

Elanco®

MANUAL FOR FISH MORTALITY CLASSIFICATION

CHILE - VERSION 3

Forever Salmon

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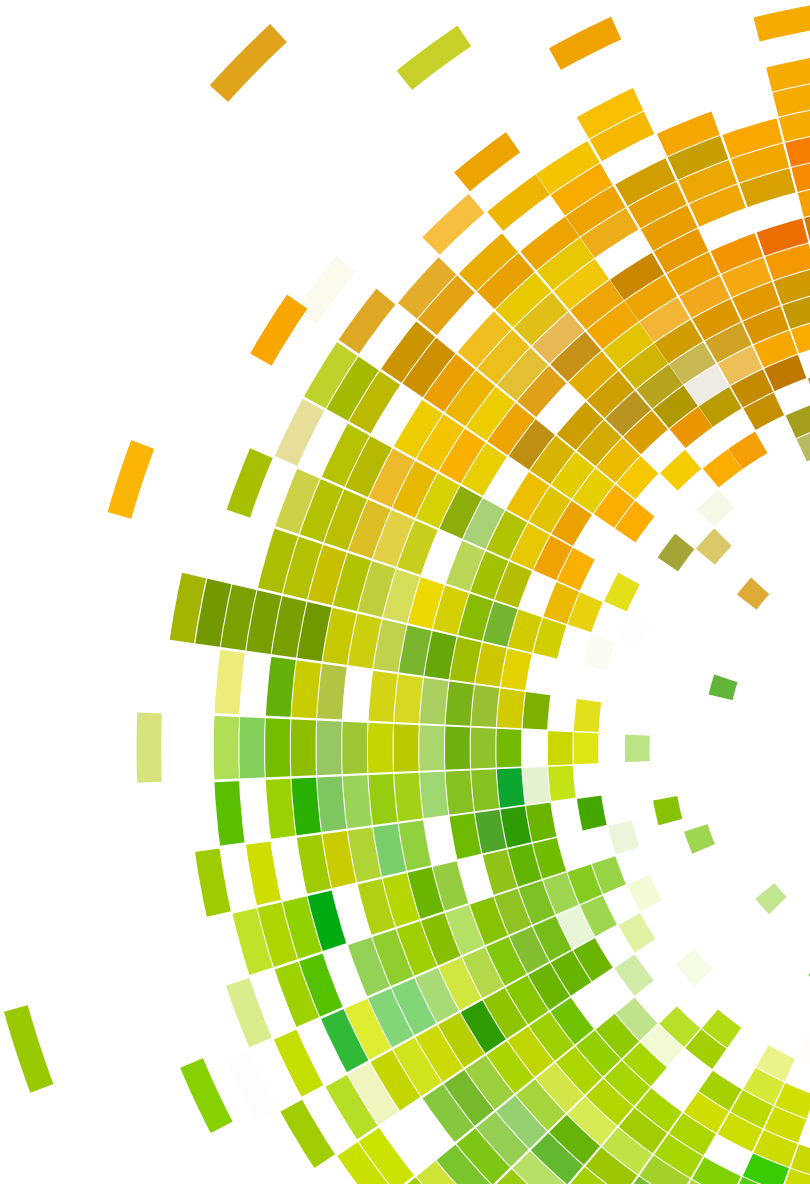
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NECROPSY

Anatomopathological examination is essential for the correct diagnosis of a disease. During this examination, adequate tissue samples are obtained for observation under the microscope, as well as for bacteriological, parasitological, and viral research analysis.

To carry out this analysis, the fish must be transferred in small containers and then euthanized with an overdose of anesthetic in the water, to later carry out the extraction of any tissue or body fluid.

The skin is in contact with the environment and is vulnerable to damage that can be produced by various causes, among which we can mention primary and opportunistic pathogens, infections by parasites, bacteria and fungi. During necropsy, it is important to pay attention to the general external condition of the fish, for example, excess mucus, areas of necrosis or bleeding, any type of associated injury, presence of discolored areas, presence of parasites, exophthalmia, skeletal deformation, and muscle atrophy. Other factors such as handling, or damage produced by nets also contribute to tissue damage but are generally secondary causes of a disease. During autopsy, some abnormalities are apparent, while others can only be observed through the microscope.

A very important part of the necropsy report refers to the correct description of the injury or abnormality, and its location on the body of the fish.

Once the fish is removed from the water, the gills must be quickly examined; in order to do this, a cut of the operculum is made looking for the presence of anemia, increased mucus, the presence of blood clots or an infection by parasites. The entire branchial arch can be removed and observed under the microscope.

The eye examination must include a description of the presence or absence of corneal opacity, presence of cataracts, or exophthalmia. The observation of cloudy eyes or exophthalmia may indicate a minor infection but may also be a sign of a more severe condition, however, its observation does not necessarily imply the diagnosis of a specific agent.

It is necessary to open the mouth of the fish and examine the oral cavity; the presence of petechiae or any abnormality associated with the mouth area should be recorded.

To observe the brain correctly, it is necessary to dissect the cranial bone. The presence of bleeding, congestion, or other discoloration of the cerebrospinal fluid or the organ itself should be recorded. If it is required to fix this organ for histological analysis, this must be done as quickly as possible in order to avoid the effect of the autolysis.

All fish must be opened in the same way, laying the fish on their right flank, allowing access to their liver, spleen, and pyloric caeca. It is required to open the ventral cavity carefully by making a longitudinal cut along the midline, paying attention not to cut the intestinal tract. This will allow us to lift the skin and muscle layer that we cut from the fish, to be able to observe the internal organs.

The observations of the anatomopathological analysis of internal organs must include presence of inflammation of the organs, congestion or bleeding, presence of fluids, adhesions, alterations in the color of the organs, presence of parasite cysts, amount of visceral fat and the presence of content in the intestinal tract.

The heart must be examined in order to observe the presence of parasites, carditis, or other lesions that surround the myocardium and are associated with blood vessels. If it is required to fix the organ, it is advisable to divide the organ longitudinally to achieve its correct fixation.

The swim bladder is generally a colorless organ; the presence of bleeding or congestion must be recorded.

The examination of the gonads will provide information about the degree of maturation of the fish.

When observing the liver, any changes regarding its normal coloration, inflammation, and the presence of nodules must be recorded.

The amount of visceral fat present in the fish must be recorded, and also if it is in a congestive or hemorrhagic state.

The examination of the pyloric caeca must include the presence of congestion or bleeding on its surface, while the description of the stomach and intestine must include the presence or absence of feed. Then the lumen of these organs must be opened to observe the mucous membrane, recording abnormalities or irregularities.

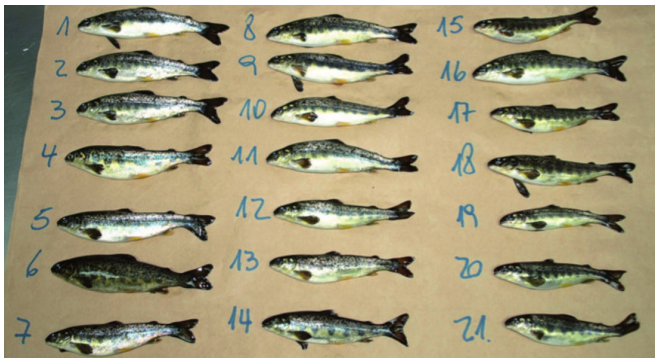
The kidney, which is located adjacent to the backbone, is commonly affected by many infectious and non-infectious conditions, therefore, the presence of unusual coloration, as well as the presence of inflammation of the organ, nodules or granuloma must be observed and recorded.

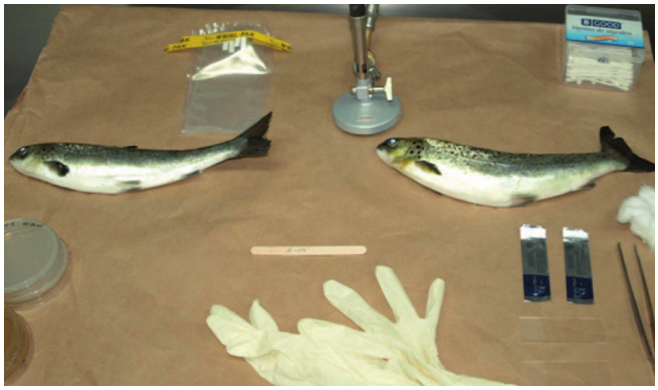
SAMPLING

Correct sampling protocols will ensure optimal conditions for the necropsy. Before starting the process, it is recommended to have the items described below:

1. Slides
2. Bags
3. Plates
4. Sheets of kraft paper to place the fish
5. Formalin
6. Labeling material
7. Permanent ink pen (or waterproof)
8. Paper form (template) to record necropsy
9. Scalpel
10. Cotton swabs
11. Absorbent paper
12. Forceps

1. Regardless of the number of fish to be necropsied, they must be arranged on the table, and the number of the cage to which they correspond must be clearly identified. At the beginning of the necropsy, it is recommended to have all the materials in order.





2. Before starting with the necropsy, it is practical to label the material for use, that is, the slides, bags, plates, bottles with formalin, etc. The labels must contain the following information:
   - Tank or cage reference
   - Analysis required
   - Organ sample identification





3. After performing the external examination of the fish, the ventral cavity is opened, making a longitudinal cut along the midline, making sure, at the end of it, not to cut the intestinal tract. This will allow the skin and muscle to be removed and leave the internal organs exposed for observation.

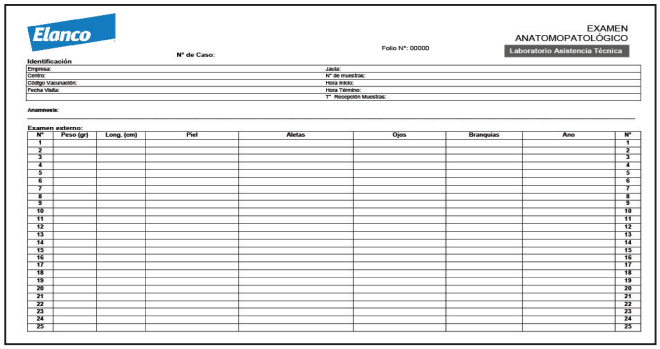








4. Once the internal organs are exposed, the necropsy can then be performed. An important component of the report is an accurate description of any abnormalities or injuries, as well as their location. A pathology examination form, similar to the one shown, allows for an accurate description of any observations.



EXAMEN ANATOMOPATOLÓGICO

Laboratorio Asistencia Técnica

N° de Caso:

Folio N°: 00000

Identificación

Empresa:		Jaula:	
Centro:		N° de muestras:	
Código Vacunación:		Hora Inicio:	
Fecha Visita:		Hora Término:	
		T°. Resguardo Muestras:	

Anamnesis:

Examen externo:

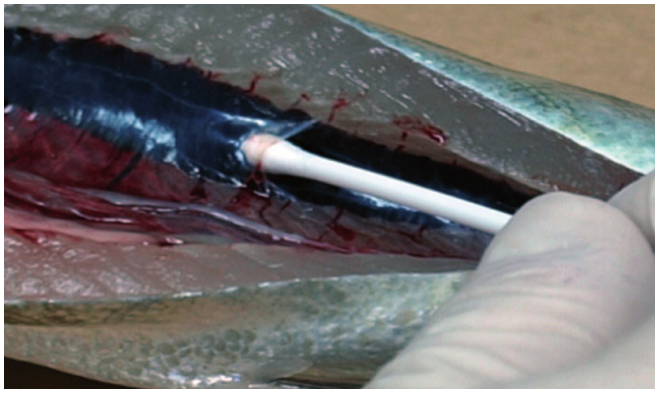
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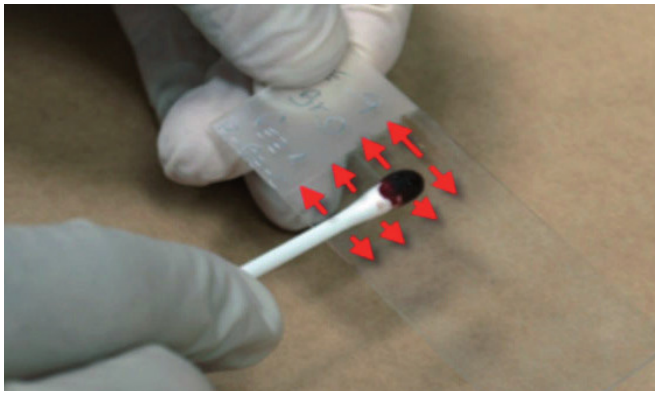
5. Data such as the following must be recorded: cage or tank reference, intended analysis (Gram stain, Acridine, IFAT, etc.), Organ sample (K-, L- S-, or B- / Kidney, Liver, Spleen or Brain) and then fish sample number.



6. To make a kidney smear, insert the swab into the posterior third of the organ, and rotate the swab ensuring to collect as much tissue as possible. Then, remove the swab avoiding contact with the abdominal cavity of the fish.



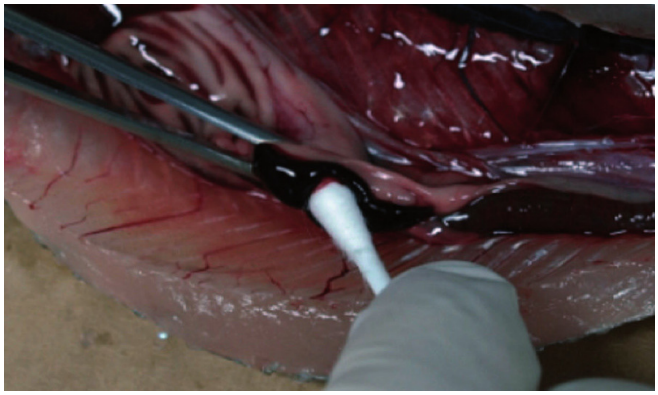
7. To smear the slide, start at the top of the slide and rub the swab from side to side and from top to bottom.

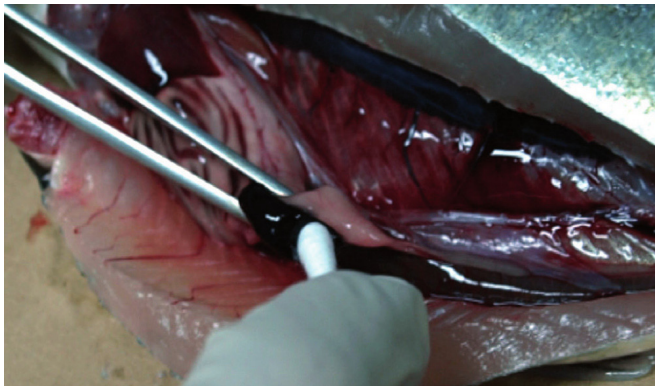


8. Once finished, leave the slide to dry at room temperature for a few minutes before storing, ensuring to avoid direct contact with other slides.

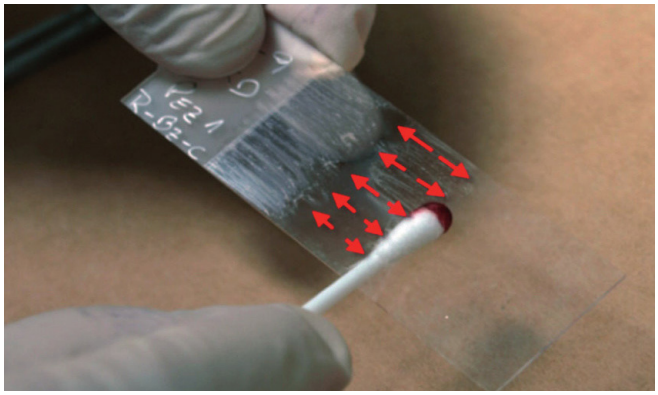


9. To make a smear from the spleen, the procedure is similar; the swab is inserted into the spleen, then rotate the swab ensuring to collect as much tissue as possible. Then remove the swab avoiding any contact with the abdominal cavity of the fish.

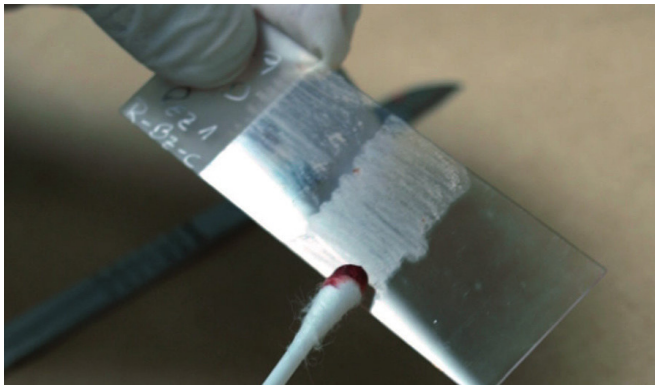




10. To smear the slide, start at the top of the slide and rub the swab from side to side and from top to bottom.

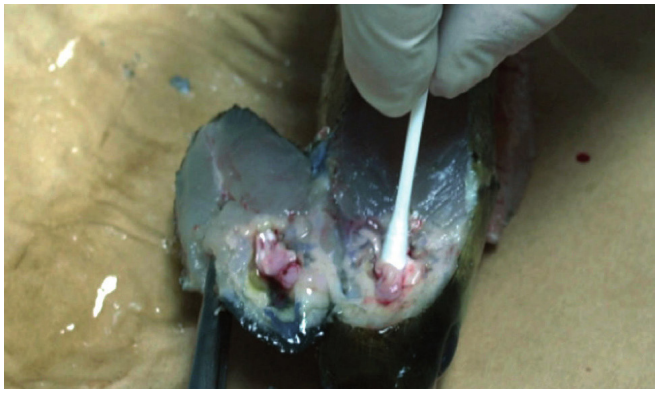


11. Once finished, leave the slide to dry at room temperature for a few minutes before storing, ensuring to avoid direct contact with other slides.

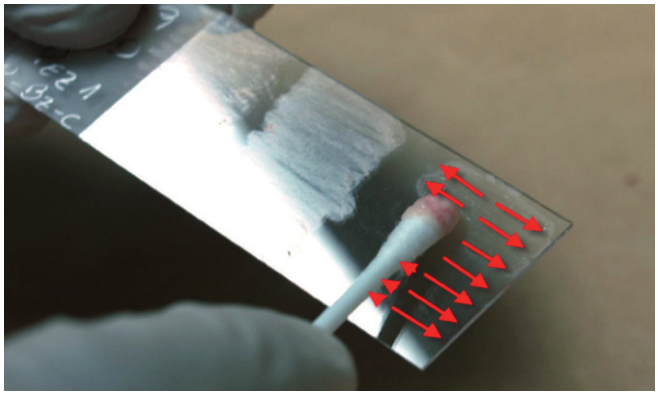


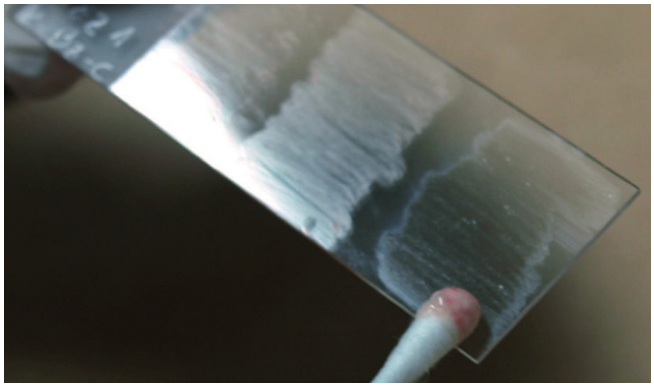
12. To make a brain smear, use a scalpel to open the cranial cavity, insert a swab into the brain and rotate to collect as much tissue as possible. Then remove the swab avoiding any contact with the cranial cavity.





13. To smear the slide, start at the top of the slide and rub the swab from side to side and from top to bottom.





MORTALITY CLASSIFICATION

The classification of mortality is key in the early detection of disease since it allows the farm and the authorities the opportunity to enact timely control measures.

Necropsies should be performed in a dedicated area, which has bio-security measures in place at the entrance and exit, adequate lighting, and predator control. Procedures should be conducted on a surface that can be washed and disinfected ensuring resulting fluids are disposed of appropriately.

Classification of mortality is graded according to a primary and secondary levels.

TYPE OF MORTALITY

PRIMARY LEVEL

Environmental, Embryonic, Mechanical damage, Deformed, Mature, Culls, Other, Predators, No apparent cause.

SECONDARY LEVEL

IPN, Furunculosis, BKD, Ichthyopthiriasis, Flavobacteriosis, Yersiniosis, Mycosis, Francisellosis, Vibriosis, SRS, ISAv, Amoebiasis, Jaundice, Streptococcosis, Tenacibaculosis, SIT, HSS, HSMI

CLASSIFICATION: PRIMARY LEVEL

The Primary level classification corresponds to the external observations that are made on all daily mortalities at a unit (hatchery or farm) or each time mortalities are removed from a cage.

Primary classification only considers the external features of the fish, which is why different assessment categories have been established, such as:

1. Environmental
2. Embryonic
3. Mechanical damage
4. Deformed
5. Mature
6. Culls
7. Other
8. Not adapted / Runts
9. Predators
10. No apparent cause

These categories associated with the Primary Level classification are described in the following pages.

1. ENVIRONMENTAL

It corresponds to those fish that die from causes associated with environmental events. This category includes two specific sub-classifications:

a. Bloom: when the cause is associated with an Algae Bloom.
b. Oxygen: when the cause is associated with the reduction of disolved oxygen.

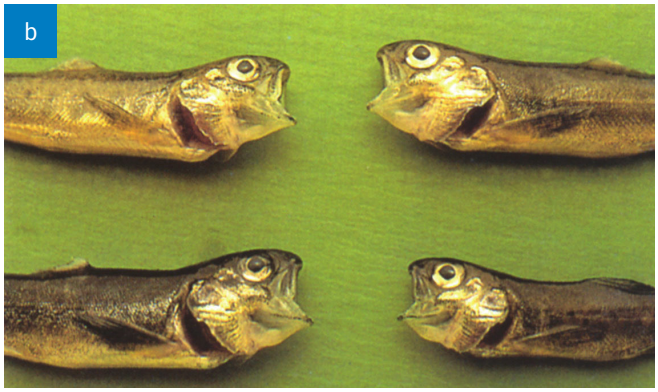
a



a



b



2. EMBRYONIC

Corresponds to mortalities during the incubation and hatching periods.



3. MECHANICAL DAMAGE

Corresponds to fish that die due to trauma associated with weather, mechanical or operational events on farms such as pressing against nets or solid structure on the farm, death by suffocation due to reduction of the water level of the tanks among others. The farm must keep a record of the occurrence of these events.

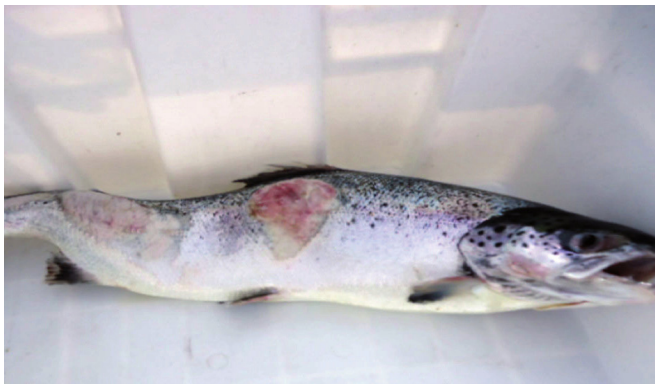






4. DEFORMED

Corresponds to mortalities where the only indication of a cause of death is evidence of a deformity, such as spinal, tail, jaw, operculum or gill arches.



5. MATURE

Corresponds to mortalities displaying the characteristics of early maturation, such as colour change, precociousness or sexual dimorphism.









6. CULLS

This cause includes two sub-classifications:

a. Health: corresponds to fish mortalities due to culling by the farm as a results of the application of health control measures for certain diseases or, as a consequence of measures applied as a component of applicable surveillance and control programs.

b. Production: corresponds to fish that are culled during handling events at the farm such as during vaccination, grading, injectable treatments, transfers, etc. and are eliminated due to poor conditions (injuries, deformities, small grade, etc). Each farm must keep a record of these culls.

7. OTHER

Corresponds to mortalities with no cause that could be classified within the other categories, and could be sub-classified into one of the following 4 sub-groups:

a. Samples: when fish are collected as samples for laboratory, anatomopathological, or other analyses.

b. Bloating: when fish present severe abdominal distention due to liquid in the stomach unrelated to an infection or disease.

c. Transport: when fish die from various non-infectious causes during transport activities.

d. Other: when the cause does not correspond to any of the above, a situation in which the associated cause must be individualized.





8. NOT ADAPTED / RUNTS

It corresponds to those dead fish whose main characteristics are being thin, long, of deficient body condition and that did not grow during the first farming stages or that later for some reason stopped doing so. Those fish that did not smoltify properly are included in this category.







9. PREDATORS

Corresponds to those fish that die as a result of predator attacks. It is divided into the following two sub-classifications:

a. Seal predation: Corresponds to those dead fish with evidence of damage associated with seal attacks or similar predators, and that are associated with seal sightings or attacks on the site. Records of these events must be maintained.







b. Bird predation: corresponds to fish that die of unilateral or bilateral, superficial or deep wounds on the body and/or at the base of the gill cavities.



10. NO APPARENT CAUSE

Corresponds to fish that otherwise look healthy following external assessment and to which no specific cause of death can be attributed. Also considered in this definition are those mortalities that display clear external characteristics associated with infectious disease.

Mortalities provisionally classified with no apparent cause will be subjected to the secondary level assessment. Mortalities may only be classified in this category when it is not possible to clearly identify the cause of death.

CLASSIFICATION: SECONDARY LEVEL



Corresponds to the classification that is conducted on mortalities provisionally classified as having “No apparent cause”.

The secondary classification will be based on the presence of typical anatomopathological signs of a disease, conducted by on site fish health personnel with specialized training or by a clinical diagnosis made by a veterinarian or by laboratory diagnosis.

In the event of an outbreak of any disease associated with an existing diagnostic history (clinical diagnosis and/or laboratory analysis), a necropsy may be performed on a representative number of the total number of fish on the farm.

And if there are mortality indicators attributable to more than one category, the criteria used to classify mortality must be an indication the health status of the farm at that time.

The veterinarian must confirm, at least monthly and in person, the correct mortality classification conducted by the responsible personnel on the farm. For this reason, a record must be kept in the veterinarians log at the farm.

1. INFECTIOUS PANCREATIC NECROSIS VIRUS (IPNv)

ETIOLOGICAL AGENT AND NAME OF THE DISEASE

IPNv is the species type belonging to the genus Aquabirnavirus and the family *Birnaviridae*.

IPNv has 2 strands of bisegmented RNA (double-stranded), made up by a small segment (Segment B) of 2784 bp and a large one (Segment A) of 3097 bp. It is a relatively small virus, only 60 µm in diameter, and has no membrane. The outer circumference of the virus is called the capsid, which is made up of proteins (VP2 and VP3).

CLINICAL SIGNS

External clinical signs include:

a. Erratic swimming (rotating on its longitudinal axis or turning violently).
b. Abdominal distension.
c. Skin darkening.
d. Exophthalmia
e. Mucoid pseudo-faeces.
f. Hemorrhage at the base of the fins and in some cases on the body surface.

Internally, the following may be observed:

a. Liver and spleen look pale.
b. The stomach and intestine do not contain food but are full of mucous material.
c. Visceral organs may have petechial hemorrhage.

1



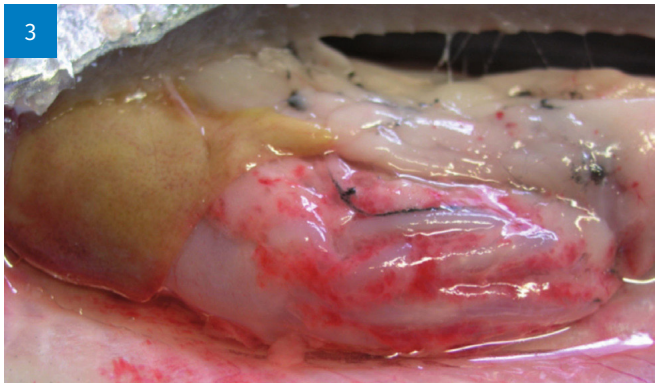
2



MACROSCOPY PATHOLOGY

1. Pale liver and petechiae in visceral fat.
2. Pale liver, petechiae in visceral fat, intestine with yellowish contents.
3. Pale liver, petechiae in visceral fat and ascites in the peritoneal cavity.

3

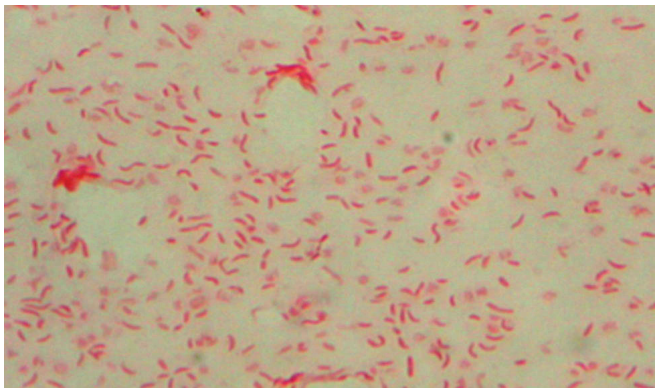


2. FURUNCULOSIS

ETIOLOGICAL AGENT AND NAME OF THE DISEASE

The etiological agent is Aeromonas salmonicida subspecies achromogenes and masoucida; the disease is known as atypical furunculosis.

Aeromonas salmonicida is a Gram Negative (-) bacillus, facultative anaerobe, immobile, non-sporulated or encapsulated, it measures approximately 1.3-2 µm long by 0.8-1.3 µm wide, it has bipolar staining, its shape is variable.



CLINICAL SIGNS

The fish that die from an acute disease generally do not present signs, or these are very mild. On the other hand, in chronic cases, it is possible to observe one or more of these characteristics:

Externally:

a. The fish look dark.
b. Lethargic, cornering.
c. Lacking appetite.
d. They present hemorrhaging at the base of the fins.
e. Furuncles are observed on the body surface, which can ulcerate, releasing remains of necrotic tissue and bacteria.
f. The gills appear very pale, but it is not uncommon to find fish in seawater that have gill hemorrhaging.

Internally:

a. The blood vessels that surround the intestine are swollen, as are those found in the pyloric caeca.
b. The intestine does not contain feed, and may present a blood exudate, composed of mucus and cellular debris.
c. The spleen is swollen, with red spots.
d. The liver has a gray-green color.
e. It is common to observe the presence of petechiae on the surface of all serosae.
f. The swim bladder looks swollen and cloudy.
g. The kidney is swollen and when cut with the scalpel, it commonly appears liquefactive.
h. The peritoneum and pericardium often have an accumulation of blood fluids.
i. The hematocrit is very low, with a low red blood cell count (hence the gills and liver are pale) and also a low number (possibly even the total absence) of white blood cells.

MACROSCOPIC PATHOLOGY

1. Hemorrhage in the ventral area and at the base of the fins.
2. Skin ulcers.
3. Presence of furuncles.
4. Internally, bleeding in the swim bladder.
5. Internally, peri-renal hemorrhaging is observed.

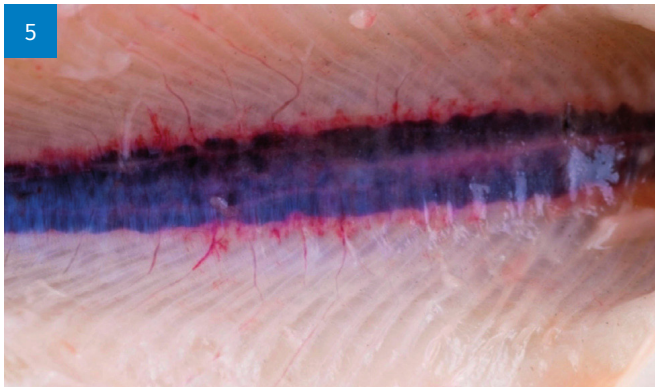
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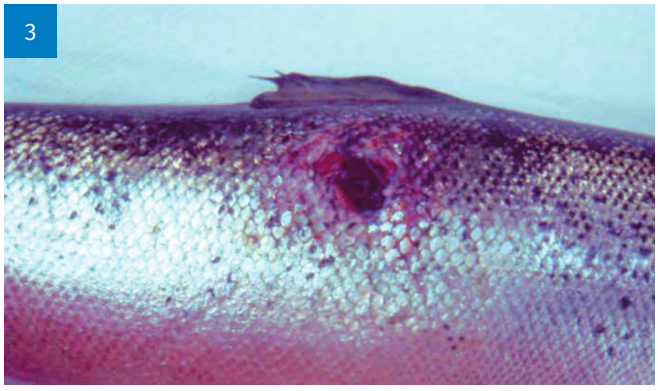
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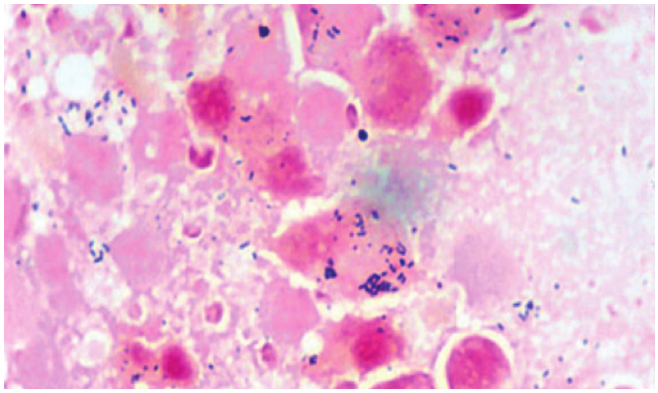
3



3. BACTERIAL KIDNEY DISEASE (BKD)

ETIOLOGICAL AGENT AND NAME OF THE DISEASE

The etiological agent of Bacterial Kidney Disease is *Renibacterium salmoninarum.* This is a short Gram Positive diplobacillus (0.3 x 1.5 µm), they are grouped alone, in pairs or in small chains, aerobic, non-sporulated and immobile.



CLINICAL SIGNS

Fish, even severely affected ones, may not show external signs.
Some of the external signs that can be observed are the following:

a. Pale gills.
b. Exophthalmia
c. Abdominal distension.
d. Blisters on the skin with a clear or cloudy fluid.
e. Superficial ulcers.
f. Petechial hemorrhaging in the abdominal area.
g. Cavities in the muscles.

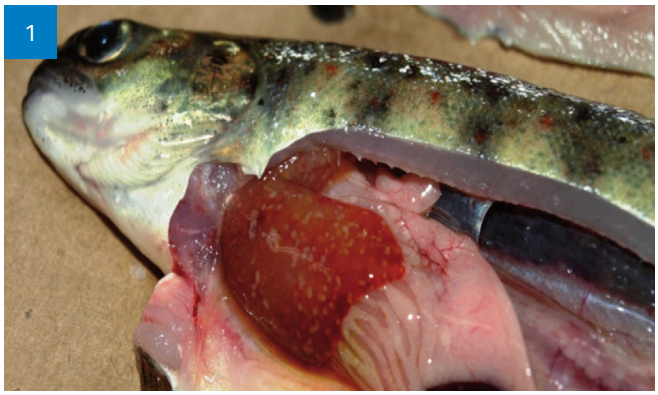
Internally, the following may be observed:

a. Cloudy fluid in the abdominal and pericardial cavity.
b. Variable hemorrhaging on the walls of the abdominal cavity and viscera.
c. A membranous layer on one or more visceral organs.
d. Hepatomegaly
e. Splenomegaly
f. Renomegaly
g. And the most characteristic sign, white-creamy granuloma injuries in the kidney and less frequently in the spleen and liver.

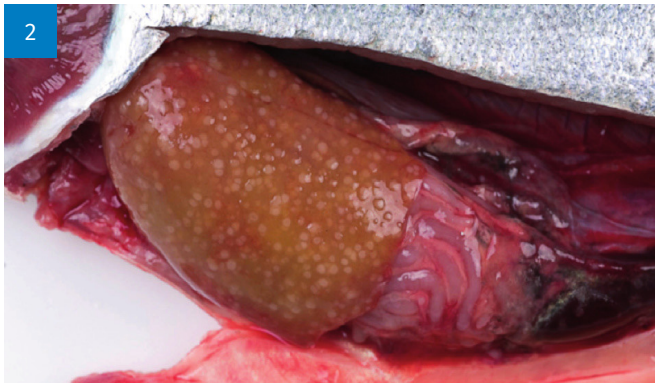
MACROSCOPIC PATHOLOGY

1. Nodules in the liver.
2. Mild hepatomegaly with nodules in the liver.

1



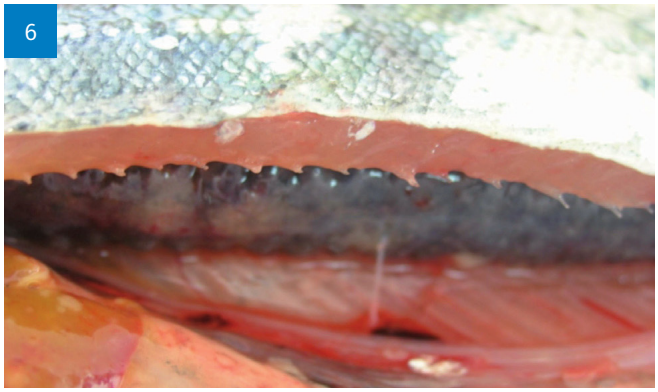
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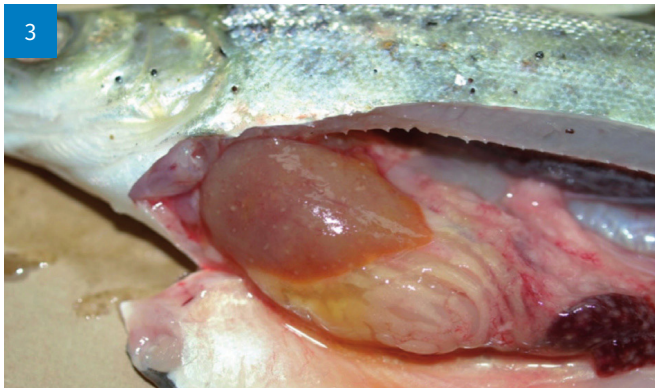
(Bacterial Kidney Disease BKD, continued)

3. Nodules in the liver and spleen, presence of ascites.
4. Nodules in the spleen.
5. Presence of whitish nodules in the kidney.
6. Severe renomegaly.
7. Presence of muscle caverns.

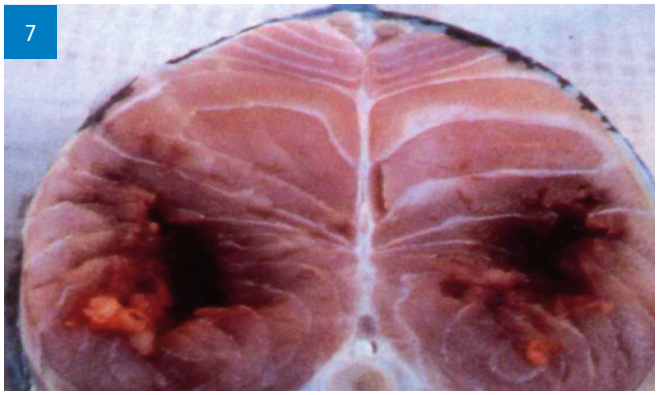
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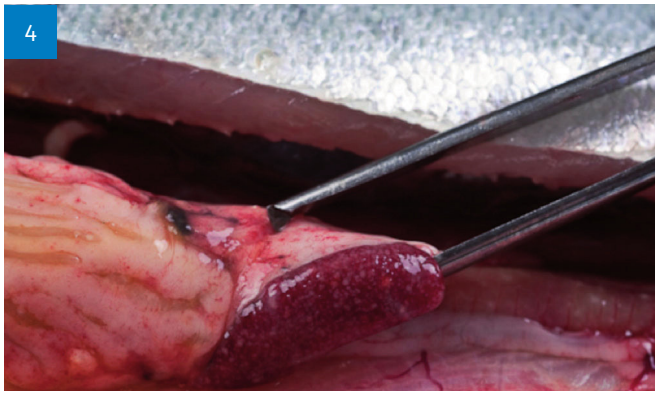
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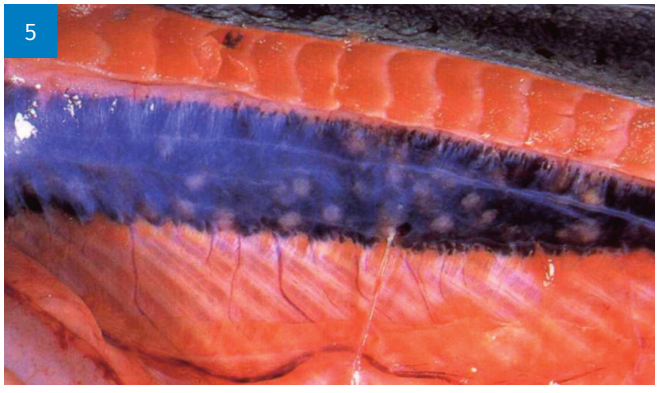
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4. ICHTHYOPHTHIRIASIS

ETIOLOGICAL AGENT AND NAME OF THE DISEASE

White spot disease, "Ichthyophthiriasis" or simply "Ich", is caused by a ciliated protozoan known as *Ichthyophthirius multifiliis*.
This ectoparasite is commonly found in small numbers in wild fish, which rarely experience a severe infection due to this protozoan. Its location is on the skin (especially in the dorsal area and on the fins) and on the gills, where it forms a white cyst approximately 1 mm in diameter.

The adult organism of this protozoan has an oval to round shape measuring between 0.5 to 1 mm in size. The adult parasite is long, dark in color (due to the fact that the cilia cover the entire body), it contains a horseshoe-shaped nucleus which is observed with 100x magnification under the microscope. This adult parasite stumbles slowly and is easily recognizable with practice. In its immature form (tomites) it is small, translucent and moves quickly.

This life cycle is highly dependent on the water temperature and can take from 5 to 7 days at 21°C to 30 to 40 days when the water has a temperature of 10°C. Water temperatures of 32°C eliminate therontes. It is distributed all over the world.

In theory, this parasite can infect any freshwater fish. Transmission of this disease is horizontally, and generally occurs (in fish farms) when alevins are mixed with older fish that are infected.

CLINICAL SIGNS

Before the appearance of the white spots, the fish show signs of irritation, weakness, loss of appetite and a decrease in their activity.

If the location of these white spots is only on the gills, the infection may be unnoticed but an increase in mortality begins to be observed. Fish can be seen gasping because of the parasite's interference with gas exchange in the gills. In these fish, the gills are pale and swollen.

The observation of the white spots on the skin or on the gills (or both) can be considered a preliminary diagnosis. The confirmatory diagnosis of "Ich" is made by taking skin or gill samples (or both) and observing them directly under a microscope, or by removing several of the white spots from infected fish and performing a fresh analysis of those samples (i.e., placing these white spots on a slide with a few drops of water and then putting a coverslip to observe them in the microscope). The observation of any of the stages of the life cycle of the protozoan constitutes a confirmatory diagnosis.

MACROSCOPIC PATHOLOGY

1. Presence of white spots on the skin.


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5. FLAVOBACTERIOSIS

ETIOLOGICAL AGENT AND NAME OF THE DISEASE

Flavobacterium psychrophilum is the causative agent of bacterial cold water disease (BCWD), which is a serious septicemic infection in alevins (especially Coho Salmon). It is one of the most frequently isolated pathogens in hatcheries. The term cold water disease is due to the fact that the most prevalent cases were found in water temperatures below 10° C.

Flavobacterium psychrophilum is part of the normal flora of the fish, it is found on the skin, fins, mucus, operculum and gills. The bacterium does not grow in traditional culture media, but only when seeding the samples in TYES (Tryptone-Yeast Extract Salts) or MAOA (Modificated Anacker & Ordal Agar) culture media, at 48-96 hours post incubation at 15-20°C, the presence of colonies of 1-3 mm in diameter, raised, convex, with a thin and irregular border, whose margins are extended, is observed.

When observing the colonies using gram stains, it is seen that they are composed of long, Gram Negative (-) bacilli, weakly retractile with rounded edges. In young cultures (14-24 hours), the bacilli measure between 0.3-0.75µm x 2-7µm, while the filamentous forms measure between 10-40µm.

The reservoir of *F. psychrophilum* is unknown. Apparently, the bacterium is able to remain in a vegetative state for years.

It does not grow in culture media incubated above 25°C. The pathogen is distributed worldwide from North America, South America, Europe, Asia to Oceania.

Its main route of transmission is horizontal, although it is also transmitted from sexually mature adults (it has been found in ovarian fluid and semen) to the eggs and from there to susceptible alevins (vertical transmission).

Some strains of *F. psychrophilum* are capable of producing extracellular enzymes that degrade components of the skin, muscle and cartilage of the fish.

When the alevins develop the disease, mortality can range from 30% to 50% and external clinical signs can be limited to skin erosion.

It is typical to find that the alevins in the first feeding present around a 20% mortality with the typical injury on the peduncle.

CLINICAL SIGNS

Fish can be observed:

a. Lethargic, cornering or swimming in spirals.
b. They have dark pigmentation on one side or both sides of the body.
c. Skin and muscle lesions are common, and are generally observed in the pedicle area, although they can also occur in other areas of the body. At the beginning of the disease, especially in Trout, a whitish discoloration is observed on the border of the adipose fin which progressively spreads until almost completely wrapping up the caudal peduncle.
d. In some cases, the adipose fin and the skin of the dorsal area of the peduncle can become necrotic, exposing the underlying muscle, this progressive degeneration of the tissue, as well as that of the muscle, can in extreme cases expose the backbone.
e. In the more advanced stages of the disease, the fish can be observed completely dark, or only in the pedicle region, or simply die without presenting external injuries.
f. In chronic cases, this bacterium can produce a spinal deformities (scoliosis and lordosis) and can be isolated from the deformed vertebrae.

g. In other cases, it is also possible to isolate this bacterium from brain tissue. These last two conditions are commonly seen in Coho Salmon (*Oncorhynchus kisutch)*.

h. The bacterium can also be isolated from gills; however, this is less frequent.

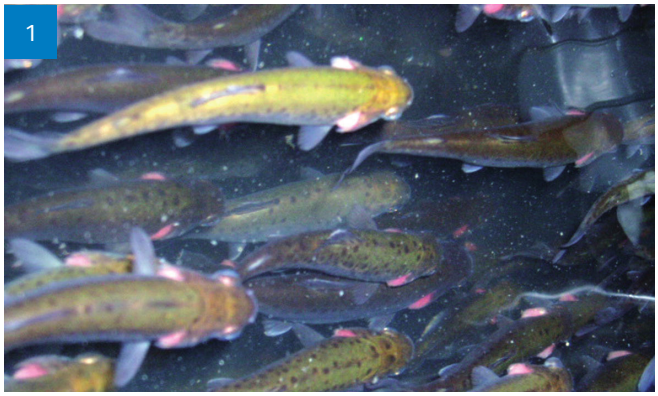
i. In Coho Salmon alevins, the surface of the yolk sac can be eroded causing it to rupture.

MACROSCOPIC PATHOLOGY

In fish, the following can be observed:

1. Presence of unilateral or bilateral exophthalmia.
2. Presence of gill lesions.
3. Presence of lesions in the lateral and dorsal area with muscle exposure.
4. Presence of lesions in the lateral and pedicle area with muscle exposure.

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ETIOLOGICAL AGENT AND NAME OF THE DISEASE

The causative agent of Red Mouth Disease or Enteric Red Mouth (ERM) is *Yersinia ruckeri*. Currently the term Yersiniosis or Yersinia Septicemia is used when there is an infection produced by Y. ruckeri, since the clinical sign of "red mouth" is not necessarily present.

This bacterium belongs to the Enterobacteriaceae family and to the Yersinia genus, the species is composed of a variety of strains, which include six serological varieties (Type I to Type VI), Type I being the most common and pathogenic. *Yersinia ruckeri* is a Gram Negative (-) bacillus, filamentous in shape, mobile with a peritric flagellum (although there are immobile strains).

Yersinia ruckeri causes low but sustained levels of mortality, which are dramatically increased when fish are stressed by due to handling or by unfavorable environmental conditions such as high densities or reduction in dissolved oxygen. The severity of the infection as well as the mortalities are lower when the fish are in systems that use temperatures below 10°C.

The most commonly affected size in Rainbow Trout is 6 to 8 cm and chronic infections occur in larger trout (> 12 cm).

About 75% of *Yersinia ruckeri* outbreak survivors become asymptomatic carriers of the disease. Cases of *Yersinia ruckeri* have been reported in the United States, Europe, and South America. Its hosts can be all species of salmonids.

The route of transmission of this disease is horizontal, and it occurs mainly through the release of the bacterium from the intestine (in infected and carrier fish) through the faeces.

CLINICAL SIGNS

The forms of presentation of this disease are the following:

I. Acute infection, fish can be externally observed:

a. Lethargic
b. Dark
c. Anorexic
d. With bilateral exophthalmia, which can progress up to the rupture of the eye.
e. Petechial hemorrhages are common on the surface of the body (but the skin remains intact).
f. Some fish may have abdominal distension.
g. Congestion at the base of the fins, in the midline and in the cranial area.
h. Some cases develop the typical symptoms of bleeding in the mouth and in the opercula.

II. In chronic infections, the fish may not show symptoms attributable to the disease, but it is found in the lymphoid tissue and in the intestine of these fish.

Internally, the findings observed in Yersiniosis are similar to other bacterial septicemias:

a. Congestion of the peritoneum.
b. There may be petechial hemorrhages in the liver, fat, and pyloric cecum.
c. The distal part of the intestine as well as the gills may be swollen.
d. The stomach may have liquid content and the intestine may have a yellowish fluid.
e. The anus can be swollen both internally and externally.
f. The spleen may be swollen and dark.
g. The kidney may be swollen.

h. The infection can have the presence of ulcers but without any internal organs being involved.
i. In relation to the blood patterns observed in Yersiniosis, it can be said that the hematocrit, as well as the total blood proteins, are decreased by about 50% in relation to normal values.

MACROSCOPIC PATHOLOGY

Fish may present:

1. Bilateral exophthalmia and eye hemorrhage.
2. Exophthalmia
3. The base of the fins is congestive, as well as the mandibular area.
4. Eye hemorrhage and gill paleness.
5. Periocular hemorrhage (unilateral or bilateral).

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(Yersiniosis continued)

6. Periocular hemorrhage (unilateral or bilateral), congestive pectoral fin base.
7. Internally, visceral fat congestion is observed.
8. Internally, congestion of visceral fat and presence of ascites are observed.

6



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7. MYCOSIS

ETIOLOGICAL AGENT AND NAME OF THE DISEASE

The disease is generically known as Mycosis, whose causative agent generally corresponds to *Saprolegnia parasitica*. This pathogen has a fast-growing colony, which develops in approximately 3 days, and is cotton-like and hyaline, without pigment on the front and back. Microscopically, coenocytic hyphae 20 to 40µm wide, moderately branched, are observed.

Saprolegniasis is caused by a pathogen of the *Saprolegniaceae* family, of the genus Saprolegnia which does not belong to the fungi kingdom, but is classified in the Chromista kingdom, which is phylogenetically closer to algae than to fungi. Despite this, they produce coenocytic hyphae, mycelium, grow in culture media for fungi and their classification is carried out according to their morphological characteristics, which is why they were previously included among fungi.

Most of the species of this family are considered opportunistic pathogens and secondary invaders, and they need some previous wound or the infection of another pathogen previously; however, the Saprolegnia parasitica species can produce systemic mycosis and is considered a primary agent.

Saprolegnia does not survive in environments with high salt concentrations, so it does not affect fish in the estuarine areas or in sea water, so its findings occur only in fresh water.

It is transmitted through biflagellate zoospores released from hyphae present in water. The susceptibility of fish to this agent is affected by stress, wounds caused by handling, poor nutrition, sudden changes in temperature, infections from external parasites and during the spawning season. Systemic infection occurs with feeding.

Its isolation is achieved on Saboraud agar, or another medium for fungi, growing well at room temperature(20°C) to 30°C; isolates can be obtained from live fish or from fresh mortality.

Infection by this pathogen mainly affects the skin, gills, fins and eggs of the fish.

The transmission route is horizontal.

Mortality is mainly due to osmoregulatory issues, although significant respiratory difficulties are also described when the infection of this fungus is associated with the gills.

CLINICAL SIGNS

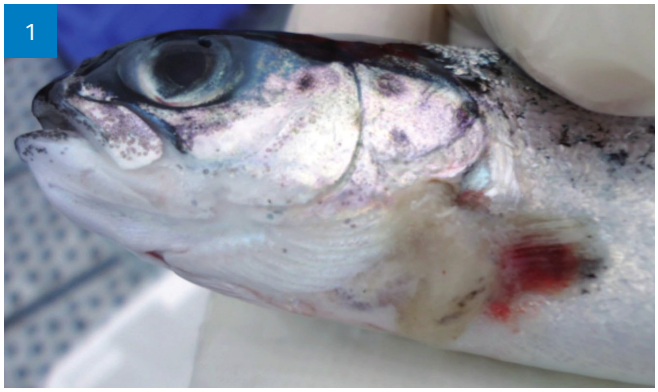
The fungal infection appears as:

a. Appearance of cotton flake-like colonies on the eggs, skin or gills of fish.
b. Whitish to brown spots focused on the surface of the skin or on the gills, which over the days evolve into spots similar to cotton patches.
c. Early lesions consist of focalized pale foci with peripheral erythematous areas and a central area raised in scale, which is frequently ulcerated, causing muscle exposure.
d. Systemic infection is characterized by masses of mycelium surrounding the stomach and viscera, causing peritonitis and extensive hemorrhaging, necrosis and adhesions.
e. In young fish, external signs such as bloating can be observed, caused by the obstruction of the stomach, which can progress to perforation of the abdominal wall.

MACROSCOPIC PATHOLOGY

In fish affected by this condition, the following is observed

1. Fungi at the base of the pectoral fins surrounded by congestive tissue.
2. Presence of fungi at the base of the pectoral fins, which can be completely eroded and where the surrounding tissue is congestive.
3. Presence of fungi in the entire anterior ventral area including the pectoral fins, even reaching the dorsal-cranial area.


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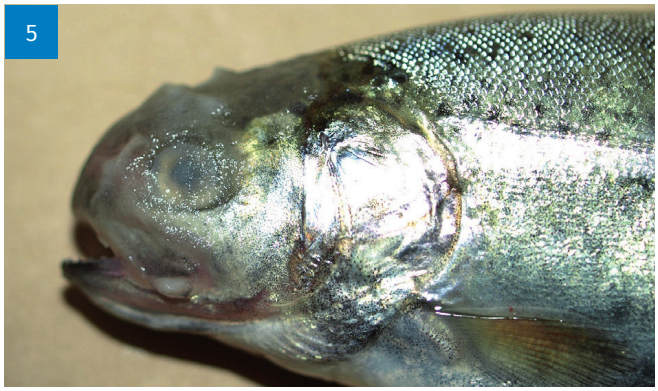
(Myosis continued)

4. Gills affected by mycosis are clearly pale.
5. Mycosis in the cranial area; it is observed even on the eyes of the fish.

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8. FRANCISELLOSIS

ETIOLOGICAL AGENT AND NAME OF THE DISEASE

At the beginning of the 1980s, the presence of a pathogen with Rickettsial characteristics but at the same time different from *Piscirickettsia salmonis* was detected. Initially it was called "UA2", that is, "unknown agent 2", in order to differentiate it from *P. salmonis* (initially known as UA).

Francisella sp. is an immobile bacterium, whose size is 1.45 x 0.35 µm within the cytoplasmic vacuoles of phagocytes, Gram Negative (-), facultative intracellular, pleomorphic, with a coccoid form even of small bacilli, and strictly aerobic.

It is classified as a fastidious microorganism, generally requiring enriched culture media (5% lamb blood, with the addition of cysteine). As for the transmission route, it is horizontal.

CLINICAL SIGNS

Some of the most characteristic external signs are:

a. Pale gills.
b. Exophthalmia
c. Dilated abdomen.
d. Petechiae in the eye chamber.
e. Bleeding in the abdomen.
f. Edema in the scales.
g. In the gills, in addition to their paleness, accumulation of blood is observed in some cases, especially in the central venous sinus of the gill filaments.

Internally, the following may be observed:

a. In the liver, there is a darkening of the organ, caused by hemorrhage necrosis.
b. In the kidney, moderate inflammation with interstitial hemorrhaging and tubular necrosis is observed.

c. The intestine appears dark red due to bleeding within the intestinal wall but not in the lumen.
d. Hematocrit <10 in the final stages (between 25 and 30 in less severe cases).
e. Blood smear with vacuolized or degenerated erythrocytes and the presence of erythroblasts with irregularly shaped nuclei. Reduction in the ratio of leukocytes and erythrocytes, with greater reductions between lymphocytes and thrombocytes.

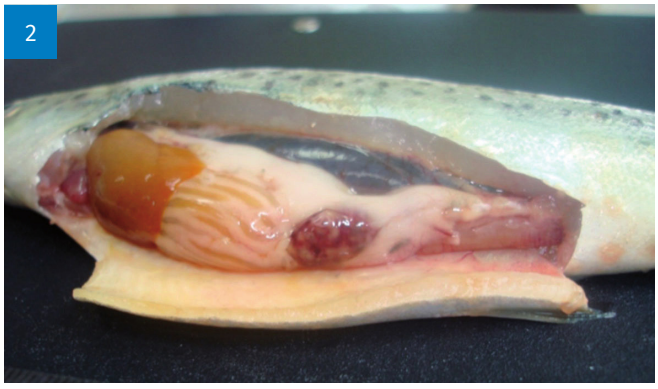
MACROSCOPIC PATHOLOGY

1. Presence of whitish nodules in the liver, with a slight congestion of visceral fat.
2. Presence of whitish nodules on the spleen, with a slight paleness of the liver.
3. Presence of whitish nodules in the spleen, in addition some ascites may be observed.

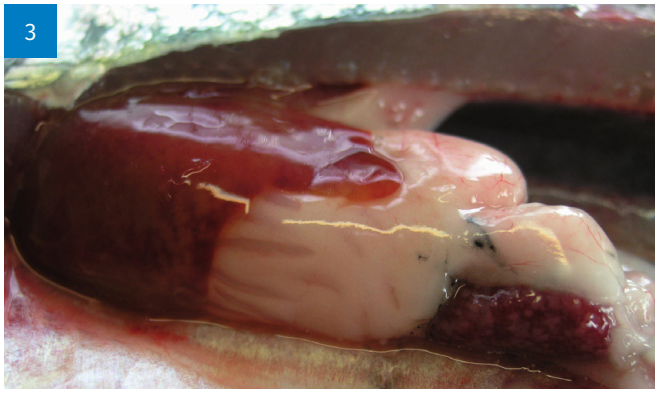
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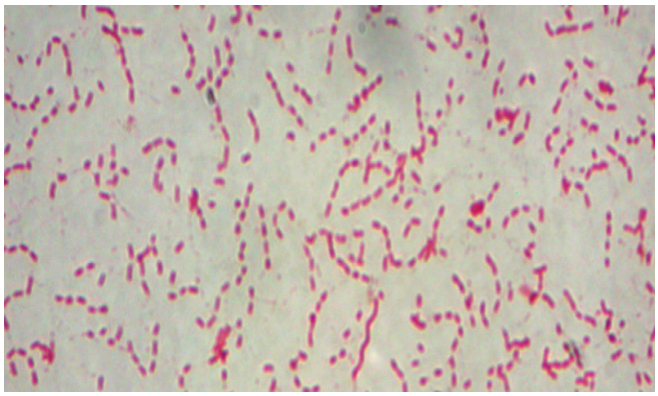
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9. VIBRIOSIS

ETIOLOGICAL AGENT AND NAME OF THE DISEASE

In Chile, the main etiological agent of vibriosis is *Vibrio ordalii.* However, there are also cases of vibriosis that are caused by *Vibrio sp.*, although these act as opportunistic pathogens. The condition caused by V. ordalii causes higher levels of mortality, the lesions are both internal and external and it differs from the disease caused by *Vibrio sp*. (opportunistic) as lesions typically are only superficial.



They belong to the genus Vibrio, they are characterized as Gram Negative Bacilli (-), from 0.3 to 1.0 x 1 to 3.5 µm in diameter, they have a comma shape (which will be more or less obvious depending on the growth medium), they are motile by means of a polar flagellum. Their metabolism is aerobic or facultative anaerobic, and from glucose, they ferment carbohydrates producing acid but not gas. They are sensitive to the vibriostatic compound 0/129, which makes it possible to differentiate Vibrio species from Aeromonas.

CLINICAL SIGNS

The following external clinical symptoms may be observed.

a. Erythema or hemorrhaging at the base of the fins, in the operculum, and around the mouth.
b. Petechiae can be seen on the muscles.
c. Gill hemorrhaging.
d. Hemorrhagic ulcerative injuries often develop in the later stages of chronic disease.

Internally, the following can be observed:

a. Erythema and hemorrhaging in all organs.
b. In severe cases, a liquefactive necrosis of the organs can be observed.
c. Splenomegaly, where the spleen appears pale.
d. Renomegaly

MACROSCOPIC PATHOLOGY

1. Multiple ulcers.
2. Multiple ulcers and splenomegaly.

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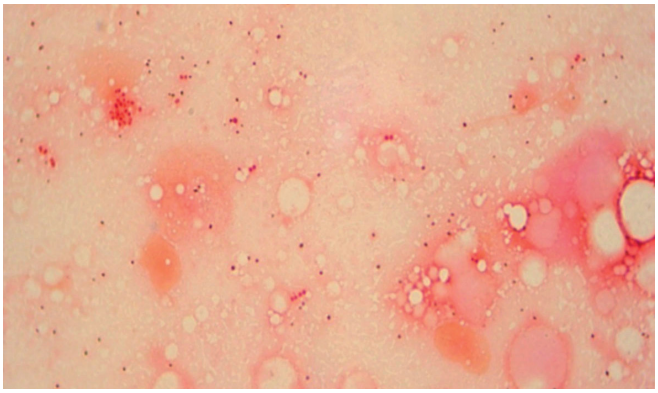
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10. SALMONID RICKETTSIAL SEPTICAEMIA (SRS)

ETIOLOGICAL AGENT AND NAME OF THE DISEASE

The etiological agent of Piscirickettsiosis is called *Piscirickettsia salmonis.*
It belongs to the Piscirickettsiaceae family, to the Piscirickettsia genus (which is composed of a single species). This corresponds to a pleomorphic but predominantly coccoid bacterium and can be observed Gram staining as a Gram Negative bacterium (-), grouped alone or in pairs (in the shape of curved bars or also as rings), and in small groups; it is approximately 0.5 to 1.5 µm in diameter, immobile, highly fastidious, aerobic, intracellular fish pathogen.



CLINICAL SIGNS

The most commonly observed external signs are:

a. Fish darkening.
b. Loss of appetite.
c. Lethargy
d. Swimming on the surface and close to the net (cornering)
e. Some fish may have lesions on the skin, appearing white spots on it which evolve to the formation of ulcers.
f. Pale gills (because of the fish anemia).

Internally, the following can be observed:

a. Inflammation and discoloration of the kidney.
b. Splenomegaly
c. Presence of ascites on the fish.
d. Bleeding in visceral fat, stomach, swim bladder, and muscle.
e. The liver may appear pale with circular, opaque, cream-colored nodules that are 5–6 mm in diameter.

MACROSCOPIC PATHOLOGY

1. Presence of ulcers on the skin.
2. Petechial hemorrhaging of the ventral area.





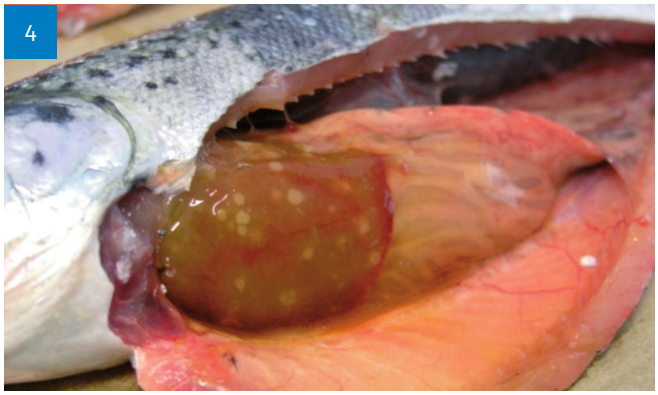
(Salmonid Rickettsial Septicaemia, SRS, continued)

3. Muscle lesions.
4. Nodules in the liver and renomegaly.
5. Severe renomegaly.

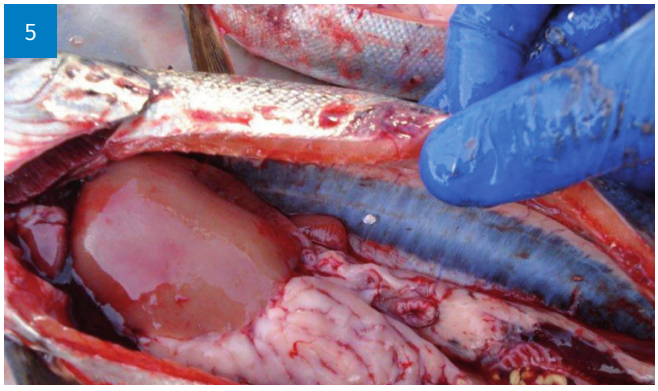
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11. INFECTIOUS SALMON ANEMIA (ISAv)

ETIOLOGICAL AGENT AND NAME OF THE DISEASE

Infectious Salmon Anemia is caused by an Orthomyxovirus, of the family *Orthomyxoviridae*, genus Isavirus. It corresponds to an enveloped virus, 100-130 µm in diameter, with a genome composed of 8 single-stranded RNA segments of negative polarity.

CLINICAL SIGNS

Some of the most characteristic external signs are:

a. Gill paleness
b. Exophthalmia
c. Dilated abdomen.
d. Petechiae in the eye chamber.
e. Bleeding in the abdomen.
f. Edema in the scales.
g. Gills may appear pale and you may in some cases observe the accumulation of blood, especially in the central venous sinus of the gill filaments.

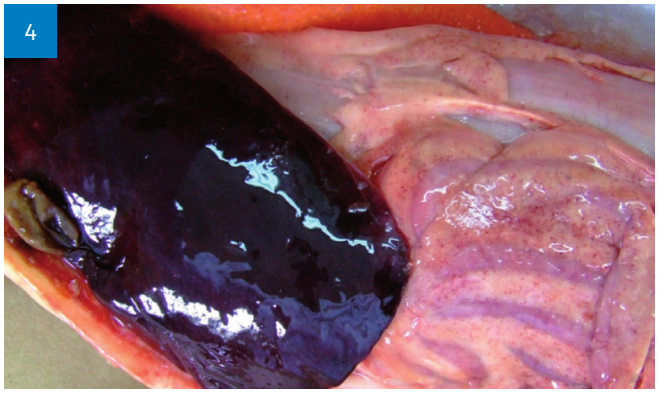
Internally, the following is observed:

a. In the liver, a darkening of the organ is observed, caused by hemorrhagic necrosis.
b. In the kidney, moderate inflammation with interstitial hemorrhaging and tubular necrosis is observed.
c. The intestine appears dark red due to bleeding within the intestinal wall but not into the lumen.
d. Hematocrit <10 in the final stages (between 25 and 30 in less severe cases).
e. Blood smear will present with vacuolized or degenerated erythrocytes and the presence of erythroblasts with irregularly shaped nuclei. Reduction in the ratio of leukocytes and erythrocytes, with greater reductions between lymphocytes and thrombocytes.

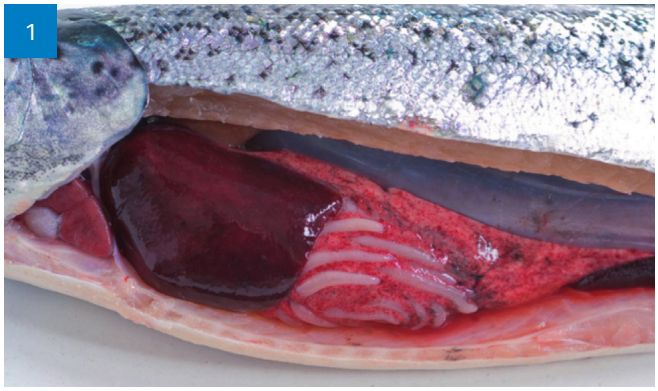
MACROSCOPIC PATHOLOGY

1. Dark liver, congestive visceral fat and ascites.
2. Dark liver with hemorrhagic pyloric cecum and intestine.
3. Dark liver, petechiated visceral fat.
4. Dark liver, petechiated visceral fat with congestive pyloric caeca.

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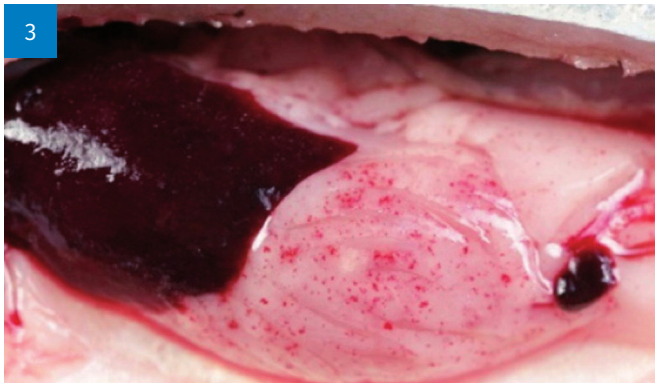
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12. AMEBIASIS

ETIOLOGICAL AGENT AND NAME OF THE DISEASE

The causal agent of Amoeba Gill Disease (AGD) in Chile corresponds to *Neoparamoeba perurans*.

In addition to Chile, cases of this agent have been described in Australia, Ireland, Japan, New Zealand, Norway, the United States, Scotland, and Spain.

N. perurans is a free-living amoeba, approximately 15–40 µm in diameter, normally found throughout the entire marine environment, but can proliferate under certain conditions and cause AGD. Among the predisposing conditions to trigger a disease due to *N. perurans*, is an increase in water temperature, increased salinity, high stocking densities, smolt quality and size, and the presence of suspended organic material in the water.

This disease is horizontally transmitted.

CLINICAL SIGNS

In fish affected by this disease, the following can be observed:

a. Lethargy
b. Respiratory stress (they breathe rapidly).
c. They tend to swim on the surface.
d. The gills are pale, with an increase in mucus secretion, in some cases the presence of small hemorrhages can be seen due to the presence of multifocal injuries on the gills.
e. Some fish may have lighter areas on the gills, which gives them a spotty-like appearance.

MACROSCOPIC PATHOLOGY

1. The gills are pale and with an abundance of mucus.
2. The presence of lighter areas on the gills is observed.
3. The presence of lighter areas in the gills is observed together with the presence of small hemorrhages in the gill filaments.

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13. JAUNDICE

ETIOLOGICAL AGENT AND NAME OF THE DISEASE

So far, the etiologic agent of this disease is unknown. It mainly affects fish of the *Oncorhynchus kisutch* species in the grow-out stage.

CLINICAL SIGNS

a. In general, the fish have a good nutrition.
b. There may be pale gills.
c. Jaundice can be observed at the base of the fins, in the periocular cartilage, operculum, and in the abdominal area.

Internally: The fish have a color that varies from yellow to brown; the heart is pale; and the fat may be yellowish.

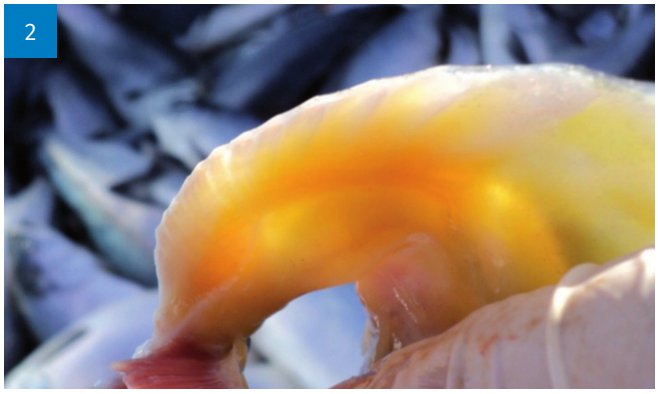
MACROSCOPIC PATHOLOGY

1. Jaundice in the ventral area, base of the fins and periocular cartilage.
2. Jaundice in the opercula.
3. Icteric visceral fat, enlarged stomach and with fluid content.
4. Internal jaundice, the abdominal cavity is yellowish, and the liver and heart are pale.
5. Internal jaundice, abdominal cavity as well as visceral fat have a yellowish coloration.

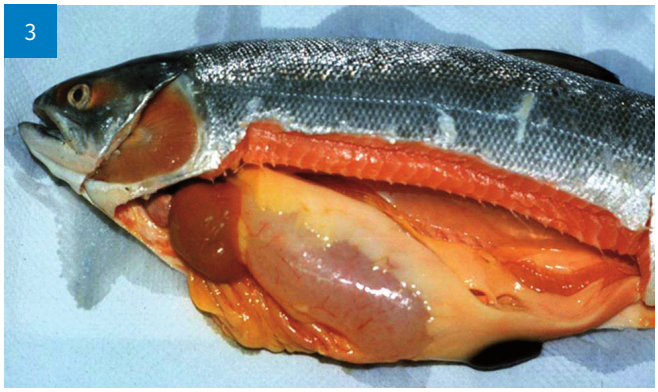
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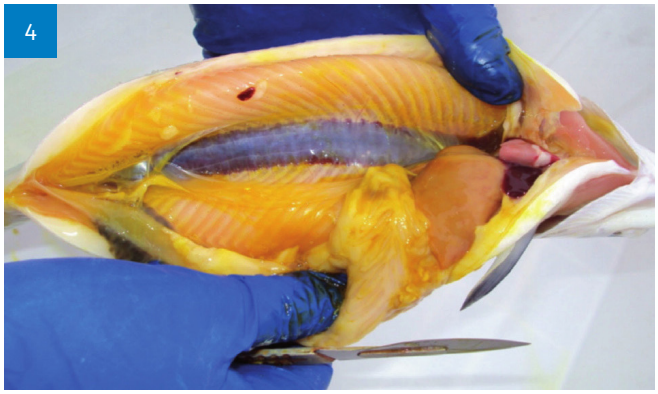
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14. STREPTOCOCCOSIS

ETIOLOGICAL AGENT AND NAME OF THE DISEASE

In Chile, the causal agent of streptococcosis in salmonids has been identified as *Streptococcus phocae*, which corresponds to Gram Positive (+) cocaceae, spherical or ovoid 0.7 x 1.4 µm, grouped alone, in pairs or small chains, immobile and non spore forming. They are facultative anaerobic, non-acid, alcohol resistant, Oxidase and Catalase Negative (-).

CLINICAL SIGNS

The external signs observed in cases of streptococcosis include:

a. Erratic swimming.
b. Body darkening.
c. Unilateral or bilateral exophthalmia.
d. Corneal opacity.
e. Hemorrhaging in the operculum and at the base of the fins.
f. Ulcerations in the body is the most common symptom. These ulcers mainly affect the dorsal area, just before the caudal peduncle, the inner side of the operculum, around the mouth and anal area.
g. Erythema around the anus.

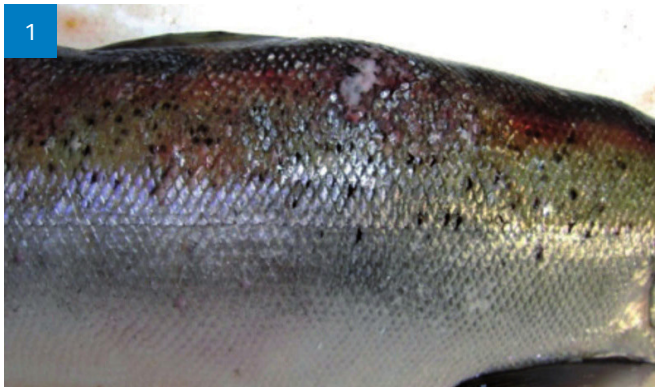
The internal signs seen in streptococcosis include:

a. The liver may appear grayish, or dark and congestive.
b. The kidney may be swollen.
c. The intestine is usually hemorrhagic.
d. There may be some degree of muscle inflammation.

MACROSCOPIC PATHOLOGY

1. Abscesses in the dorsal area.
2. Muscle sores.
3. Gray liver.

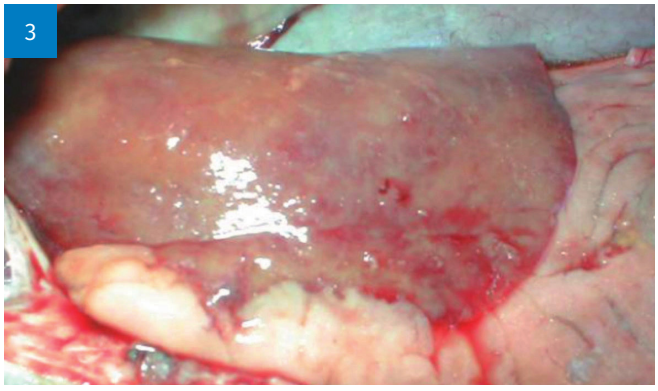
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15. TENACIBACULOSIS

Tenacibaculosis is a term used to refer to an infection caused by bacteria of the genus *Tenacibaculum spp.* The following species associated with fish diseases are described (*T. maritimum, T. dicentrarchi, T. discolor, T. soleae, T. ovolyticum and T. finnmarkense*).

Tenacibaculum spp. It is a Gram Negative bacterium (-), aerobic, thin, filamentous, without a flagellum and whose colonies present with yellow pigmentation.

This genus is part of the marine flora so it is possible to find it free as planktonic cells, associated with different marine organisms and also in organic detritus, which suggests that the main source of transmission is at the environmental level. In particular, *T. maritimum* can be associated with jellyfish, and also with sea lice (*L. salmonis*) which would increase the persistence of *Tenacibaculum spp*. in water. However, the mode of transmission and pathogenesis have not been described.

T. maritimum affects a wide variety of farmed and wild anadromous fish species, including Turbot, Rainbow Trout and Atlantic Salmon. Its distribution is global and has been isolated in all continents; T. dicentrarchi affects European Seabass, Conger Eel, Rainbow Trout and Coho Salmon, it has been associated with mortalities of Atlantic Salmon both in Norway and Chile.

Photographs courtesy of ADL Diagnostic Chile.

DIFFERENTIAL DIAGNOSIS

Piscirickettsiosis

CLINICAL SIGNS

The most prominent external clinical signs include macroscopic lesions on the body surface, such as:

a. Ulcers
b. Necrosis
c. Eroded mouth (bacterial stomatitis).
d. Fin Rot.
e. Eroded fins.
f. Sometimes gill and eye necrosis (corneal infection that can lead to eye rupture).
g. Inflammation and erosion of the oral cavity with the appearance of yellowish plaques in oral sores and gills (due of the high number of bacteria present in these lesions).

Internally, the following is observed:

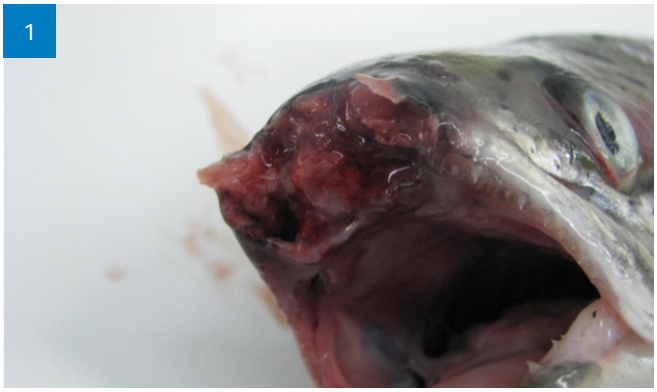
a. Petechia
b. Hemorrhaging of the peritoneum
c. Congestion of the meninges, intestine and accumulation of blood fluid in the abdomen of the fish.

There is variation in presentation, depending on species, age and when found in a mixed infection with other pathogens.

MACROSCOPIC PATHOLOGY

1. Jaw (mouth) hemorrhages.

1

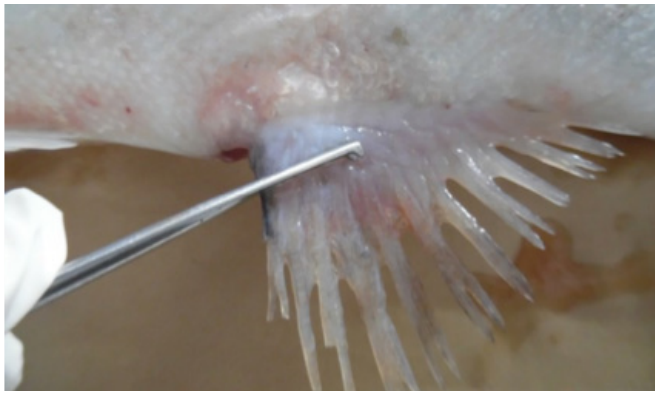


(Tenacibaculosis continued)

2. Degradation of the inter-radial tissues of the caudal and marginal fins.
3. Presence of skin ulcers in different areas of the body.

2





3



16. IDIOPATHIC TROUT SYNDROME

Idiopathic Trout Syndrome, also called Trout Anemic-Anasarca Syndrome, corresponds to a pathology with an unknown etiology that exclusively affects Rainbow Trout (*Oncorhynchus mykiss*) at the grow-out stage. It is characterized by being present in the most robust and corpulent fish within the population, frequently weighing more than one kilogram.

Photographs courtesy of ADL Diagnostic Chile.

CLINICAL SIGNS

Affected fish generally present the following internal clinical signs:

a. Bilateral exophthalmia.
b. Clots on the liver and visceral fat.
c. Scarce ascites.
d. Anasarca (generalized edema).
e. Bleeding in the abdominal cavity.
f. Hydropericardium.
g. Protruded and hemorrhagic anus.

MACROSCOPIC PATHOLOGY

1. Protruded and hemorrhagic anus.

1

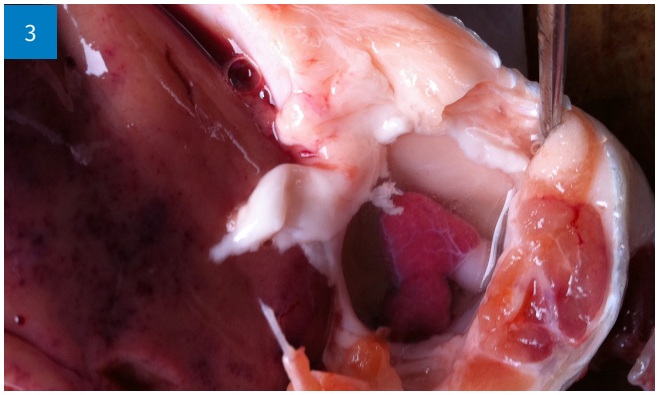


2. Hydropericardium.
3. Clots and ascites in the peritoneal cavity.
4. Opaque, thickened swim bladder with fluid.

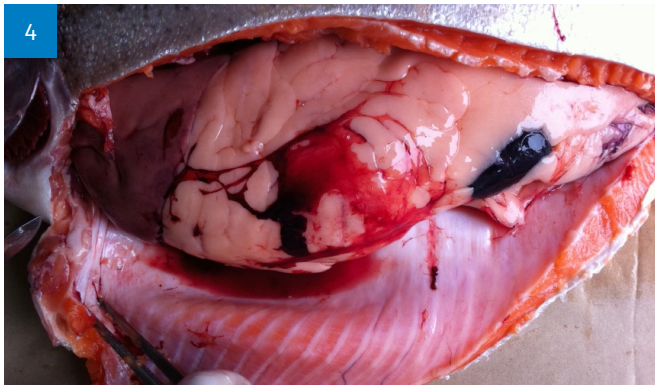
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17. HEMORRHAGIC SMOLT SYNDROME (HSS)

HEMORRHAGIC SMOLT SYNDROME (HSS)

The causative agent of the disease has not yet been isolated, in some publications it has been associated as an etiological agent to viral particles of the Togavirus type, while others mention viral particles of the Rhabdovirus type.

CLINICAL SIGNS

Externally, the following can be observed:

a. In general, fish in good condition with normal behavior.
b. Some fish are lethargic.
c. Difficulty to maintain a horizontal position.
d. Pale gills.
e. Petechiae on the belly.
f. Edema on the skin.
g. Bleeding at the base of the fins and on the inner side of the operculum.
h. Hemorrhaging in the gills (in arches and lamellae).

Internally, the following can be observed:

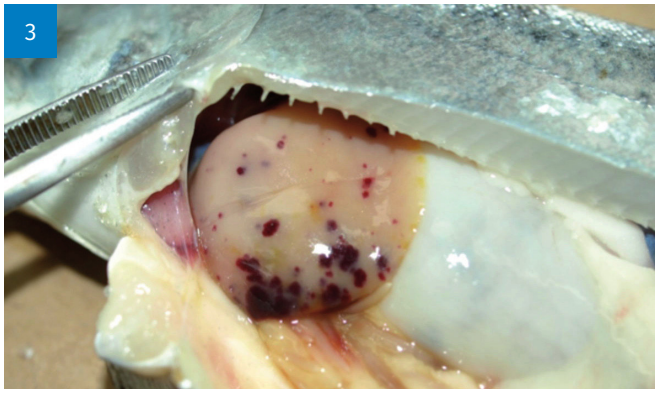
a. Internal bleeding.
b. Accompanied by an ascitic fluid.
c. Petechiae and ecchymosis in viscera and muscle.
d. The liver is bright yellow and sometimes ecchymosis.
e. The spleen looks dark and swollen.
f. There may be multiple foci of bleeding in the swim bladder and hindgut.
g. The contents of the intestine are yellow (catarrhal enteritis).
h. The pericardial cavity is filled with fluid.

i. The heart is clearly petechiated.
j. The hematocrit in affected fish is less than 10% and in the most critical cases even less than 2%.
k. Blood smears show a very high number of immature red blood cells and also a high number of white blood cells. An increased permeability of endothelial tissue is observed and there is clearly a failure of normal coagulation.

MACROSCOPIC PATHOLOGY

1. Bleeding at the base of the fins.
2. Muscle bleeding.
3. Multiple hemorrhages in the liver.
4. Muscle bleeding, pale liver, ascites.
5. Pale gills, muscle bleeding, pale liver, intestine with yellowish contents.

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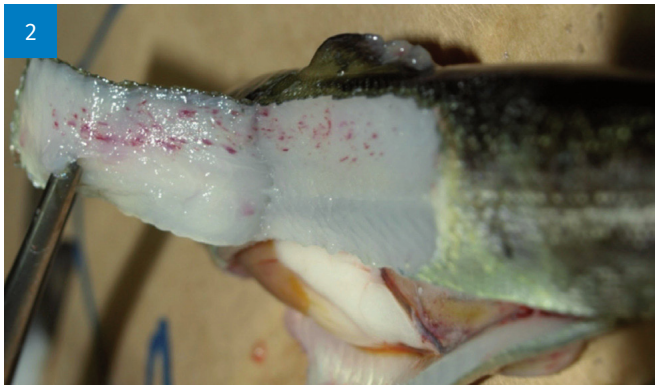
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18. HEART AND SKELETAL MUSCLE INFLAMMATION (HSMI)

ETIOLOGICAL AGENT AND NAME OF THE DISEASE

A new reovirus called Piscine Reovirus (PRV) has been identified as the causative agent of the disease. This virus belongs to the *Reoviridae* family. It is an icosahedral virus, not enveloped, with a double strand of RNA composed of 10 to 12 segments.

CLINICAL SIGNS

The most prominent external clinical signs include abnormal swimming, anorexia, darkening, lethargy and cornering.

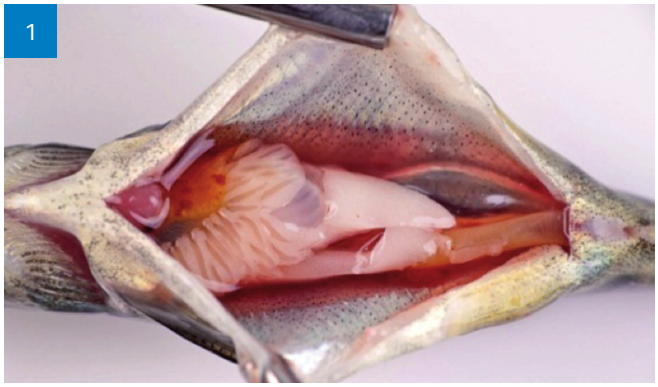
Internally, the following is observed:

a. Pale heart with loss of texture.
b. Swollen spleen.
c. Pericardial hemorrhage.
d. Ascites
e. Pale or spotted liver.
f. Petechiae in visceral fat.
g. Hemopericardium

MACROSCOPIC PATHOLOGY

1. Slightly pale heart, liver with hemorrhagic foci, ascites.

1



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