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

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

**Gyrodactylus**
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
Cut out a few gill arches and place on a clean slide.
Do the same if you have a larger fish.
Place the gills on a slide. Use the scalpel to cut out the gill arch.
Add enough water to cover the sample.
GILL EXAMINATION

Add a coverslip.
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.*
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

Cut from the vent up to the pectoral fin, in a half-moon shape.
Open the fish up to expose the internal organs.
INTERNAL EXAM

Make note of any hemorrhage or other abnormalities.
Use a scalpel and cut around the posterior portion of the kidney.
Using forceps extract a sample of kidney tissue.
Blot the kidney tissue to remove excess red blood cells.
Place the tissue on a clean slide.
Take another clean slide and press down on the tissue.
KIDNEY IMPRINTS

Separate the slides. Use forceps to remove excess tissue from each slide.
Label each slide with the source, species, and tissue type.
Put kidney imprints and any other slides in a slide box for shipment to the lab.
HISTOLOGY SAMPLES
Use a freshly dead fish, fix tissues as soon as possible after removing from the water.
Open the fish up as described before to expose internal organs.
Use a scalpel to cut around the kidney.
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.
To fix gills, cut gill arches out.
Place gills in fixative.
To fix whole viscera of fish, cut it open as shown previously.
Disconnect the organs from the body by cutting the esophagus anterior to the heart.
Using forceps, pull the entire viscera out of the body cavity.
Cut the descending intestine at the vent.
HISTOLOGY SAMPLES

Place the viscera in the fixative.
To fix a whole fish, cut from the vent to the pectoral fins.
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.
Package freshly dead fish with no water in the bag.
Do not put ice packs on top of the samples.
Do 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 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.
Fill out a Hatchery Sample Submission Form and include it with your samples that you are sending to the lab.
# Hatchery Sample Submission Form

**Hatchery Name:**

**Lot Number:**

**Address:**

**Collector:**

## Sample Information:

<table>
<thead>
<tr>
<th>Location</th>
<th>Water Conditions</th>
<th>Sample Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatchery</td>
<td>Temperature</td>
<td>Healthy</td>
</tr>
<tr>
<td>Concrete</td>
<td>Nitrogen</td>
<td>Moribund</td>
</tr>
<tr>
<td>Circular Tank</td>
<td>Other (list below)</td>
<td>Dead</td>
</tr>
<tr>
<td>Dirt Pond</td>
<td>Other (list below)</td>
<td>Abnormal</td>
</tr>
<tr>
<td>Other</td>
<td>Other</td>
<td>Other</td>
</tr>
</tbody>
</table>

### General Appearance and Behavior of Fish

<table>
<thead>
<tr>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal with a few mortalities</td>
</tr>
<tr>
<td>Sluggish</td>
</tr>
<tr>
<td>Flashing</td>
</tr>
<tr>
<td>Spooky</td>
</tr>
<tr>
<td>Corkscrewing</td>
</tr>
<tr>
<td>Swimming upside down</td>
</tr>
<tr>
<td>Sinking to the bottom</td>
</tr>
<tr>
<td>Listless</td>
</tr>
<tr>
<td>Feeding normally</td>
</tr>
<tr>
<td>Reduced feeding</td>
</tr>
<tr>
<td>Refuse food</td>
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</tbody>
</table>

### Fish Arrangement in Water

<table>
<thead>
<tr>
<th>Arrangement</th>
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</thead>
<tbody>
<tr>
<td>Normal distribution</td>
</tr>
<tr>
<td>Near surface</td>
</tr>
<tr>
<td>Schooling</td>
</tr>
<tr>
<td>Crowding at water outlet</td>
</tr>
<tr>
<td>Crowding at water inlet</td>
</tr>
<tr>
<td>Sinking to the bottom</td>
</tr>
</tbody>
</table>

### Gill Condition

<table>
<thead>
<tr>
<th>Condition</th>
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</thead>
<tbody>
<tr>
<td>Covered in mucus or debris</td>
</tr>
<tr>
<td>Swollen</td>
</tr>
<tr>
<td>Covers open more than normally</td>
</tr>
<tr>
<td>Opaque</td>
</tr>
<tr>
<td>Spots or hemorrhage in lens</td>
</tr>
<tr>
<td>Pop-eye</td>
</tr>
</tbody>
</table>
### Body surface

<table>
<thead>
<tr>
<th></th>
<th>Cysts</th>
<th>Spots</th>
<th>Bluish film</th>
<th>Grayish-white patches or tufts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swollen areas</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Open lesions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necrotic areas</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Granulations</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

### Other symptoms or conditions noted:

### Services Requested:

<table>
<thead>
<tr>
<th>Categories</th>
<th>Frozen Fish</th>
<th>Formalin Preserved</th>
<th>Fresh Fish</th>
<th>Live Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necropsy</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Bacteriology</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Whirling Disease Analysis (PTD)</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Virology</td>
<td></td>
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<td>X</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>PCR</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

### 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.
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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