

## **CHAPTER 4**

# **Standard Necropsy Procedures for Finfish**

**Theodore R. Meyers  
Alaska Fish & Game  
C.F. Division  
Juneau & Anchorage**

## I. Introduction

This chapter describes the general procedure for performing a diagnostic necropsy on finfish. While the majority of sampling conducted for the Survey will be performed on normal fish, identification of characteristic behaviors and clinical signs of infected or diseased fish are important components of any pathogen screening program. Proper dissection techniques during necropsy will optimize pathogen detection and subsequent determination of disease status.

Live fish should be examined for behavioral abnormalities (spiral swimming, flashing, flared gill opercula, prostration, etc.) then anesthetized to avoid tissue artifacts caused by alternate methods of euthanasia such as pithing or a blow to the head. Some external abnormalities (whitened or eroded fin tips, cloudy cornea, body discoloration, excessive mucus) are best observed while the fish is submerged in water. In many cases postmortem change in fish that are received dead will prevent this latter opportunity.

## II. General Necropsy Procedure

- A. Necropsy subjects should first be examined for external abnormalities or lesions that could include: pugheadedness or otherwise poor body condition; exophthalmia; cloudy cornea or lens opacity; hemorrhaging within the anterior chamber of the eyes, fins, body surface or body orifices (anus, nares, mouth, gill chamber), frayed or missing fins; gas bubbles within the fin rays or connective tissues of the eyes; ulcerations, abscesses, abrasions; body discoloration; excessive mucus; trailing fecal casts or rectal prolapse; external foreign bodies such as fungus, metazoan or protozoan parasites, cysts or tissue growths; potbelly or other protrusion or body malformations (spinal deformities, cranial swelling, shortened opercula, pugheadedness, microeye).
- B. External lesions such as ulcerations or abrasions should be inoculated onto BHIA (see Chapter 5 - Bacteriology). Use of BHIA with 1% NaCl may be necessary depending upon case information and whether fish are in saltwater and a halophilic bacterial pathogen is suspected.
- C. A peripheral blood smear can be made by excising the caudal peduncle (for small fish) and allowing a drop of blood to be deposited near the frosted end of a clean glass slide. The blood is smeared before clotting with a second glass slide by touching the drop with the slide at a 45° angle to the first slide and pushing the angled slide to the end of the first slide. Capillary action draws the smear across the first slide and the narrower the angle the thinner the smear (Figure 2, page 11). Stain the smears in Diff-Quik® (see staining procedures in section V.) and observe on the microscope at 1000X for bacteria, erythrocytic inclusion bodies (EIB) and viral erythrocytic (VEN) cytoplasmic inclusions, necrobiotic bodies (IHNV) and erythroblastosis or other blood abnormalities in cell composition and morphology. Larger fish may be bled by caudal vein puncture into a heparinized syringe or Vacutainer® and blood expressed onto a slide for subsequent smearing. For blood collection, the needle should be inserted at the location just below the lateral line that intersects with the rear margin of the anal fin. The needle should be inserted until just penetrating the vertebra (hemal canal) as indicated by slight resistance. Blood will automatically begin to flow when the Vacutainer® is punctured by the needle.

base or when the plunger of the syringe is pulled back.

- D. Fish should be placed on their right sides for performance of the remaining necropsy procedures. Skin scrapes of normal and lesion areas mounted with a drop of PBS and coverslip on a glass slide should be made by using either the edge of the coverslip as the scraping instrument, or a scalpel. Bacteria or fungus from lesion areas or protozoan parasites such as *Ichthyobodo* and *Trichodina* are common subjects to look for beginning at 40x and then at 200-400X on a compound microscope (if phase contrast is not available on the microscope, the condenser and diaphragm can be adjusted to increase contrast ).
- E. Wet mounts of gill filaments are made by using a small pair of surgical scissors to remove a portion of one gill arch. Gill filaments should be slightly teased apart for good viewing of filament and lamellar profiles and mounted in PBS with or without a coverslip. These should be examined immediately since branchial epithelium rapidly deteriorates causing postmortem artifact. Look for gas bubbles in the capillaries, telangiectasia, hyperplasia, external parasites (bacterial, protozoal, fungal, metazoan), or other foreign bodies. Should bacteria be observed or suspected the coverslip may be removed and used to mince the gill tissue. This is allowed to air dry for later Gram staining (see Chapter 5 – page 5). After staining, the gill tissue is removed with forceps for viewing of the stained slide for bacteria by oil immersion.
- F. Disinfect the outer surface of the fish by flooding with 70% ethanol. Disinfect a pair of scissors, forceps and scalpel by immersion in 100% ethanol and passing the instruments through a Bunsen flame allowing the alcohol to ignite and burn off. Repeat one or two more times. Wipe instruments clean of any organic matter beforehand for effective disinfection.
- G. The abdominal cavity is entered by pulling the pectoral fin with sterile forceps while cutting into the abdominal wall at the base of the pectoral fin with a pair of small sterile scissors. The cut is continued dorsally to just below the lateral line where resistance is encountered. Start again at the base of the pectoral fin and continue the incision towards the posterior of the fish along the ventral abdominal wall to the vent. Stay slightly above the intestinal tract when making the incision so that it is not punctured, thereby contaminating the tissues. At the vent continue dorsally to just below the lateral line and continue cutting anteriorly to connect with the first incision. Remove the flap of abdominal tissue, thus exposing the internal viscera and cavity. When done correctly on a moribund specimen the air bladder should remain inflated and the GI tract completely intact. Instruments may need wiping of organic material and flaming repeatedly during this procedure.
- H. Visually examine viscera (heart, liver and gall bladder, kidney, pancreas, adipose tissue, spleen, air bladder, pyloric caecae and entire GI tract) for abnormalities such as: discoloration or mottled appearance; enlargement (hypertrophy); hemorrhage or erythema; abscesses or cysts; fluid in the abdominal cavity (ascites causing potbelly); foreign bodies such as fungus, metazoan parasites or tissue growths, etc.

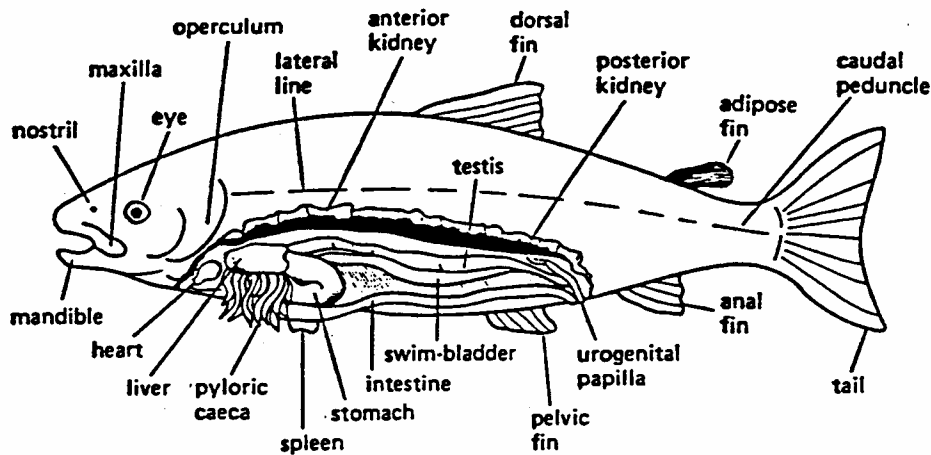
- I. If bacteria samples are to be taken they should be inoculated onto BHIA or other appropriate growth medium (i.e., TYES from the kidney, spleen, visceral lesion, or other tissues if indicated. See Chapter 5 – Bacteriology).
- J. Tissues to be taken for viral assay of larger fish (kidney/spleen pool) should also be placed into sterile tissue culture fluid for refrigeration and homogenization at a later time. Fry are generally processed whole for virology (see Chapter 11 - Virology).
- K. Kidney smears for FAT and tissues (kidney or kidney/spleen) for ELISA detection of the *Renibacterium salmoninarum* should be taken at this step. Generally, bacterial problems due to Gram-negative bacteria such as furunculosis and ERM agents can be detected more efficiently by isolation on prepared media.
- L. If the spleen has not been completely removed for virus assay, a spleen squash can be made by placing a cut section of the tissue with a drop of PBS on a glass slide and covering with a coverslip. Whole spleen squashes will be necessary when small fish are examined. Look for the presence of motile or non-motile bacterial rods and fungal hyphae. The coverslip may be removed and the squash Gram stained for confirmation of bacteria as described for gill tissues.
- M. A squash of a small section of the lower intestine (rectum) should also be made on a glass slide using PBS and a coverslip. Look for presence or absence of food and *Hexamita* or amoebae. Bacteria should obviously be abundant as part of the normal gut flora. Also look for fungal hyphae within the gut wall.
- N. A squash of lesion material from a visceral organ or organs may be warranted if present and if its cause is not readily discernible. Gram stains (Chapter 5) and/or Diff-Quik® stains (section V) of this material may also be warranted. An example would be stained impression smears of kidney tissue to examine for possible BKD, PKD or *Enterocytozoon salmonis*.
- O. If the cause of mortality or morbidity is in question as to whether or not the above procedures will provide an answer, histology samples should be taken as a backup measure, but only if moribund fish are available. Fish that have been dead for several hours or longer are generally not suitable for histology due to postmortem tissue autolysis. If fry are involved, whole fish may be dropped into Davidson's, or a non-formalin based fixative. Fingerlings should have the abdomens opened with scissors for better fixative penetration (refer to Chapter 13 – Histology, for more information on fixing tissues).
- P. If clinical signs suggest a central nervous system disorder the top of the cranial cavity should be opened and the brain included in bacteriologic sampling using BHIA and TYES agar. Heads from additional affected fish should be severed behind the gill opercula and placed into whirlpak bags for later testing for *Myxobolus cerebralis*. Heads can be halved for PTD and an archive sample for corroborative testing by PCR or histology.

Q. During necropsy, occasional serial sectioning of skeletal muscle using a razor blade may be necessary should a lesion within that tissue be suspected. Examples would include abscesses, hematomas, neoplasms or encysted parasites causing a protrusion of the musculature. Depending upon the nature of the lesion, bacteriological sampling, Gram staining or fixation for histology may be necessary.

If clinical signs are present, or fish are moribund, include at least 5-10 moribund for proper diagnosis. Control or healthy fish should also be examined and compared to determine whether abnormalities perceived in the population are real, or not. The number of control fish processed will depend upon availability and the particular case and may range from 10 to none.

Necropsies are best performed as a 2-3 person team effort in which a microbiologist and/or technician can make gross external and internal observations and the bacteriologic and tissue preparations. The pathologist in charge can devote his or her time to interpreting the sample preparations on the microscope. In this approach a case can be processed in a minimum amount of time and provides further pathology experience to the support staff.

**Figure 1 – Salmonid Anatomy**



### III. Results and Report of Findings

In summary, a standard necropsy should include all the information contained on the NWFHS Submission Form (Case History Number, location, species, examination date and number of tissues/samples submitted). In addition to the Submission Form, a complete Necropsy Form, containing the following information, should be attached:

- External and internal gross observations recorded on a necropsy worksheet.
- Wet mounts or squashes of:
  - ◊ gills
  - ◊ skin
  - ◊ spleen
  - ◊ lower gut
  - ◊ lesions (if any)
- Peripheral blood smear stained with Diff-Quik® (Optional)
- Gram Stain – Type and Number of tissues
- Overall evaluation / findings

### V. Fish Diseases: Causative Agents and Signs

#### A. BACTERIA

##### 1. Bacterial Kidney Disease (*Renibacterium salmoninarum*)

**External signs:** exophthalmia; abdominal swelling; sometimes blisters in skin filled with clear amber to cream colored purulent fluid. In advanced disease, large muscle lesions may be present.

**Internal signs:** kidneys pale and swollen; abscesses in kidney, liver or spleen; may have ascitic fluid in abdomen; intestine distended, fluid filled.

##### 2. Cold Water Disease (*Flavobacterium psychrophilum*)

**External signs:** tail darkening, white or bluish areas behind dorsal or adipose fins; loss of epidermis on dorsal or posterior surface; erosion of the dermis on the peduncle exposing skeletal muscle; loss of caudal peduncle; erosion of jaw or snout; gill hemorrhages and anemia. In some cases, no external signs are observed.

**Internal signs:** generally not remarkable but sometimes has enlarged spleen with myriad number of filamentous rods; petechial hemorrhages of adipose tissues.

##### 3. Columnaris (*Flavobacterium columnare*)

**External signs:** white to yellow lesions that may have a red periphery on the head, jaw, back (saddleback lesion), and/or fins, especially caudal fin. Gills may also be infected; disease begins at the tips of the lamellae and causes a progressive necrosis that may

extend to the base of the gill arch. Bacteria are gliding and often form clumps that appear like a column or “haystack.”

4. **Edwardsiella tarda Septicemia (*Edwardsiella tarda*)**

**External signs:** small cutaneous lesions that become large abscesses within the muscle, and become necrotic. May also have loss of dermal pigmentation.

**Internal signs:** generalized septicemia, ascitic fluid in abdominal cavity, protruding hemorrhaged anus, opaqueness in eyes; small white nodules may be present in the kidney, liver, spleen, and gills.

5. **Enteric Redmouth (*Yersinia ruckeri*)**

**External signs:** hemorrhaging or erosion around mouth; pale gills; exophthalmia; swollen abdomen; reddened opercula and fin bases; inflamed hemorrhagic vent.

**Internal signs:** inflammation and hemorrhaging in most visceral organs; edema in spleen, liver and kidney; liver may be pale; fluids may accumulate in abdominal cavity, stomach and intestine; inflamed, hemorrhagic lower intestine with bloody diarrhea.

6. **Enteric Septicemia (*Edwardsiella ictaluri*)**

**External signs:** Fish refuse feed and swim at the surface. External lesions with hemorrhage around the mouth and lateral and ventral portions of the body and fins; pale gills; exophthalmia; and small ulcerations on the body. Ulceration in the fontanelle of the frontal bones.

**Internal signs:** generalized septicemia with petechiae throughout the visceral mass, in the peritoneum and musculature. Ascites and enlargement of the liver, kidney and spleen.

7. **Furunculosis (*Aeromonas salmonicida*)**

**External signs:** skin blisters or furuncles which may ulcerate; erythemia of eyes, base of fins and anal vent. In acute cases, bleeding from the gills may be seen.

**Internal signs:** kidney necrosis; petechiae in mesenteries around pancreatic tissue; localized hemorrhages in intestine and liver; dark, hypertrophied spleens.

8. **Citrobacter infection (*Citrobacter freundii*)**

**External Signs:** Ulcerative lesions may be seen on skin, eye and base of fins.

**Internal Signs:** Hemorrhaging in the peritoneum and gastro intestinal tract; swollen kidney with multiple lipid granuloma in some fish species.

## B. PARASITES

1. **Asian tapeworm (*Bothriocephalus acheilognathi*)**

**External signs:** abdominal swelling if heavily infected.

**Internal signs:** little abdominal fat due to starvation, presence of tapeworm in stomach.

2. **Ceratomyxosis (*Ceratomyxa shasta*)**

**External signs:** loss of appetite, hemorrhaging and swelling of urogenital opening.

**Internal signs:** ascites; swelling and hemorrhaging of the intestine; swollen vent. Developing parasites incite a diffuse granulomatosis in many host tissues, including intestine, liver, kidney, spleen, gonads, and muscle. The abdomen is often distended because of granulomatous peritonitis.

3. **Whirling Disease (*Myxobolus cerebralis*)**

**External signs:** black tail in 3-6 month old fish; impaired balance and a frenzied, tail-chasing behavior. Older fish that survive often develop spinal curvature, pug-headedness, or an undershot jaw from cartilage damage.

**Internal signs:** none except histological.

## C. VIRUSES

1. **Infectious Hematopoietic Necrosis Virus (IHNV)**

**External signs:** exophthalmia, body darkening, abdominal distension, pale gills, trailing white fecal cast; lethargic swimming, riding high in the water column.

**Internal signs:** ascites, viscera paleness, anemia, petechial hemorrhages, pale kidney; little or no food in the intestinal tract.

2. **Infectious Pancreatic Necrosis Virus (IPNV)**

**External signs:** whirling, agonal swimming, anorexia, dorsal darkening, abdominal distension, and/or trailing white feces.

**Internal signs:** petechial hemorrhages and yellow exudate in gut of older fish; fry will have pale viscera with few petechiae.

3. **Infectious Salmon Anemia Virus (ISAV)**

**External signs:** appear 2-4 weeks after infection, few external signs other than exophthalmia, pale gills and lethargy. Mortality of up to 3% per day in some cases. Infected facilities may see a predictable rise in mortality by 0.05% per day for three consecutive days.

**Internal signs:** hemorrhaging on the kidney and other organs; swollen eyes; fluid in body cavity; swelling of kidney; darkening of posterior gut and swollen spleen.

4. **Largemouth Bass Virus (LMBV)**

**External signs:** moribund fish lose equilibrium and float at the surface due to enlarged swim bladder.

**Internal signs:** Gas gland excessively red; air bladder lesions consist of a yellow to brown waxy residue in the lumen of the air bladder.

5. **Oncorhynchus Masou Virus (OMV)**

**External signs:** epithelioma (tumors of the epithelial layer) occurring mainly around the mouth, ulcers on skin.

**Internal signs:** intestinal hemorrhages, white spots on liver.



6. **Spring Viremia Carp Virus (SVCV)**

**External signs:** lethargy, sluggish breathing, concentration in slow waters. A darkening of skin and gills, bloody mucus or fecal casts from hemorrhaging vent.

**Internal signs:** Fluid in the body cavity, swollen spleen, blood in swim bladder and a general hemorrhagic condition.

7. **Viral Hemorrhagic Septicemia Virus (VHSV)**

**External signs:** lethargy, avoid current, listless, hang suspended or drop to bottom; body darkening, exophthalmia with hemorrhaged orbit, external hemorrhage especially base of fins and in roof of mouth, pale gills with focal hemorrhage.

**Internal signs:** empty gastrointestinal tract; scattered hemorrhages of connective tissue, adipose tissue, swim bladder, and intestine; kidney red and thin in acute stage, gray and swollen in chronic stage.

8. **White Sturgeon Iridovirus (WSIV)**

**External signs:** emaciation, go off feed: swollen gills.

**Internal signs:** hyperemic areas on abdomen, necrosis of epidermis.

9. **White Sturgeon Herpesvirus (WSHV-2)**

**External signs:** few to severe hemorrhagic signs in young fish; hemorrhages around ventral scutes and mouth; small ulcers with petechial hemorrhaging. Chronic form will have reoccurring blisters, especially after stress, often starts on the head.

**Internal signs:** none, except for ulceration in the mouth area.

## V. Staining Procedures

A. GRAM STAIN - See Chapter 5 – Bacteriology (page 5), for Gram Staining procedure.

B. DIFF-QUIK® STAIN:

1. Make smears of blood, fluids, or tissues and air-dry.
2. Dip 5 times in Diff-Quik® solution 1, one second each time, and drain.
3. Dip 5 times in Diff-Quik® solution 2, one second each time, and drain.
4. Dip 5 times in Diff-Quik® solution 3, one second each time, and drain.
5. Rinse in tap water and drain.
6. Air-dry and examine using 10x, 40x, or 100x objective lens.

**NOTE:** Diff-Quik® solution 3 can become weakened with age or use. Check stains intensity on slides periodically. Slides may be re-stained with fresh solution 3 if necessary. Periodically pass solutions 2 and 3 through separate 0.45-µm filters to remove precipitates and contaminating bacteria.

## Figure 2. Preparation of a Thin Blood Smear.

1. On slide "A" express a drop of blood about one-half inch from the end.
2. The edge of a second slide "B" is placed on the surface of slide "A" at about a 45° angle and is moved backward (to the right in the diagram) until contact with the drop of blood.
3. Contact with the blood will cause the drop to spread along the edge of slide "B" due to capillary action. Slide "B" is then pushed forward (left in the diagram), being careful to keep the edge pressed uniformly against the surface of slide "A".
4. The size of the drop of blood and acuteness of the angle formed between the slides will determine the thickness of the film. A more acute angle results in a thicker film.
5. The smear is allowed to air dry for transport in a slide box and later staining.

