling of Finfish

4.1 Introduction:

This template is intended for use by instructors to train the Department of Fisheries & Oceans (DFO) staff and students in the blood sampling of finfish. Templates are used to provide the minimum requirements necessary in a training exercise, but the instructor may add additional material as deemed necessary.

An experienced instructor must demonstrate the methods outlined in this template and trainees must be deemed qualified in carrying out one or more of the procedures before they are permitted to blood sample without an instructor present. Hands-on training of staff is a requirement for facility approval by the Canadian Council on Animal Care (CCAC), of which DFO is a member. This template is part of a comprehensive DFO Science Branch series on training for users of aquatic research animals.

4.2 Rationale:

Scientific study often requires that fish be blood sampled for reasons such as haematology, clinical chemistry parameters, bacteriology, parasitological investigation, packed cell volume, etc. Investigators have an ethical obligation to minimize the pain and/or distress of all laboratory animals undergoing laboratory-sampling techniques. Only those methods deemed acceptable by the CCAC should be employed.

4.3 Authority:

The staff/consultant veterinarian or Animal Care Committee is responsible for providing information about blood sampling methods of the fish species used for scientific study in their respective regions. The Animal Care Committee may delegate training of these procedures to an instructor who has demonstrated knowledge and experience in the areas of anatomy and anaesthesia and has demonstrated excellent survival of fish that have been blood sampled using the techniques outlined in this training template. Staff must be trained in the proper blood sampling techniques for the fish species and size being studied prior to initiation of any blood sampling procedure(s).

4.4 Goal of this training exercise:

1. Learn the methods to humanely remove blood samples from anaesthetized fish.
2. Understand the consequences of each method of blood sampling on the fish and thus the potential impact of the method selected and the sample collected.
3. Understand the function/physiology of the circulatory system within a fish.
4. Demonstrate gentle handling techniques of fish when live fish are used in this section.
5. Understand proper disinfection techniques to use after completing the procedure (provide biosecurity SOPs).

4.5 Theoretical Training – to be completed before hands on session

1. Completed 'The Experimental Fish'.
2. CCAC guidelines: Section H Experimental Procedures 6: Collection of body fluids.
3. Completed the anatomy & physiology, anaesthesia and euthanasia templates or has experience or training in all three areas.
4. WHMIS training.
5. Summary theory material provided with this training template (Appendix A: Fish blood and the circulatory system).

4.6 Details of the Procedure:

Introductory:

Methods of blood sampling, that may be included in the training session, are:

1. Blood sampling by tail ablation of small fish that have been euthanized.
2. Blood sampling by caudal venous puncture.
3. Blood sampling by dorsal aorta puncture.

Advanced:

4. Blood sampling by heart puncture.

4.6.1. Time estimate for completing the hands on exercise

Set up: 1 hour

Instruction and Training: 2 hours

4.6.2 Equipment Required:

- Fish: number will depend on the number of trainees in a session; species used will depend on availability and requirements of the facility.
- TMS™ (plus $NaHCO_3$ buffer if anaesthetizing in freshwater)
- Dip net
- Container for anaesthetic bath
- Air stones and compressed air or oxygen
- Thermometer
- Gloves and splash glasses to be worn when handling fish or anaesthesia water
- Disinfectant for clean up after blood sampling
- Soft sponge or mat to prevent mucous sloughing during blood sampling
- Scalpel or sharp knife for tail ablation
- Paper towel for containment of blood from ablation
- Sterile needles or vacutainer needles (size will be dependent on the size of the fish)
- Syringe or vacutainer tube for collecting blood samples (size will depend on the amount of blood to collect and the size of the fish)
- Vacutainer holder

- Tube racks for blood collection tubes
- Hematocrit tubes
- Critoseal
- Sharps container

4.6.3 Procedure:
The instructor should demonstrate all the procedures prior to allowing the trainees to attempt them. Fish sacrificed in the euthanasia template make excellent teaching specimens for the trainees to practice their techniques.

Instructors will need to use their own best judgement in choosing which procedures are appropriate with the common species used at their research facility. It is recommended that all procedures be taught to trainees so that they have multiple options at their disposal when faced with the need to sample the blood of fish of different species.

Introductory:

4.3.6.1 Equipment for Blood Collection:

Human Safety Measures

- Discuss human safety measures while discussing the equipment used for blood sampling.

- Wear gloves at all times when handling blood collection equipment. While this will not prevent accidents, it may reduce the risk of contamination.

- Never attempt to re-sheath needles. Always discard them into a sharps container immediately after use.

- Never overfill the sharps container. Adhere to the recommended fill line on the sharps container.

- Place the sharps container in the area you are working to avoid transporting used needles outside of your work area.

- Ensure the area you are working is clean and all the equipment need to carry out the procedure is readily available. Allow enough workspace to safely obtain the blood sample without contaminating the blood sampling supplies should the fish exhibit common reflex movements upon needle insertion. For blood collection in the field it is suggested to spread a fine mesh net under the entire work area to prevent loss of supplies (particularly sharps) that may accidentally drop while sampling.

Equipment Options:

- Present equipment options available for blood collection (i.e. needles of various gauges and length, multiple syringe types and sizes, haematocrit tubes and critoseal, vacutainer needles and tubes, etc.).

- Emphasize the relationship between needle gauge and needle size (e.g. a 20 gauge needle is larger than a 30 gauge needle).

- Discuss safety equipment and how it is to be used (i.e. tube racks are for holding tubes after filling with blood, sharps containers are for discarding used needles, etc.).

- Identify the proper needle gauge and length required for blood sampling the fish used in this training session.

- Discuss the common types and sizes of vacutainer tubes and chose the type and size appropriate for the fish used in this training session. Trainees should consult with the principal investigator to determine the appropriate tubes to use when blood samples are collected for research purposes. Examples of vacutainer tubes include, but are not limited to the following:

    - Red top: contains no additives. Used for serum collection, bacteriology, and parasitological examination.

    - Green top: contains the anticoagulant lithium or sodium heparin. Intended for clinical chemistry parameters and plasma collection.

    - Purple top: contains the anticoagulant EDTA. Intended for whole blood collection for haematology. EDTA will interfere with some tests and is therefore not a suitable sample tube for blood chemical analysis.

    - Grey top: contains the additive sodium fluoride. Intended for glucose measurements.

    - Light blue top: contains the additive sodium citrate. Intended for coagulation studies.

- Select the appropriate tube or syringe size for the fish used in the training session. Too large a tube or syringe can significantly reduce the blood reserve in smaller fish causing excess stress and/or death. Paediatric tubes or 1 ml syringes should be used when blood sampling smaller fish.

- Instruct trainees in the proper method to attach and detach needles to syringes or vacutainer tubes.

- Stress the importance of feeling comfortable with the bleeding apparatus prior to insertion into a living fish.

- Trainees should practice moving the plunger up and down in the syringe. A good practice tool is having the trainees insert a syringe with attached needle into an orange and extract a small amount of juice.

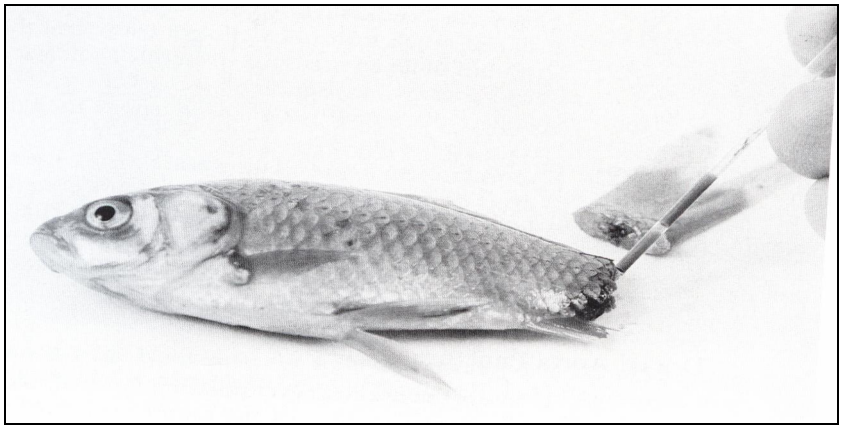
- Trainees should practice inserting a vacutainer into the vacutainer holder (with attached needle) without breaking the vacuum seal. A break in the vacuum seal in air makes the tube useless for blood collection, as the blood will not aspirate once in the vein or aorta of the fish.

Note: The following procedures and photographs have been adapted with permission of the author from Chapter 4. Aseptic Bacterial Examination of Finfish in: Finfish and Shellfish Bacteriology Manual-Techniques and Procedures, 2004. Whitman K.A. (author); Blackwell Publishing, Iowa State Press, Iowa, USA.

4.6.3.2. Tail Ablation:

- Fish that are too small to bleed with a syringe and needle or a vacutainer system may necessitate lethal sampling by tail ablation.

- Euthanize a fish. Refer to the Euthanasia Template for accepted methods of euthanasia.

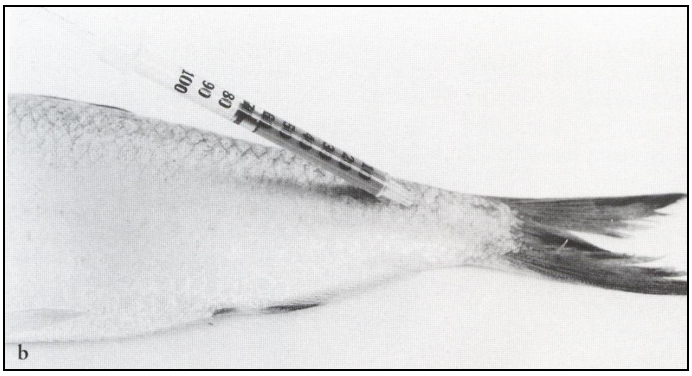


Blood collection from *Carassius auratus.* (Photo by R. Hebb)

- Sever the caudal peduncle with a scalpel blade or sharp knife.
- Fill a hematocrit tube with the blood as it flows from the caudal vein.
- Plug one end of the haematocrit tube with critoseal.
- Dispose of the fish carcass and waste as outlined in facility SOP.

4.6.3.3 Caudal Venous Puncture:

(a) Blood collection from an American shad (*Alosa sapidissima*) using the caudal vein (ventral view). (Photo by R. Hebb)



(b) Lateral view. (Photo by R. Hebb)

- Insert a needle attached to a syringe or a vacutainer system under the skin of the ventral midline of the caudal peduncle of an anaesthetized or freshly euthanized fish.
- Alternatively, a lateral approach can be used by inserting the needle under the scales of the mid portion of the tail just below

the lateral line at a 45° angle to the long axis of the fish in a cranial direction.

- Ease the needle toward the vertebral column until you reach the base of the column.
- Withdraw the needle a fraction of a millimetre, and obtain the blood sample.
- Remove and discard the needle in a sharps container.
- Return the fish to a recovery bath or its home tank and monitor the recovery process.
- Prepare the blood sample for processing as instructed by the instructor.
- Clean the work area as outlined in the facility SOP.

4.6.3.4 Dorsal Aorta Puncture:

Blood collection from an Atlantic salmon (*Salmo salar* L.) using the dorsal aorta. (Photo by R. Hebb).

- Insert the needle attached to a syringe or a vacutainer system, bevel pointing upward, along the dorsal midline of the mouth, just past the juncture of the second gill arch of an anaesthetized or freshly euthanized fish.
- Collect a blood sample.
- Remove and discard the needle in a sharps container.

- Return the fish to a recovery bath or its home tank and monitor the recovery process.
- Prepare the blood sample for processing as instructed by the instructor.
- Clean the work area as outlined in the facility SOP.

Advanced:

- Blood collection using the heart puncture method is often performed on euthanized fish, though it can be performed successfully on anaesthetized fish with proper training.
- Instructors should demonstrate this procedure on several fish to ensure the trainee is cognitive of the potential risks associated with this method.
- Trainees should not be permitted to perform this procedure on live fish without a firm grasp of the anatomy and physiology of the heart.
- It is helpful for trainees to dissect a sacrificed specimen to observe the location (bulbous arteriosus) where the needle is to be inserted and determine the potential trauma a fish's heart can undergo after blood sampling.
- Trainees should practice on a number of euthanized specimens to practice their technique and gain confidence prior to attempting a live specimen.

4.6.3.5 Heart Puncture:

Blood collection from an American shad (*A. sapidissima*) using the heart puncture technique. (Photo by R. Hebb).

- Hold the needle attached to a syringe or vacutainer system perpendicular to the skin and insert the needle slightly below the tip of the V-shaped notch formed by the gill cover and the isthmus of an anaesthetized or freshly euthanized fish.
- Collect the blood as the needle enters the bulbous arteriosus.
- Remove and discard the needle in a sharps container.
- Return the fish to a recovery bath or its home tank and monitor the recovery process.
- Prepare the blood sample for processing as instructed by the instructor.
- Clean the blood sample area as outlined in the facility SOP.

4.6.4 After the training session:

- Increase monitoring for 2 – 3 weeks after handling if live fish were used for the training.
- Trainees should have clear instructions for needle/sharps disposal and carcass disposal.
- Anaesthesia baths must be disposed of in accordance with local waste management regulations.
- Disinfect the area where fish were handled (provide trainees with site biosecurity SOP).
- Trainees must wash hands with disinfectant soap.
- Update inventory records to reflect the number of fish euthanized for this session (if any).
- Update drug use records to include anaesthetic use.

4.7 ACC Notes

- Locally significant differences required in training (e.g. species).

- Authorization required to teach/list of possible instructors for your region.

- Any other requirements for your region.

APPENDIX A: Review Theory: Fish Blood and the Circulatory System

References:

CCAC guidelines on: the care and use of fishes in research, teaching and testing.

Bone, Q. and Marshall, N.B. 1982. Biology of Fishes. Blackie & Sons Ltd., New York, USA.

Evans, H.E. 1992. Anatomy of tropical fishes in: Aquariology Master Volume, The Science of Fish Health Management. J.B. Gratzek and J.R. Matthews (Eds.) Tetra Press Publication; N.J., USA.

Iwama, G.K. and Farrell, A.P. 1998. Disorders of the Cardiovascular and Respiratory System in Fish Diseases and Disorders: Volume 2, Non-infectious disorders ed. J.F. Leatherland and P.T.K. Woo, Oxford University Press, CABI International

Reinert, R.E. 1992. Fish physiology in: Aquariology Master Volume, The Science of Fish Health Management. J.B. Gratzek and J.R. Matthews (Eds.) Tetra Press Publication; N.J., USA.

Whitman, K.A. (2004). Finfish and Shellfish Bacteriology Manual – Techniques and Procedures. Published by Blackwell Scientific, Iowa State Press, Iowa, USA.

Definitions:

- **Anticoagulant:** Chemical additive which inhibits clotting.
- **Erythrocyte:** Red blood cell or RBC, the nucleated (non-mammalian), a-granular blood cells containing the oxygen-carrying pigment haemoglobin and responsible for the red colour of fresh blood.
- **Haematocrit:** Volumetric relationship of the cellular elements of blood to the total blood volume; also referred to as packed cell volume.
- **Haematopoiesis:** Formation and growth of blood cells.

- **Leukocytes:** Collective term referring to any non-pigmented, nucleated blood cell. Leukocytes in fish blood include 4-40% granulocytes (subdivided into neutrophil, acidophil, and basophil cells according to their staining properties) and granular lymphocytes, monocytes, and thrombocytes.

- **Lymphoid:** Resembling the tissue of lymph glands.

- **Lymphocyte:** Granular leukocyte of the peripheral blood formed in the lymphatic tissue.

- **Monocyte:** Large mononuclear leukocyte with a deeply indented nucleus, slate grey cytoplasm and azurophilic granulation that are form a macrophage upon migration into tissue.

- **Myeloid:** Relating to the spinal cord or bone marrow.

- **Plasma:** The fluid fraction of the blood containing dissolved salts and proteins, as distinguished from corpuscles.

- **Serum:** The fluid portion of the blood that remains after the blood is allowed to clot and the cells are removed.

- **Thrombocyte:** Blood cell that is involved in blood clotting.

Fish Blood and the Circulatory System

- Blood cells are formed primarily in the kidney and spleen and to a lesser extent in the liver, intestinal submucosa and thymus.

- The erythrocytes are oval and nucleated.

- Leukocytes are morphologically and functionally diverse and all are nucleated. There are at least five different subgroups of white blood cells.

- Some arctic fishes lack haemoglobin and thus have clear blood. At lower temperatures oxygen can be carried in simple solution in the plasma.

- Lymphatic vessels are present in teleosts, but there are no lymph nodes.

- Capillary networks or retia are present in the gas bladder for the introduction or removal of gas (oxygen, nitrogen).

- Within the red muscle strip (aerobic slow contracting) of the lateral body wall, the capillary network functions for gas exchange and for heat exchange in large fish such as tuna.

- In the oviduct and ovarian walls of live-bearing fishes, there are networks for nutrient exchange, which can be considered placentae.

- Fishes have a one-way/single circulatory pathway through the heart. All the blood leaving the heart passes through at least two sets of capillaries; the capillaries of the gills and the capillaries of the body organ.

- The blood in the circulatory system transports oxygen to the tissues, and removes carbon dioxide (CO_2) from them. It varies in its properties in different fishes depending on the metabolic demands and the way in which the fish obtains oxygen and excretes CO_2.

- Hypobranchial vessels from the gill rather than from the dorsal aorta supply the heart wall. A few fish species have a coracoid artery that arises from the dorsal aorta and supplies blood to the caudal aspect of the heart.

- The pseudobranch also receives a special blood supply from a branch of the efferent branchial artery called the mandibular artery.

- All venous blood from the organs, body wall, and the fins returns to the heart via superficial and deep veins.

- The sinus venosus serves as a receiving chamber for venous blood returning from the body and it helps to assure a smooth flow of blood into the atrium. The hepatic veins and anterior jugular vein empty directly into the sinus venosus. The anterior and posterior cardinal veins join to form the common cardinal vein (Cuverian duct) before emptying into the sinus venosus.

- Blood flows from the atrium to the ventricle, which, with each contraction (called the systolic period of the heartbeat), ejects blood into the bulbous arteriosus in teleosts and the conus arteriosus in other fish species.

- As the ventricle relaxes (the diastolic period in the heartbeat), the high pressure persists in the bulbous arteriosus and serves to maintain an even flow of blood into the ventral aorta.

- Valves between the bulbous and the ventricle prevent back flow of the blood during the diastolic period.

- From the bulbous arteriosus the blood flows into the short ventral aorta (it is the outflow path to the gills). The ventral aorta gives off paired afferent branchial arteries to the branchial arches.

- From the capillaries in the gills the oxygenated blood is collected by efferent branchial arteries and is emptied into the dorsal aorta above. These roots join immediately to form the dorsal aorta, which is the major distributing vessel of fish circulation.

- The dorsal aorta passes caudally beneath the vertebral column, giving rise along the way to paired segmental intercostal, renal, and spinal arteries as well as unpaired arteries to the gas bladder and viscera.

- Posterior, where the paired ribs fuse to form the haemal arches, the dorsal aorta enters the haemal arch and passes into the tail.

- The caudal vein also lies within the haemal arches beneath the aorta.

- Branches of the dorsal aorta supply arterial blood to the head, body musculature, swim bladder, and all internal organs such as the gut, liver, gonads, and kidney.

Blood Collection Guidelines:

- No more than 0.1% of the fish's body weight (i.e. 1ml/kg) should be removed from a fish that will be recovered from blood collection.

- Fish greater than 200 grams can be recovered successfully following blood collection (dependant on the health status of the fish prior to blood sampling).

- Fish smaller than 200 grams may have to be sacrificed to ensure an adequate blood volume is collected.

- Fish must be allowed adequate time to recover and regenerate blood volume if serial blood samples are to be collected.

- Blood collected for haematology and clinical chemistry parameters must be taken from living fish. Care must be taken to avoid contamination of the sample with tissue fluid.

- Fish blood clots very rapidly. The type of anticoagulant used should be determined in consultation with the principal investigator. For some parameters an anticoagulant will not be required. Examples of anticoagulants include:

- **EDTA** (dipotassium ethylenediamine tetraacetic acid): acidifies blood, binds divalent cations lowering calcium measurements in the sample.

- **Disodium oxlalate:** raises blood pH and sodium levels.

- **Trisodium citrate:** raises blood pH and sodium levels.

- **Lithium or Sodium heparin:** can cause erythrocyte clumping but does not interfere with divalent cation measurements and has less effect on blood pH. Lithium heparin does not interfere with sodium levels.

- Equipment used for blood collection should be scaled to the size of the fish and the expected blood volume to be collected.

- The length and gauge of a needle used for blood collection will depend on the size and species of the fish being sampled. (E.g. a 22 gauge, 1 ½ inch needle is most commonly used for salmonids averaging 1 kg).

- Only sterile needles should be used for blood collection.

- The choice of using a vacutainer system or a syringe and needle is based purely on preference.

Blood Sample Handling:

- Blood samples should be processed within 30-60 minutes after collection. The time between blood sampling and laboratory evaluation of the sample collected is critical. Cells can swell and rupture, and some parameters may not be stable during the time between collection and processing.

- The handling and storage of samples after processing will depend on their purpose. Check with the principal investigator to determine how to process the samples.