

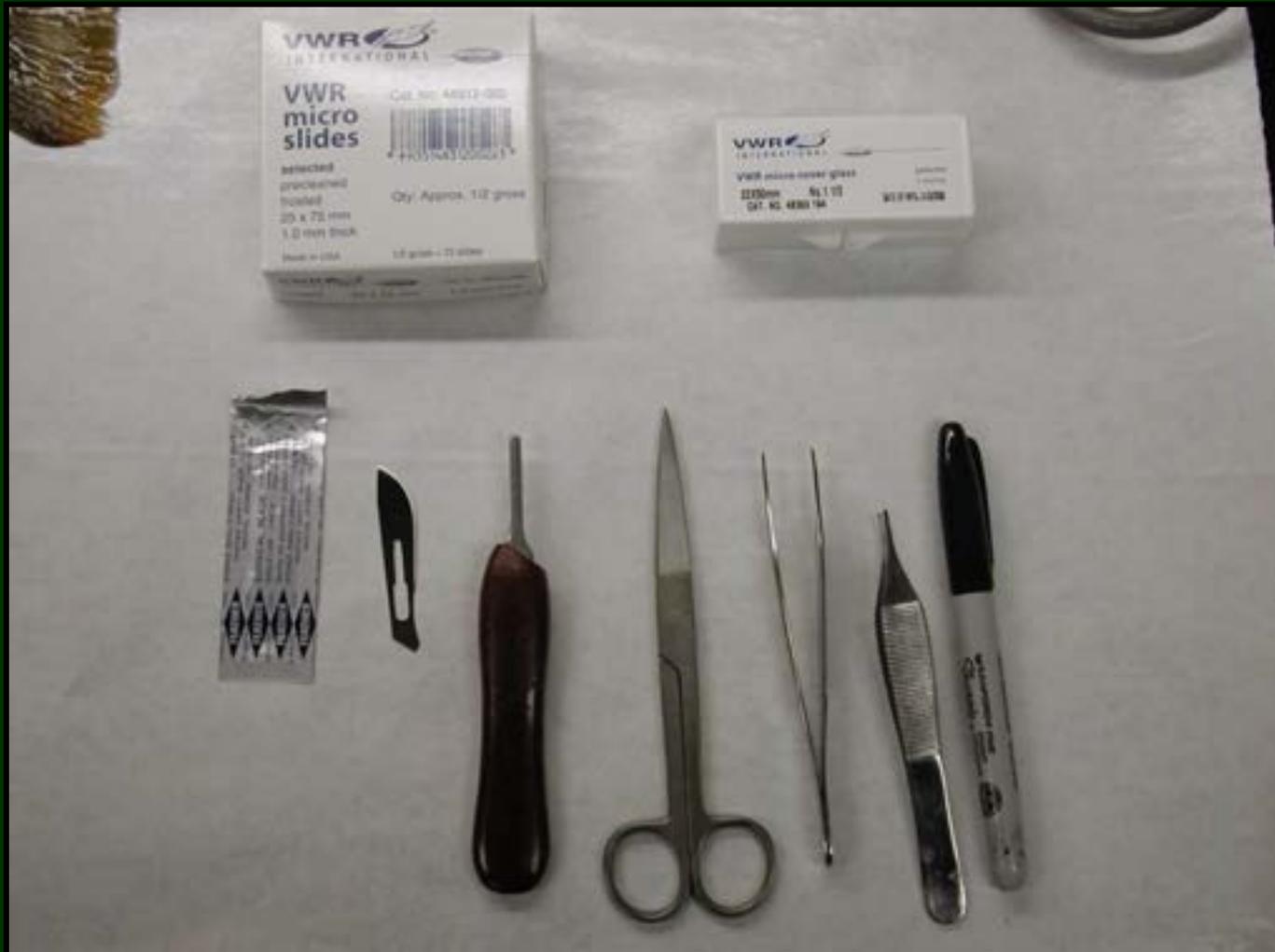
Fish Health Sample Collection Procedures

Wyoming Game and Fish Laboratory

How to do a general examination of your fish at the hatchery.



EQUIPMENT NEEDED



USE FRESHLY DEAD FISH

Examine ASAP after death



EXTERNAL EXAM



EXTERNAL EXAM



Look at the eyes for cloudiness or hemorrhage

EXTERNAL EXAM



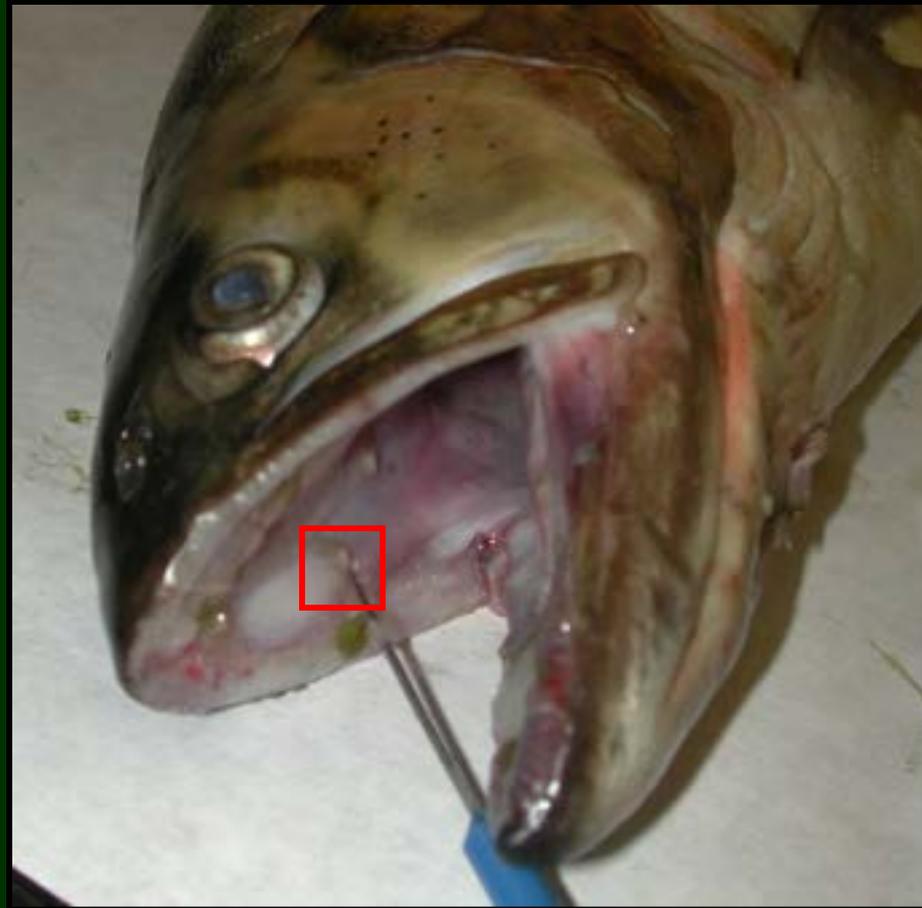
Or for gas bubbles behind the eye.

EXTERNAL EXAM



Make note of fin condition, look for visible external parasites.

EXTERNAL EXAM



Look in the mouth for parasites.
The copepod *Salmincola* is shown in the above photo.

EXTERNAL EXAM



Look for any external lesions.

EXTERNAL EXAM



If lesions are present, do a scraping across their surface.

EXTERNAL EXAM



Put the scraping on a slide, let air dry and label appropriately.

SKIN SCRAPE



A method to look for any external parasites.

SKIN SCRAPE



Gently scrape the edge of the scalpel blade across the skin above the lateral line.

SKIN SCRAPE



Scrape behind the pectoral fin down toward the tail.

SKIN SCRAPE



Wipe the mucus on the scalpel blade on a clean slide and add water.

SKIN SCRAPE



Place a coverslip on the slide.

SKIN SCRAPE



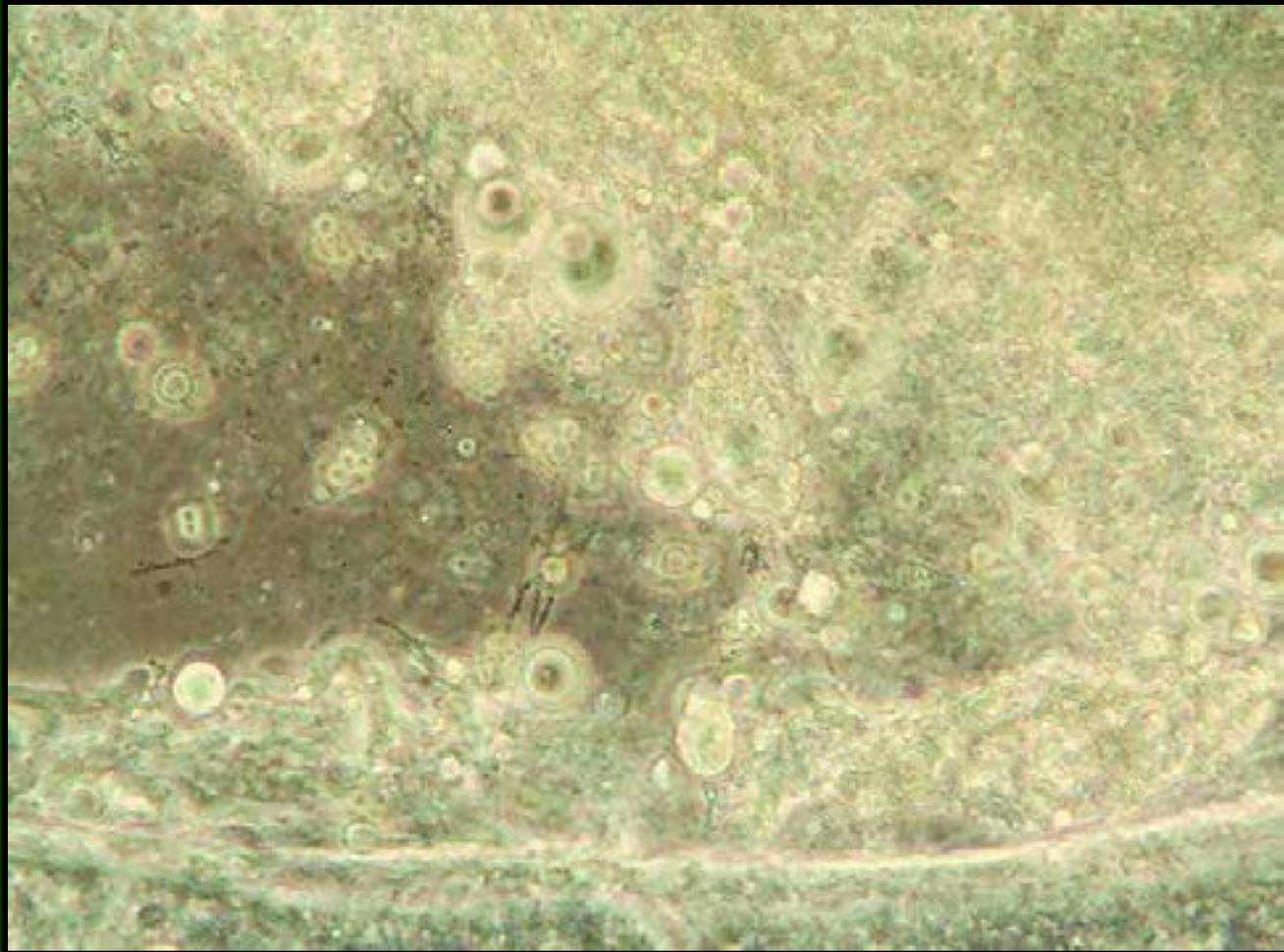
Examine the slide using bright-field microscopy.

Examine for parasites



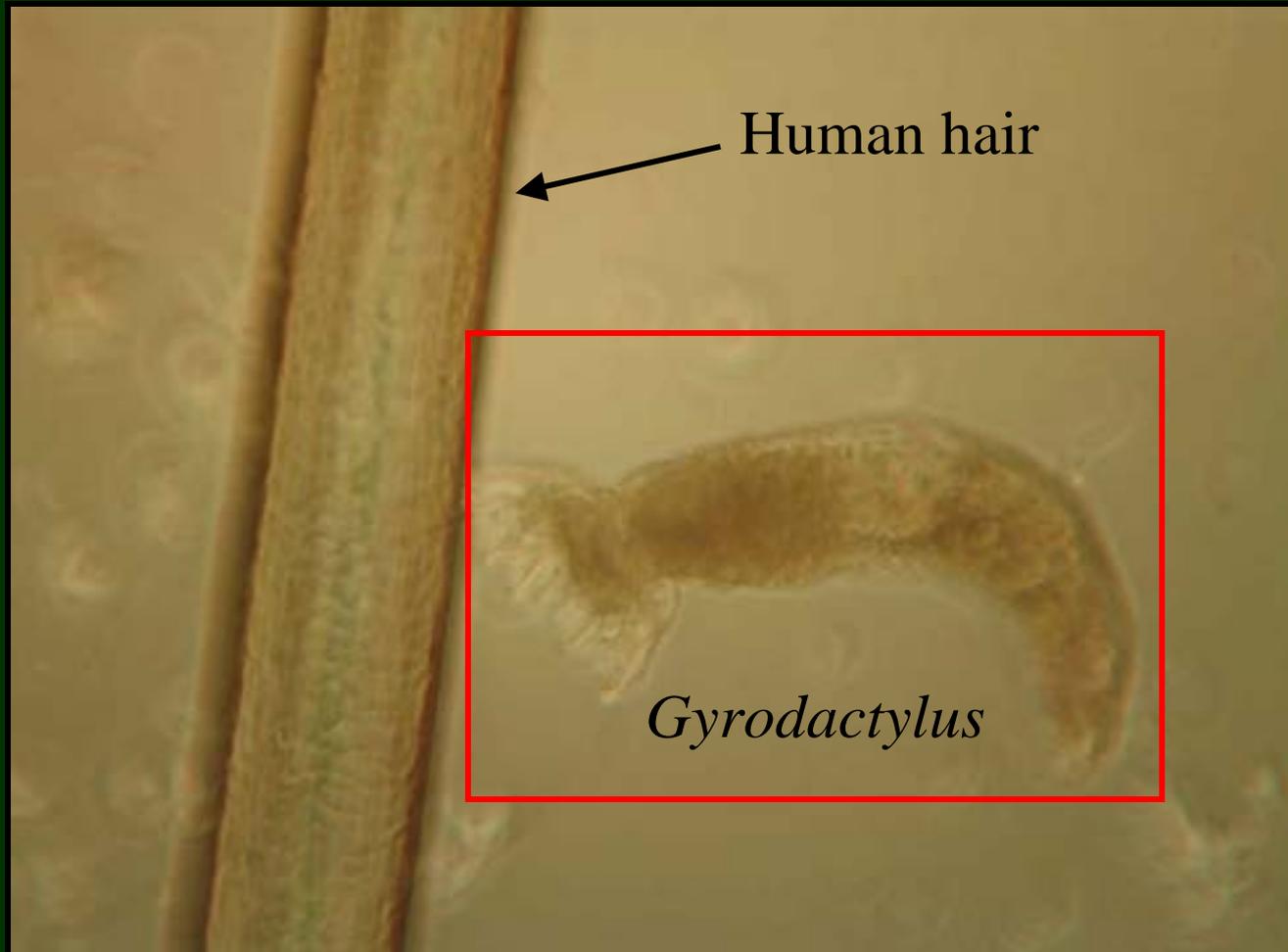
You will see lots of cells, debris, and air bubbles on the slide.

Examine for parasites



Parasites may be hard to see.

Here are some examples of common skin parasites.



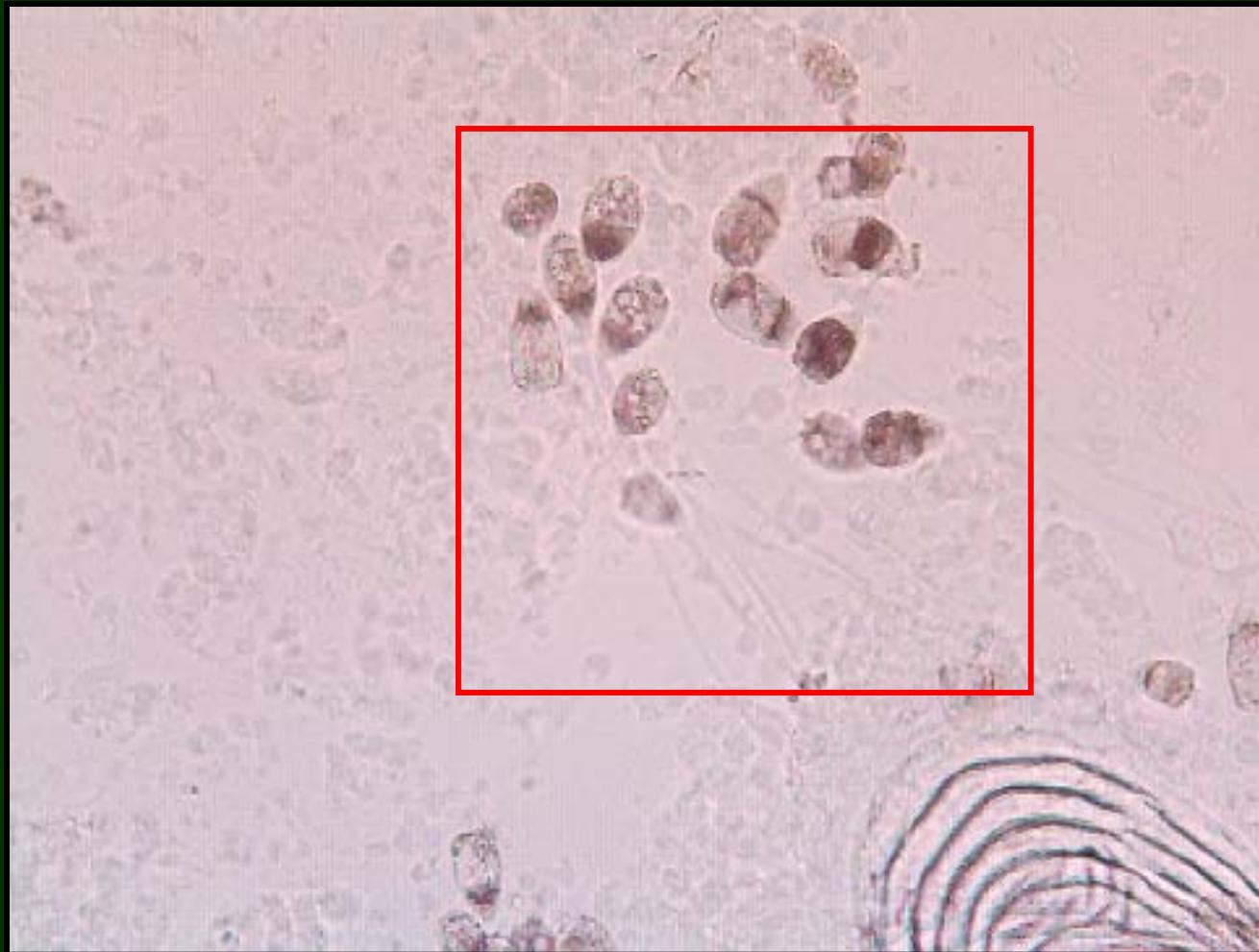
A human hair shows approximate size

Here are some examples of common skin parasites.



Trichodina

Here are some examples of common skin parasites.



Epistylis

If fish is not fresh, parasites can degrade and will not be as noticeable.



Gyrodactylus haptors (mouthparts)

GILL EXAMINATION



GILL EXAMINATION



Cut out a few gill arches and place on a clean slide.

GILL EXAMINATION



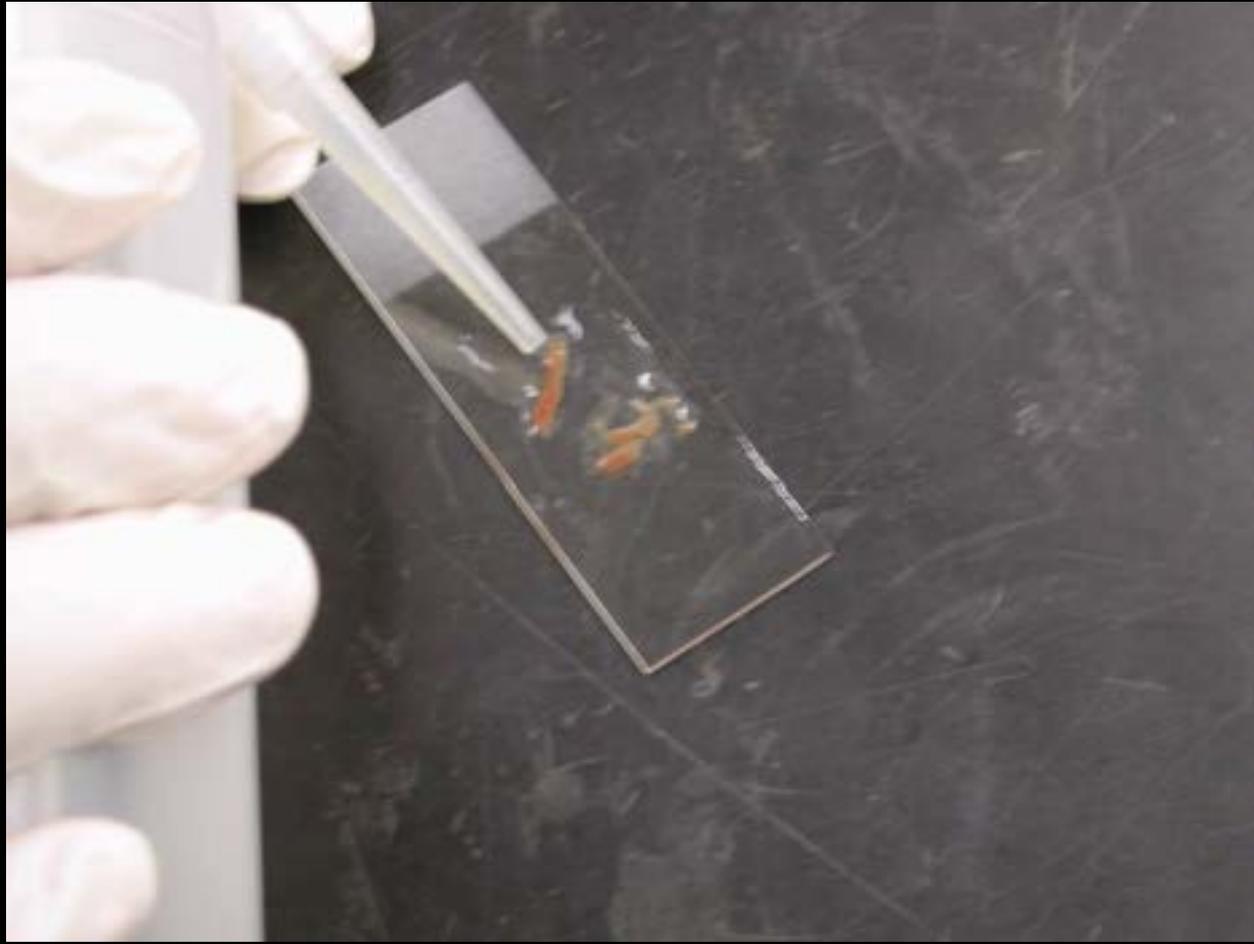
Do the same if you have a larger fish.

GILL EXAMINATION



Place the gills on a slide. Use the scalpel to cut out the gill arch.

GILL EXAMINATION



Add enough water to cover the sample.

GILL EXAMINATION



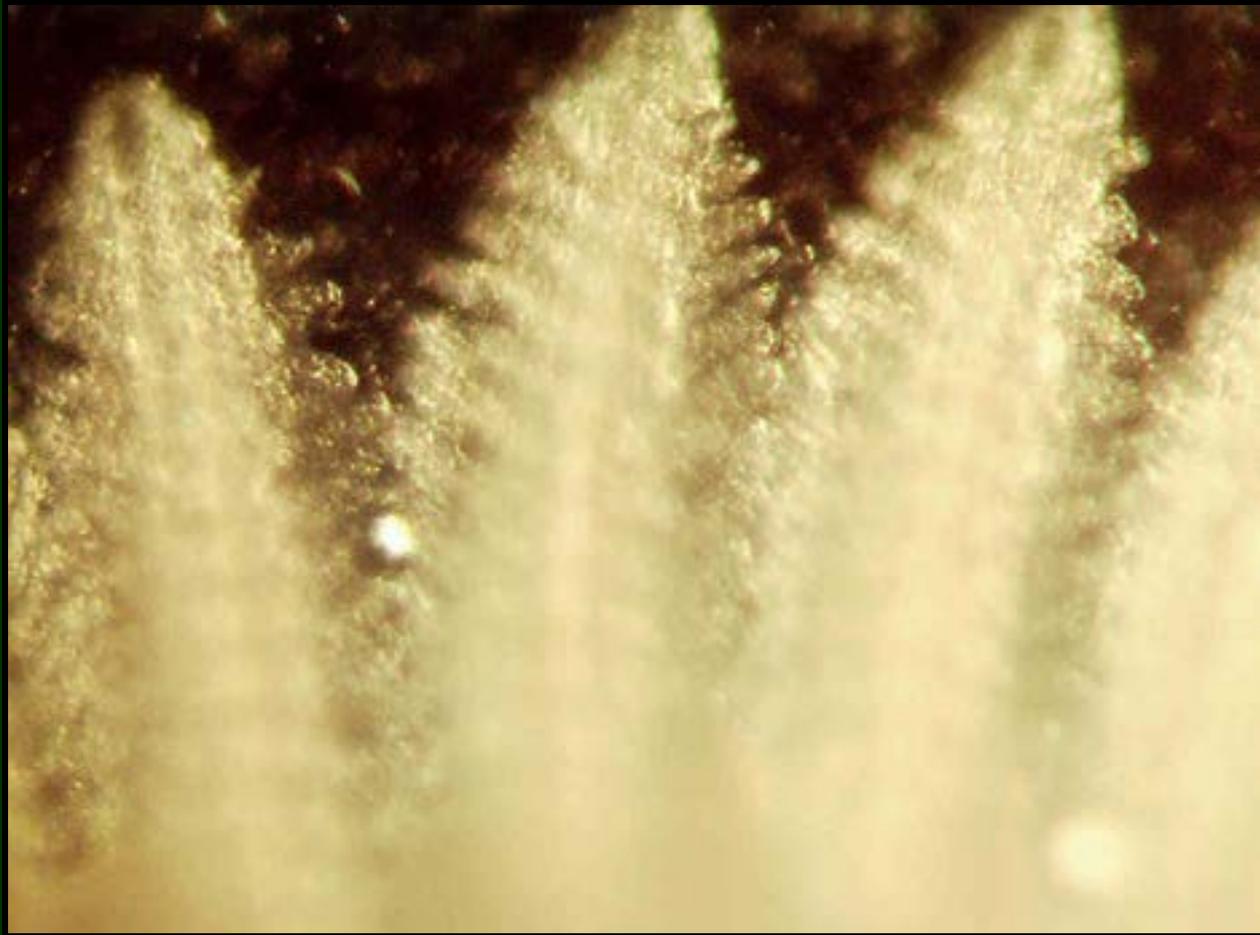
Add a coverslip.

GILL EXAMINATION



Examine using bright-field microscopy.

Healthy gills will look nice and feathery...



These gills show signs of gill disease.



Look for any unusual cysts; these are Xenomas formed by the microsporidian parasite *Loma salmonis*.



GILL EXAMINATION



It can be hard to see the fine structure of the gills using bright-field microscopy on large fish.

GILL EXAMINATION



In this case, place gill tissue in a petrie dish with water.

GILL EXAMINATION



Examine under the dissecting microscope.

INTERNAL EXAM



INTERNAL EXAM



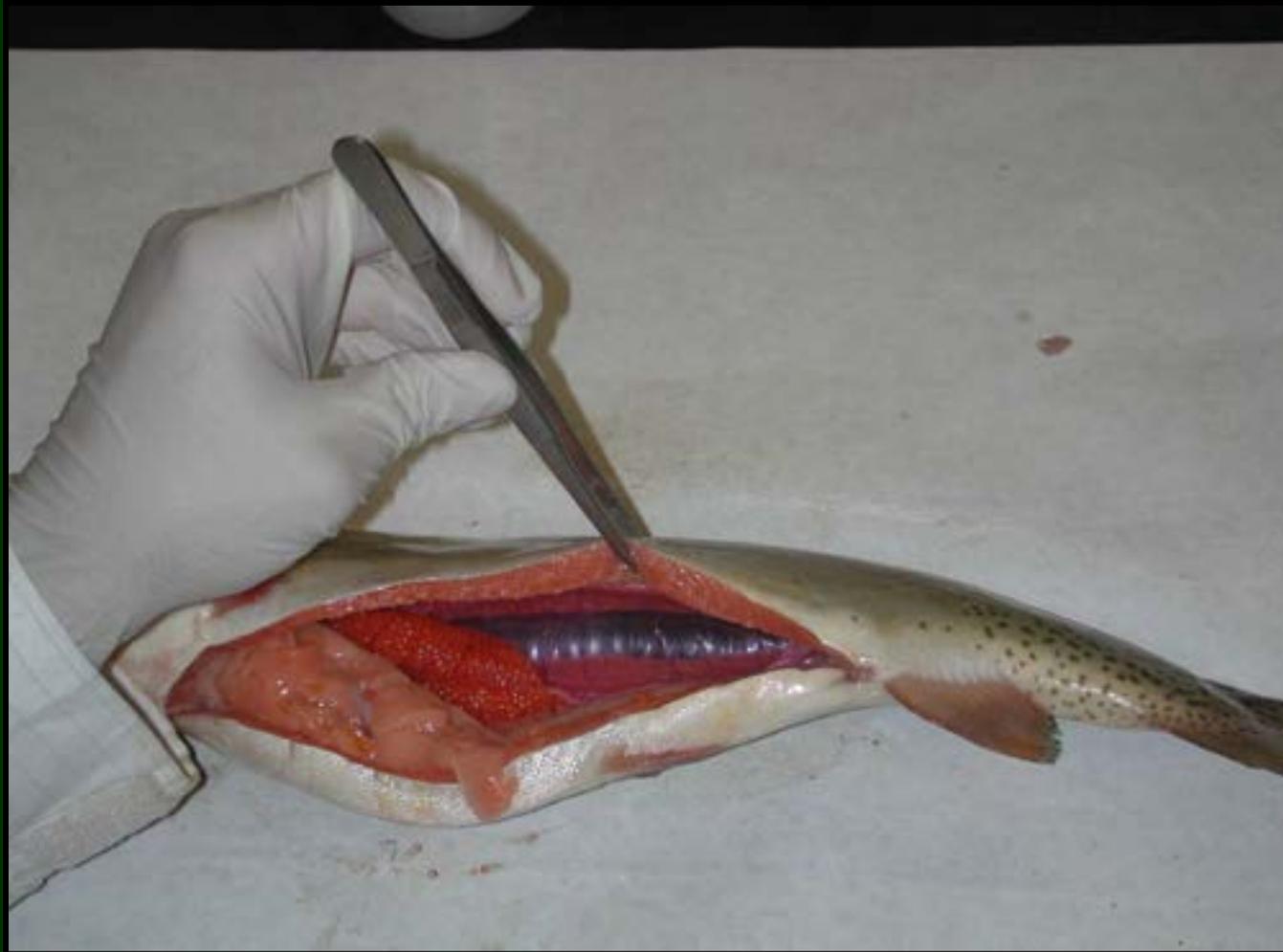
Cut from the vent up to the pectoral fin, in a half-moon shape.

INTERNAL EXAM



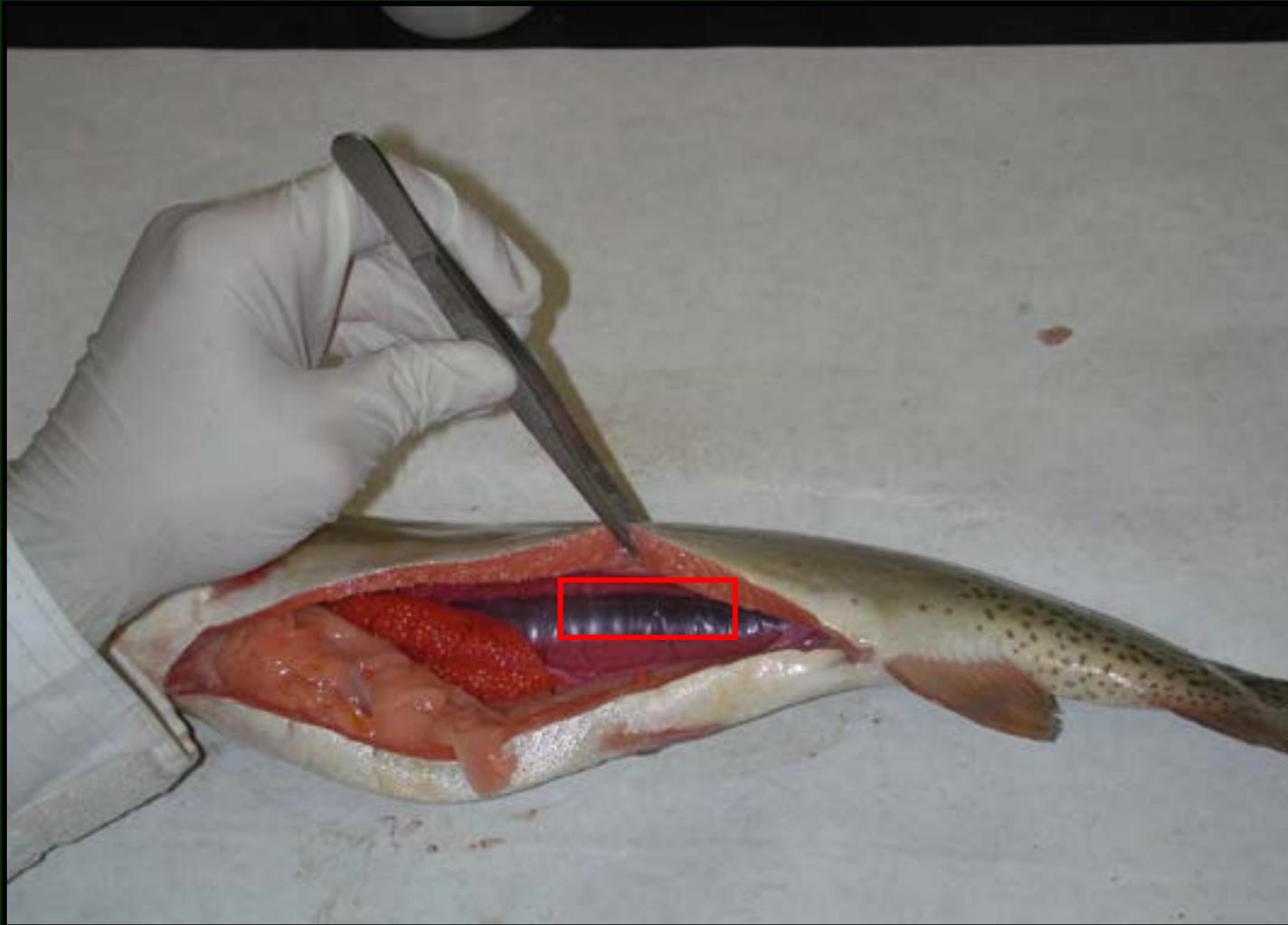
Open the fish up to expose the internal organs.

INTERNAL EXAM



Make note of any hemorrhage or other abnormalities .

KIDNEY IMPRINTS



Use a scalpel and cut around the posterior portion of the kidney.

KIDNEY IMPRINTS



Using forceps extract a sample of kidney tissue.

KIDNEY IMPRINTS



Blot the kidney tissue to remove excess red blood cells.

KIDNEY IMPRINTS



Place the tissue on a clean slide.

KIDNEY IMPRINTS



Take another clean slide and press down on the tissue.

KIDNEY IMPRINTS



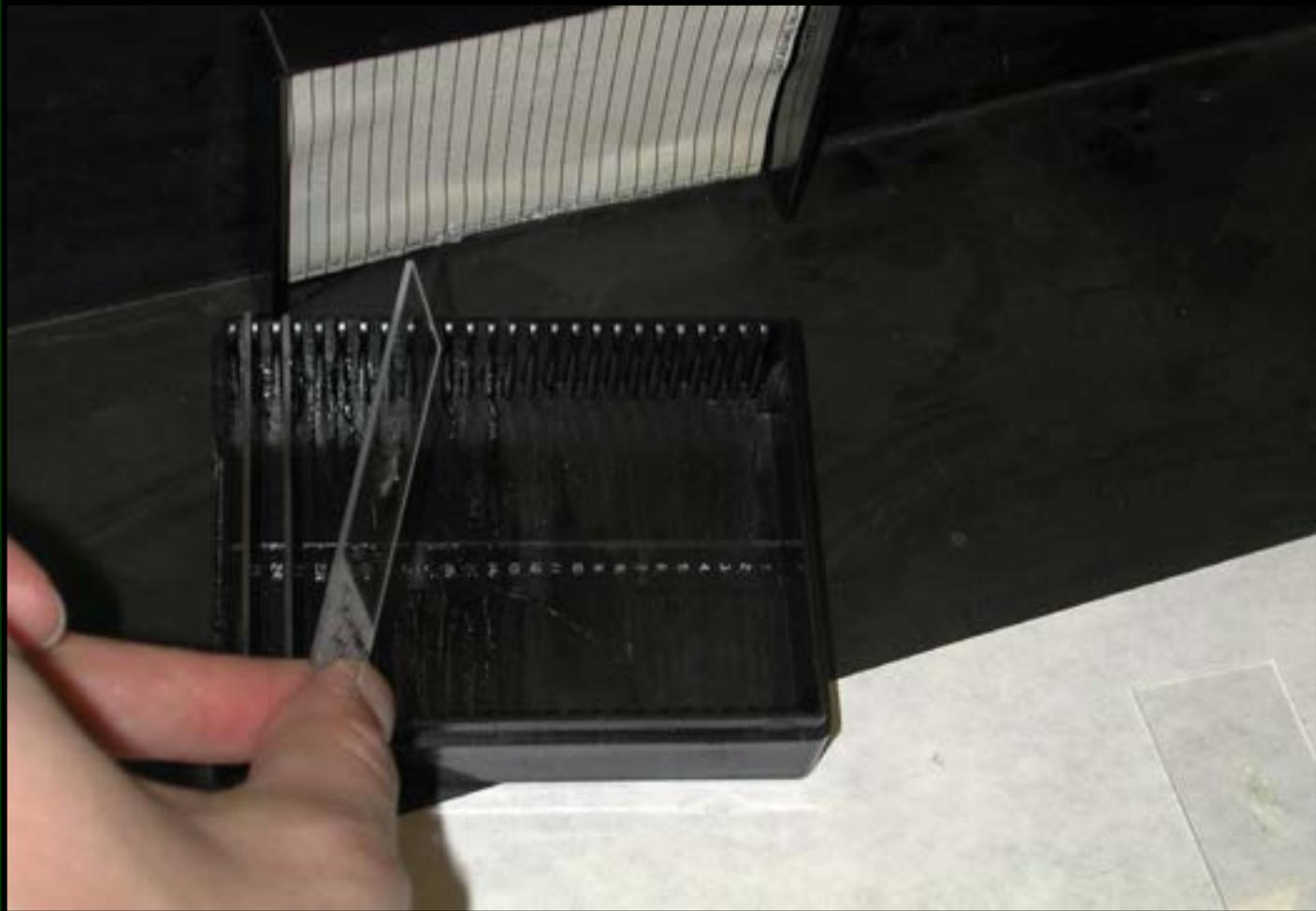
Separate the slides. Use forceps to remove excess tissue from each slide.

KIDNEY IMPRINTS



Label each slide with the source, species, and tissue type.

KIDNEY IMPRINTS



Put kidney imprints and any other slides in a slide box for shipment to the lab.

HISTOLOGY SAMPLES



HISTOLOGY SAMPLES



Use a freshly dead fish, fix tissues as soon as possible after removing from the water.

HISTOLOGY SAMPLES



Open the fish up as described before to expose internal organs.

HISTOLOGY SAMPLES



Use a scalpel to cut around the kidney.

HISTOLOGY SAMPLES



Remove the portion of kidney, keeping it whole, and place it in the fixative.

HISTOLOGY SAMPLES



Remove the spleen and any other organs of concern and place in the fixative.

HISTOLOGY SAMPLES



To fix gills, cut gill arches out.

HISTOLOGY SAMPLES



Place gills in fixative.

HISTOLOGY SAMPLES



To fix whole viscera of fish, cut it open as shown previously.

HISTOLOGY SAMPLES



Disconnect the organs from the body by cutting the esophagus anterior to the heart.

HISTOLOGY SAMPLES



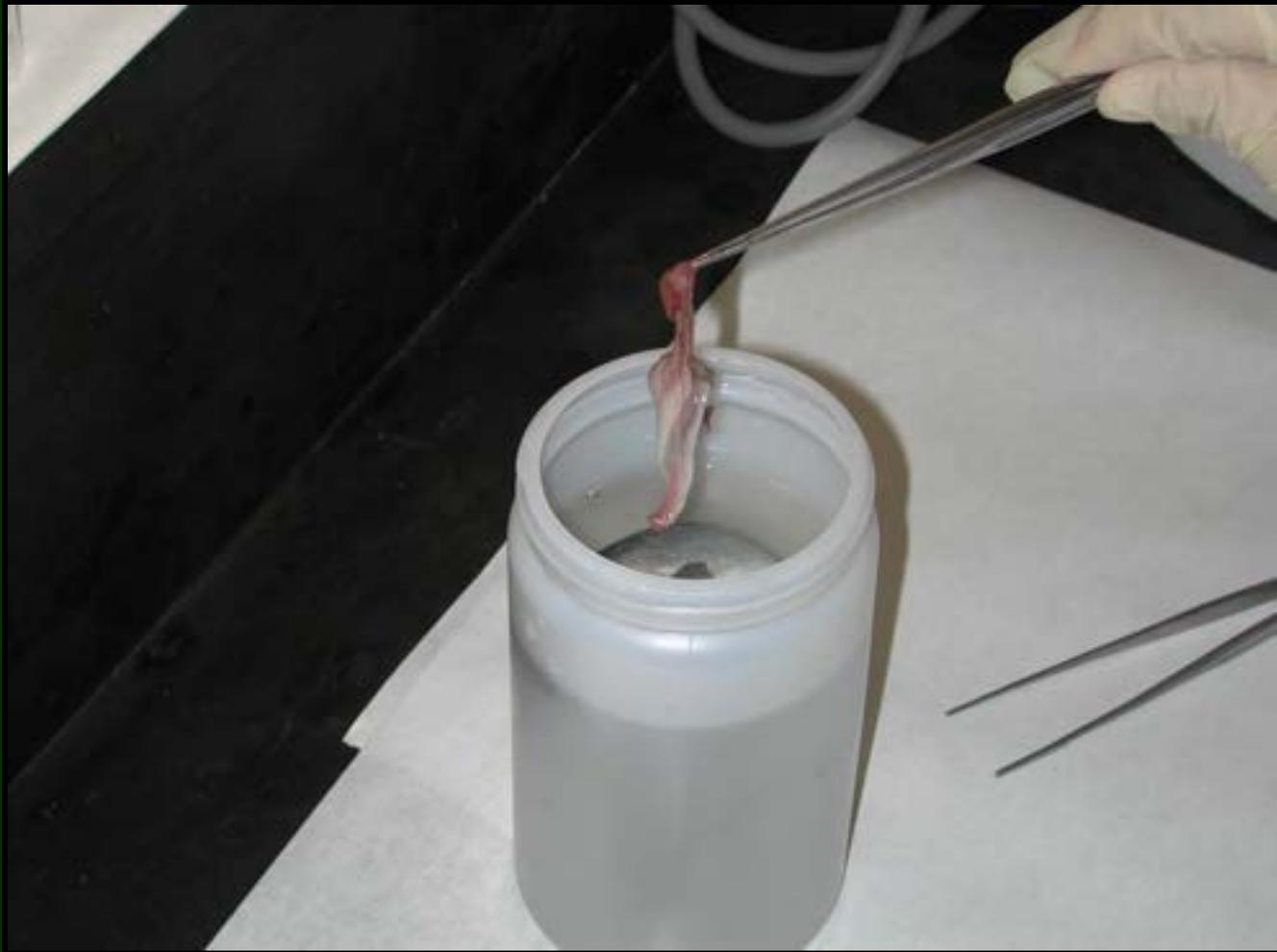
Using forceps, pull the entire viscera out of the body cavity.

HISTOLOGY SAMPLES



Cut the descending intestine at the vent.

HISTOLOGY SAMPLES



Place the viscera in the fixative.

HISTOLOGY SAMPLES



To fix a whole fish, cut from the vent to the pectoral fins.

HISTOLOGY SAMPLES



Make another cut into the muscle tissue above the lateral line. These cuts allow the fixative to penetrate all of the fish tissues.

HISTOLOGY SAMPLES



Place the fish into a jar of fixative.

HISTOLOGY SAMPLES



Make sure the entire fish is covered by fixative.

SHIPPING SAMPLES



Package freshly dead fish with no water in the bag.

SHIPPING SAMPLES



Do not put ice packs on top of the samples.

SHIPPING SAMPLES



Do not put samples on top of ice preferably with newspaper or other insulation between the samples and ice so they don't freeze during shipment.

SHIPPING SAMPLES



Shipping fixed samples: It is a good idea to put fixative jars in a plastic bag (or two) before placing in the cooler to prevent leakage. Pack them so they will stay upright while shipping.

SHIPPING SAMPLES

Hatchery Sample Submission Form

Hatchery Name: _____
 Lot Number: _____
 Address: _____
 Collector: _____

Sample Information:

Location	Water Conditions	Sample Condition	Number of fish
Hatchery	Temperature	Healthy	
Concrete	Nitrogen	Moribund	Previous treatment (list below)
Circular Tank	Other (list below)	Dead	
Dirt Pond		Abnormal	
Other		Other	

General Appearance and Behavior of Fish

Normal with a few mortalities	Sinking to the bottom
Sluggish	Listless
Flashing	Feeding normally
Spooky	Reduced feeding
Corkscrewing	Refuse food
Swimming upside down	

Fish Arrangement in Water

Normal distribution	Crowding at water outlet
Near surface	Crowding at water inlet
Schooling	Sinking to the bottom

Gill Condition

Covered in mucus or debris	Opaque
Swollen	Spots or hemorrhage in lens
Covers open more than normally	Pop-eye

Body surface

Swollen areas	Cysts
Open lesions	Spots
Necrotic areas	Bluish film
Granulations	Grayish-white patches or tufts

Other symptoms or conditions noted: _____

Services Requested:

Categories	Frozen Fish	Formalin Preserved	Fresh Fish	Live Fish
Necropsy	X	X	X	X
Bacteriology	X		X	X
Whirling Disease Analysis (PTD)	X		X	X
Virology			X	X
Histology		X		X
PCR	X		X	X

Submitters

* Fish submitted for laboratory diagnosis are preferably collected prior to any chemical treatment, packaged live, or freshly killed (iced) for shipment to the lab within 24 hours. When individual tissues are collected, these should be kept cold with ice but should not be allowed to freeze (insulated from direct contact with ice). If a parasite infection is suspected fix fish or tissues in 10% formalin and make arrangements for transportation to the lab. If whole fish are fixed make an incision from the vent to the pectoral fins to allow for proper fixation. In some situations it may be necessary to submit both fresh and fixed samples to aid in diagnosis

Fill out a Hatchery Sample Submission Form and include it with your samples that you are sending to the lab.

SAMPLE FORM

Hatchery Sample Submission Form

Hatchery Name: _____

Lot Number: _____

Address: _____

Collector: _____

Sample Information:

Location		Water Conditions		Sample Condition	
Hatchery		Temperature		Healthy	Number of fish
Concrete		Nitrogen		Moribund	Previous treatment (list below)
Circular Tank		Other (list below)		Dead	
Dirt Pond				Abnormal	
Other				Other	

General Appearance and Behavior of Fish

Normal with a few mortalities		Sinking to the bottom	
Sluggish		Listless	
Flashing		Feeding normally	
Spooky		Reduced feeding	
Corkscrewing		Refuse food	
Swimming upside down			

Fish Arrangement in Water

Normal distribution		Crowding at water outlet	
Near surface		Crowding at water inlet	
Schooling		Sinking to the bottom	

Gill Condition

Eye Condition

Covered in mucus or debris		Opaque	
Swollen		Spots or hemorrhage in lens	
Covers open more than normally		Pop-eye	

SAMPLE FORM (continued)

Body surface				
Swollen areas		Cysts		
Open lesions		Spots		
Necrotic areas		Bluish film		
Granulations		Grayish-white patches or tufts		
<u>Other symptoms or conditions noted:</u>				
Services Requested:				
Categories	Frozen Fish	Formalin Preserved	Fresh Fish	Live Fish
Necropsy	X	X	X	X
Bacteriology	X		X	X
Whirling Disease Analysis (PTD)	X		X	X
Virology			X	X
Histology		X		X
PCR	X		X	X
Submitters				
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DISCLAIMER

Although this presentation was developed to assist hatchery personnel with the basic principals for parasite examinations and sample collection procedures, we recognize that fish disease diagnosis is often difficult and requires an experienced fish health professional and full service testing facilities.

Contact us!

We welcome any and all feedback on the usefulness of this presentation. Please contact us and let us know what you think.

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