Devils Lake, Red River, and Lake Winnipeg Parasite/Pathogen Monitoring

INTERIM REPORT FOR LAKE WINNIPEG FISH HEALTH SURVEY (FALL 2006)
- LIGHT MICROSCOPY

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Executive Summary

The objectives of the Canadian portion of the project were to;

- 1. Determine the presence and estimate the prevalence of fish parasites and pathogens in resident fish from Lake Winnipeg;
- 2. Provide a comprehensive, and scientifically credible survey of fish parasites and pathogens in fish from Lake Winnipeg that may be used in performing risk analysis associated with transfer of fish parasites and pathogens from the outlet on Devils Lake to aquatic ecosystems in the Red River basin including Lake Winnipeg; and 3. Use the comprehensive survey of fish collected during this proposed survey to meet the overall framework for biological monitoring in the Red River basin that is included in the "Work Plan" of the International Red River Board.

The purpose of this particular portion of the project (and the subject of this interim report) was to perform a fish health survey of 10 species of fish from Lake Winnipeg using light microscopy. Sixty fish from each species (with the exception that only 7 channel catfish were caught) were examined. This is an interim report, and only the results of the survey are included. The final report will follow a similar format but will include additional fish ('spring 07' collection), the methods used, and interpretation based on the literature.

For this interim report, summaries for each species are included at the beginning of each segment of the report. The summary includes the tissues examined, common lesions present that were not systematically evaluated and any issues peculiar to a species. Next, the notable lesions that were systematically evaluated are described as well as the number of fish affected. For each of these a morphologic diagnosis (summary phrase) is also included. The majority of these lesions/organisms are illustrated with a photograph (powerpoint file for each species). These photographs are low resolution images to allow easy transmission. These will be reproduced at higher resolution for the final report. The remainder of the document is a list of the morphological diagnoses for each individual fish. Occasionally, a short description is given for an additional lesion affecting a fish that wasn't common or more noteworthy. Rarely, these have an accompanying photo.

The vast majority of agents found were metazoan or protozoan (used loosely including myxosporeans and microsporeans) that caused local tissue reactions. In the majority of instances, parasites that are well-adapted to their host cause relatively minimal mortality but may impair growth, reproduction, etc. depending on location and numbers. Parasites that have moved to a new host/location typically cause the greatest impact. A relevant example to this study is the discovery of the Asian tapeworm in fish from Lake Winnipeg. Almost all of the species of fish examined by light microscopy here had intestinal cestodes. The majority of the intestinal cestodes produced no, or limited, histological lesions and this is not unusual. The effects of the presence of the Asian tapeworm will vary markedly between species but is not best assessed by tissue lesions and/or overt mortality. Unless fortuitous sections reveal discriminating features, cestodes are typically not easy to identify using light microscopy to a family, let alone species, level. This is also true for trematodes and less so for nematodes, crustaceans, myxosporeans, protzoans, etc. Detailed light microscopic examination with a micrometer, special stains and in some cases electron microscopy can speciate some protozoans, myxo- and microsporeans. Nematodes can often be identified to a family based only on visible characteristics. For this interim report organisms were only identified to the level of class.

Lesions associated with bacterial and viral agents were rare. These were limited to epitheliocystis (ricketsia-like bacteria) (in white bass, yellow perch and fathead minnow) that are visible in light microscopic sections and lymphocystis (in walleye), a piscine iridovirus that produces characteristic histological lesions and is known to exist in Lake Winnipeg.

There were numerous lesions present in these fish, as would be expected for most wild fish. Many of these lesions however, were not associated with a visible organism. The majority of the lesions not directly associated with an agent, not surprisingly, are most likely remnants of metazoan migrations, etc. or lesions left after the inflammatory/immune response has removed the agent.

The walleye was the only species in which a neoplastic lesion was noted (dermal sarcoma). This is a well-recognized lesion in walleye and has been recorded from Lake Winnipeg previously. A few hepatic altered foci were noted and they can be considered to be potential pre-neoplastic lesions, however these were rare. Lesions that are most likely to have a significant impact on fish health are (as judged by the lesions produced and number of fish affected); branchial myxosporeans of emerald shiners; branchial epitheliocystis of white bass; branchial trematodes in white bass; and the intracardial trematodes of walleye and sauger. A single agent that would likely have the greatest impact on fish health (but this could still be relatively minimal) is listed for each species below.

Of those agents specifically noted, the intracardial trematodes of walleye (and to a lesser extent sauger) are likely to have the most substantial impact on fish health. The majority of walleye had significant lesions present in the ventricle, atrium and bulbous arteriosis (in that order). If anything, the number of fish affected as determined here is an underestimate as the heart was not collected from several walleye and was not always sectioned exactly across the location of the parasite. The heart valves were often involved to an extent that would interfere with function. Many of these fish are likely anemic (due to red cell breakdown – as evidenced by splenic hemosiderin accumulation) and would be less fit. A morphologically similar trematode and lesion was also seen in the sauger (a similar fish) however the number of fish affected and the severity of the lesions were less. There were trematodes (one had a haptor and they are therefore most likely monogenetic trematodes) found on the gills of the fathead minnow. *Gyrodactylus* sp. are somewhat similar (Gyrodactylus are monogenetic trematodes) however classification can best be performed with skin scrapes.

Those agents judged to have the most significant impact on fish health for each species are; Emerald Shiner (ES - Notropis atherinoides) – branchial myxosporeans, Brook stickleback – intralenticular trematode, Fathead minnow – hepatic nematodes (also present in BS) Yellow perch – intestinal cestodes, Northern pike – not clearly a single choice, Walleye and Sauger – intracardial trematode, Channel catfish – unknown – only 7 examined Goldeye – intestinal cestodes, White bass – branchial monogenetic trematodes.

Brook Stickleback (BS - Culea inconstans)

The sections consists of multiple sections whole fish including skin, gill, heart, liver, spleen, pancreas, head kidney, caudal kidney, gastrointestinal tract (stomach and intestine), thymus, swim bladder, brain, spinal cord, eye and ovary/testis. All tissues are not present on each slide.

The notable lesions and/or organisms are described in detail and pictures of these have been provided in most cases. Less notable alterations are occasionally included in the summary of lesions for each individual fish (see later).

The majority of the organisms are identified to the level of class with some exceptions, notably for the emerald shiner. Further work with available materials could narrow this further for the other species. Due to time constraints this work will not be included in the interim report for all species. For example, myxosporeans could be identified to genus in many cases by more detailed light microscopy including a broader range of special stains and electron microscopy of micro/myxosporeans would better identify these agents. Further sectioning of some of the metazoans would likely enable better anatomical detail and allow more precise classification. All measurements are approximations based on comparison with normal anatomic structures.

Branchial Trichodina - Trichodinosis, branchial cavity.

Within the branchial chamber there are two sections of round, approximately 45μm in diameter ciliated protozoan possessing a circular denticular ring – *Trichodina sp.* (Phylum Ciliophora, Class Litostomatea, Family Trichodina). ---- 6 of 60 affected

See BS Figure 1.

Endomeningial trematode - Trematodiasis, endomeningial, encysted Within endomeningial connective tissue and adipose tissue of the cranium, sections of a trematode organism is surrounded by one to two layers of flattened macrophages/fibrocytes. The encysted trematode is approximately 80μm to 100μm in diameter and characterized by a thin eosinophilic tegument and a parenchymatous matrix with no calcareous corpuscles (Phylum Platyhelmintheses, Class Trematoda). ---- 2 of 60 affected

See BS Figure 2.

Eye fluke – Trematodiasis, intraocular.

Embedded under the cuboidal epithelium of the lens, there are sections of a trematode parasite characterized by thin eosinophilic tegument and lack of body cavity and calcareous corpuscles (Phylum Platyhelmintheses, Class Trematoda). There is mild compaction of lenticular cells surrounding the parasite. ---- 2 of 60 affected

See BS Figure 3.

Intra-hepatic nematodes – Hepatitis, multifocal to regionally extensive, mild to moderate with intra-lesional nematodes.

There are multifocal parasitic granulomas replacing normal hepatic parenchyma. The granulomas are characterized by large numbers of swirling macrophages admixed with lesser numbers of eosinophilic granular cells surrounding either sections of a nematode organism or bright eosinophilic material and cellular debris. The nematodes are approximately 50µm in diameter are characterized by a bright eosinophilic cuticle, hypodermis, digestive tract and lateral alae (Phylum Nemathelminthes, Class Nematoda). There is mild compression of unaffected hepatocytes surrounding the granulomas. ---- 6 of 60 affected

See BS Figure 4.

Extra-hepatic nematodes / Granulomatous coelomitis – Coelomitis, granulomatous, focal to multifocal, mild to severe with intra-lesional nematodes.

There are multifocal cross- sections of a nematode organism within the peritoneum of several specimens. The nematodes are characterized by a thick eosinophilic cuticle, hypodermis and sections of a digestive tract and lateral chords embedded within a pseudocoelom (Phylum Nemathelminthes, Class Nematoda). Small to large numbers of macrophages and lesser numbers of eosinophilic granular cells surround both sections of the nematodes and fill spaces between surrounding fibrous connective tissue. ---- 6 of 60 affected

See BS Figure 5a and 5b.

Intramuscular nematodes –Myositis, focal to multifocal, mild to moderate with intralesional nematodes.

Sections of a nematode organism morphologically similar to those found within the ceolom (above) are encysted in the skeletal musculature of the body wall. The nematodes possess broadly similar characteristics but lateral alae are also visible. (Phylum Nemathelminthes, Class Nematoda) ---- 1 of 60 affected (no photo)

Intrasplenic nematodes – Splenitis, multifocal, mild to moderate with intra-lesional nematodes.

There are multiple cross sections of a nematode parasite surrounded by small to moderate numbers of macrophages and lesser numbers of lymphocytes. The nematodes are similar morphologically and in size to those previously described in the liver and coelom (Phylum Nemathelminthes, Class Nematoda). ---- 1 of 60 affected (no photo).

Encysted, intestinal nematodes – Enteritis, intramuscular, multifocal, low numbers Embedded within the circular and longitudinal muscular layers, there are multiple encysted metazoan organisms characterized by bright eosinophilic cuticle, hypodermis, digestive tract and lateral alae (Phylum Nemathelminthes, Class Nematoda). ---- 1 of 60 affected (no photo)

Intradermal nematodes - Nematodiasis, dermal, focal

Embedded within the dermis of a caudal spine, there is a cross section of a solitary nematode organism characterized by lateral alae, a thin eosinophilic cuticle and a psuedocoelom (Phylum Nemathelminthes, Class Nematoda). Little to no host inflammatory reaction is associated with the nematode. ---- 1 of 60 affected

See BS Figure 6.

Pancreatic duct myxosporeans (suspect) – Myxosporidiosis, pancreatic duct, multifocal cysts, moderate numbers

Within the lumen of the duct, there are moderate accumulations of cyst like structures filled with small, 5-7µm in diameter protozoan organisms that possess 1 or 2 small round eosinophilic and or basophilic nuclei (Myxosporidia). In some sections the organisms appear to be plasmodium-like in appearance with multiple, round, nucleated structures within a membrane. ---- 1 of 60 affected

See BS Figure 7.

Inter-neuronal protozoan - Protozoan, inter-neuronal, focal

Within the cytoplasm of a single large neuron of the spinal cord, there are multiple accumulations of small protozoan organisms (presumptive microsporidian) ranging in size from $2-5~\mu m$ in diameter. A single dark basophilic nucleus like organ (~1 μm in diameter) is present within variable numbers of these organisms. ---- 1 of 60 affected

See BS Figure 8.

Intestinal cestodes – Cestodes, intra-luminal, intestinal.

Within sections of the intestine there are sections of a metazoan organism characterized by a lack of digestive tract and a loose parenchyma surrounded by smooth outer eosinophilic tegument (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda). -- -2 of 60 affected (no photo).

BS 1 -

Caudal kidney - There are multifocal, large granulomas within the renal interstitium. The granulomas are characterized by concentric rings of macrophages and lesser numbers of eosinophilic granular cells surrounding central areas of wispy mucous and small amounts of cellular debris.

Peritoneum – Attached to the body wall, there is a focal swirling accumulation of macrophages and lesser numbers of eosinophilic granular cells surrounding remnants of a metazoan organism.

Morphological diagnosis

- 1. Granulomas, parasitic, renal, multifocal.
- 2. Ceolomitis, mild to moderate with intra-lesional remnants of a metazoan organism (presumptive nematode).

BS 2 - NSF

BS3-NSF

BS 4 - NSF

BS 5 - NSF

BS 6 -

Morphological diagnosis

- 1. Coelomitis, granulomatous, multifocal, moderate with intra-lesional nematodes (Phylum Nemathelminthes, Class Nematoda).
- 2. Myositis, focal, mild with intra-lesional nematode (Phylum Nemathelminthes, Class Nematoda). .

BS 7 -

Morphological diagnosis

- 1. Coelomitis, granulomatous, focal, mild with intra-lesional nematode (Phylum Nemathelminthes, Class Nematoda).
- 2. Trematodiasis, intraocular, focal (Phylum Platyhelmintheses, Class Trematoda).

BS 8 -

Eye – A small granuloma is present within the choriodal rete. The granuloma is characterized by a rim of thin macrophages surrounding several layers of concentrically arranged wispy, basophilic substance and a central core of amorphous material. Morphological diagnosis

1. Granuloma, choroidal rete, focal, small

BS9

Morphological diagnosis

1. Nematodiasis, dermal, focal (Phylum Nemathelminthes, Class Nematoda).

BS10

Morphological diagnosis

 Cestodes, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda)

BS 11 - NSF

BS 12 -

Morphological diagnosis

- 1. Hepatitis, multifocal, mild to moderate with intra-lesional nematodes (Phylum Nemathelminthes, Class Nematoda)
- 2. Coelomitis, granulomatous, multifocal, mild with intra-lesional nematodes (Phylum Nemathelminthes, Class Nematoda)

BS 13 - NSF

BS 14-

Morphological diagnosis

1. Trichodinosis, branchial cavity, small numbers (Phylum Ciliophora, Class Litostomatea, Family Trichodina).

BS 15 -

Morphological diagnosis

- 1. Trematodiasis, endomeningial, encysted, solitary (Phylum Platyhelmintheses, Class Trematoda).
- 2. Trichodinosis, branchial cavity, small numbers (Phylum Ciliophora, Class Litostomatea, Family Trichodina).

BS 16 -

Morphological diagnosis

1. Coelomitis, granulomatous, focal, mild with intra-lesional nematodes (Phylum Nemathelminthes, Class Nematoda).

BS 17 -

Ceolom_— Surrounding several islands of exocrine pancreas, there increased numbers of macrophages and lesser numbers of eosinophilic granular cells expanding spaces between connective and adipose tissue.

Morphological diagnosis

- 1. Trichodinosis, branchial cavity, small numbers (Phylum Ciliophora, Class Litostomatea, Family Trichodina).
- 2. Ceolomitis, histiocytic, multifocal, mild

BS 18 - NSF

BS 19

Morphological diagnosis

1. Hepatitis, multifocal to regionally extensive, mild to moderate with intra-lesional nematodes (Phylum Nemathelminthes, Class Nematoda).

BS 20 -

Morphological diagnosis

- Splenitis, multifocal, mild to moderate with intra-lesional nematodes. (Phylum Nemathelminthes, Class Nematoda)
- 2. Enteritis, intramuscular, multifocal, low numbers multifocal, low numbers (Phylum Nemathelminthes, Class Nematoda).

BS 21-

Oral cavity – There are multifocal cross-sections of metazoan organisms embedded within the connective tissue underlying the oral epithelium. The metozoans are characterized by a thin eosinophilic tegument and a parenchymatous body matrix (trematode).

- 1. Trematodiasis, intraocular, focal (Phylum Platyhelmintheses, Class Trematoda).
- 2. Trematodiasis, pharyngeal, dermal encysted, multifocal (Phylum Platyhelmintheses, Class Trematoda).

BS 22 - NSF

BS 23 -

Morphological diagnosis

- 1. Cestodes, intra-luminal, intestinal. (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).
- 2. Coelomitis, granulomatous, focal, mild.

BS 24 - NSF

BS 25 -

Morphological diagnosis

1. Branchitis, multifocal, mild.

BS 26 -

Morphological diagnosis

- 1. Hepatitis, histiocytic, focal mild.
- 2. Granuloma, parasitic, choroidal rete, focal.
- 3. Branchitis, multifocal, mild with multifocal epithelial hyperplasia.
- 4. Coelomitis, granulomatous, focal, mild to moderate with intra-lesional nematode (Phylum Nemathelminthes, Class Nematoda).
- 5. Granuloma, renal interstitial, focal, small.

BS 27 -

Morphological diagnosis

- 1. Enteritis, histiocytic, focal, mild.
- 2. Coelomitis, granulomatous, focal, mild with intra-lesional nematode (Phylum Nemathelminthes, Class Nematoda).

BS 28 -

Morphological diagnosis

1. Branchitis, multifocal, mild.

BS 29 -

Morphological diagnosis

1. Protozoan, interneuronal, focal. (Presumptive microsporean)

BS 30 -

- 1. Myxosporidiosis, pancreatic duct, multifocal cysts, moderate numbers (Phylum Myxozoa, Class Myxosporea).
- 2. Pancreatitis, duct wall, multifocal.

BS 31 - NSF

BS 32 - NSF

BS 33

Morphological diagnosis

- 1. Branchitis, multifocal, mild.
- Trichodinosis, branchial cavity, small numbers (Phylum Ciliophora, Class Litostomatea, Family Trichodina).

BS 34

Morphological diagnosis

 Hepatitis, multifocal, mild to moderate with intra-lesional nematodes (Phylum Nemathelminthes, Class Nematoda).

BS 35

Morphological diagnosis

 Branchitis, regionally extensive, mild to moderate with inter-lamellar epithelial hyperplasia.

BS 36

Morphological diagnosis

1. Branchitis, multifocal, mild.

BS 37

Morphological diagnosis

 Trematodiasis, retro bulbar, encysted, solitary (Phylum Platyhelmintheses, Class Trematoda).

BS 38

Morphological diagnosis

 Trichodinosis, branchial cavity, solitary (Phylum Ciliophora, Class Litostomatea, Family Trichodina).

BS 39

Morphological diagnosis

1. Branchitis, multifocal, mild.

BS 40 - NSF

BS 41

Morphological diagnosis

Granuloma, intra-ovarian, large, solitary.

BS 42 - NSF

BS 43 - NSF

BS 44

Morphological diagnosis

- 1. Trichodinosis, branchial cavity, small numbers (Phylum Ciliophora, Class Litostomatea, Family Trichodina).
- 2. Branchitis, multifocal, mild.

BS 45

Morphological diagnosis

- 1. Branchitis, multifocal to regionally extensive, mild to moderate.
- 2. Trematodiasis, intraocular, focal (Phylum Platyhelmintheses, Class Trematoda).

BS 46

Morphological diagnosis

1. Branchitis, multifocal, mild.

BS 47 - NSF

BS 48 - NSF

BS 49

Mouth – Within the epidermis of the lip, there is a single small focus of cellular debris within the cytoplasm of a greatly distended macrophage

Morphological diagnosis

- 1. Branchitis, multifocal, mild.
- 2. Oral cellular degeneration, focal,

BS 50

Muscle – Within the epaxial muscles of the tail there are multiple accumulations of macrophages replacing and surrounding degenerative muscle fibers.

Morphological diagnosis

- 1. Myositis, multifocal, mild.
- 2. Branchitis, multifocal to regionally extensive, mild.

BS 51

Morphological diagnosis

1. Branchitis, multifocal to regionally extensive, mild with intra-lesional trematode.

BS 52

Peritoneum – There is moderate, regionally extensive, expansion of connective tissue between the swim bladder and kidney that is marked by numbers of macrophages, polymorphonuclear cells and eosinophilic granular cells and multiple empty slightly encapsulated granulomas. There are multifocal cross sections of a metazoan organism

associated with the peritoneal inflammation. The metazoans are characterized by a bright eosinophilic cuticle, hypodermis, and digestive tract (nematode)

Morphological diagnosis

1. Branchitis, multifocal to regionally extensive, mild.

2. Coelomitis, granulomatous, multifocal, moderate with intra-lesional nematodes (Phylum Platyhelmintheses, Class Trematoda).

BS 53 - NSF

BS 54 - NSF

BS 55

Pancreas – Surrounding a large blood vessel within the spleen, there are moderate accumulations macrophages,

Morphological diagnosis

- 1. Hepatitis, multifocal, moderate.
- 2. Pancreatitis, focal, mild to moderate.

BS 56

Morphological diagnosis

No fish / slide 56

BS 57

Morphological diagnosis

 Hepatitis, multifocal to regionally extensive, with intra-lesional nematodes (Phylum Nemathelminthes, Class Nematoda).

BS 58 - NSF

BS 59

Morphological diagnosis.

- Hepatitis, regionally extensive, severe with intra-lesional nematodes (Phylum Nemathelminthes, Class Nematoda).
- Coelomitis, multifocal, moderate.

BS 60

Morphological diagnosis.

 Hepatitis, multifocal to regionally extensive, severe with intra-lesional nematodes (Phylum Nemathelminthes, Class Nematoda).

Fathead minnow (FHM – *Pimephales promelas*)

Each slide consists of multiple sections of whole fish including skin, gill, heart, liver, spleen, pancreas, head kidney, caudal kidney, gastrointestinal tract (stomach and intestine), thymus, swim bladder, brain, spinal cord, eye and ovary/testis. All tissues are not present on each slide.

The notable lesions and/or organisms are described in detail and pictures of these have been provided in most cases. Less notable alterations are occasionally included in the summary for lesions of each individual fish (see later).

The majority of the organisms are identified to the level of class with some exceptions, notably for the emerald shiner. Further work with available materials could narrow this further for the other species. Due to time constraints this work will not be included in the interim report for all species. For example, myxosporeans could be identified to genus in many cases by more detailed light microscopy including a broader range of special stains and electron microscopy of micro/myxosporeans would better identify these agents. Further sectioning of some of the metazoans would likely enable better anatomical detail and allow more precise classification. All measurements are approximations based on comparison with normal anatomic structures.

Intra-muscular myxosporidiosis. Myxosporidiosis, intra-muscular, focal to multifocal. Replacing sections of skeletal myofibres, there are single to several myxosporean cysts (ranging is size from 100μm to 200μm) consisting of both immature multinucleate sporoplasts and maturing spores (approx 7x15μm). The spores are ellipsoid to oval (valvular view) in shape with two pyriform polar capsules. There is only limited or no inflammatory reaction in most lesions. (Phylum Myxozoa, Class Myxosporea) ----- 38 of 60 affected.

FHM Figure 1.

Branchial trematodiasis: Branchitis, multifocal to regionally extensive, mild to moderate, with intra-lesional trematodes, likely a monogenetic trematode. Within several fish, cross sections of trematodes within the branchial cavity are associated mild to moderate, multifocal to regionally extensive accumulations of macrophages and lesser numbers of eosinophils. The trematodes, ranging is size from 100μm to 175μm in diameter, are characterized by a non-segmented thin tegument supported by a row of sub-tegumental cells and loose reticular parenchymatous matrix. At least one of these trematodes has a haptor visible. At least one fish also had a section with some characteristics of a monogenetic trematode on the skin (Phylum Platyhelminthes, Class Trematoda). ----- 9 0f 60 affected.

FHM Figure 2.

Endomeningial trematodiasis: Trematodiasis, endomeningial, encysted, focal and multifocal.

Cross sections of trematode organisms ranging in size from 50 µm to 200µm in diameter are expanding endomeningial spaces surrounding multiple sections of the brain. The trematodes are encysted by single to few layers of flattened macrophages and characterized by a thin eosinophilic tegument and a parenchymatous matrix with no calcareous corpuscles. (Phylum Platyhelmintheses, Class Trematoda) ----- 31 of 60 affected. Note: Four fish that possessed identical endomeningial lesions but lacked cross sections of a trematode were included in the number of fish affected because the lesions are histomorphologically identical but the trematode was missed during sectioning. Similar lesions with intra-lesional trematodes were present within the semicircular canal of small numbers of fish.

FHM Figure 3a.

Intramuscular trematodiasis: Myositis, multifocal, small and large with intra-lesional trematodes.

Single to multiple cross sections (200µm to 500µm) of a trematode parasite are encysted within various sections of epaxial and hypaxial skeletal myofibers. The trematodes are characterized by a thin eosinophilic tegument surrounding a parenchymatous matrix with the absence of calcareous corpuscles. In some sections, a digestive tract is present and in still others, reproductive organs are also present. The trematodes are surrounded by multiple layers of plump and attenuated macrophages (cyst wall), and variable number of melanin-laden macrophages. (Phylum Platyhelminthes, Class Trematoda) ----- 15 of 60 affected. Note 12 of 15 affected fish possess histomorphologically similar parasitic granulomas without cross sections of the trematode parasite but due to the similarity of the lesion they are included in the number of fish affected with this condition.

FHM Figure 3b.

Hepatic and extra-hepatic nematodiasis. Hepatitis, multifocal to regionally extensive, mild to moderate, with intra-lesional nematodes and/or; Ceolomitis, multifocal to regionally extensive, mild to moderate, with intra-lesional trematodes. Multiple sections of histomorphologically dissimilar nematodes are embedded within hepatic tissue or are free within the peritoneal cavity. In the liver, there is mild to moderate, multifocal to regionally extensive accumulations of plump amphophilic macrophages and lesser numbers of eosinophils surrounding one to several spherical cross sections (ranging in size from 75 µm to 120 um in diameter) of a nematode parasite admixed with multifocal accumulations of degenerative and necrotic cellular debris. Within the peritoneum, the nematodes, which are larger, (~100 to 200um) are associated within mild to moderate accumulations of macrophages and mild to moderate accumulations of cellular debris. The nematodes have a highly eosinophilic cuticle possessing lateral alae attached internally to lateral chords and a psuedocoelom containing a digestive tract and occasional reproductive organs. (Phylum Nemathelminthes, Class Nematoda) ---- 12 of 60 affected. Note: All affected fish do not possess sections of nematodes within the lesions. However the lesions are histomorphologically similar to those with nematodes and are therefore included in the number of fish affected with the condition of nematodiasis.

FHM Figure 4 + 5.

Renal myxosporidiosis. Myxosporidiosis, multifocal, mild.

Within the interstitium, there is a small myxosporean cyst ($70\mu m$ to $95\mu m$ in diameter) consisting of both immature multinucleate sporoplasm and maturing spores. The spores are ellipsoid to oval (valvular view) in shape and are histomorphologically similar to those described in the muscle. No or few numbers of inflammatory macrophages, eosinophilic granular cells, lymphocytes or plasma cells surround the myxosporidian cysts. 5 of 60 affected.

FHM Figure 6.

Myocardial and Renal intravascular myxosporidiosis. Myxosporidiosis, multifocal, myocardial, nephric, inter-vascular.

Heart - Attached and abutted against sections of the endocardium, there are small to moderate numbers of multinucleate plasmodium (15μm to 40μm in diameter). Within some sections the plasmodia are surrounded by moderate numbers of active, hypertrophied endocardial cells. Kidney – Small to moderate numbers of multinucleate plasmodium are scattered throughout large renal vascular channels transecting sections of the kidney. (Phylum Myxozoa, Class Myxosporea; suspect) ----- 6 of 60 affected (5 of 60 with myocardial plasmodia and 2 of 60 with renal vascular plasmodia).

FHM Figure 7a + 7b.

Branchial myxosporidiosis. Branchitis, multifocal, mild to moderate with intra-lesional myxsporeans.

Between lamellae on scattered filaments, there are multifocal myxosporean cysts (60µm to 100µm in diameter) consisting of both immature multinucleate sporoplasm and maturing spores. Small numbers of macrophages, lymphocytes and lesser numbers of eosinophilic granular cells surround these cysts. In some sections, small multinucleate plasmodia are within interstitial spaces of the filaments and hypodermis of the branchial arch. (Phylum Myxozoa, Class Myxosporea) ----- 5 of 60 affected.

FHM Figure 8.

Pericardial myxosporidiosis. Myxosporidiosis, regionally extensive, pericardial, large numbers.

Large numbers of mostly mature myxosporean spores are filling spaces between connective tissue of the pericardium. The spores are ellipsoid to pyriform in shape and approximately $14\mu m$ in length and $7\mu m$ at the widest diameter. Little to no inflammatory cells surrounds the accumulation of spores. (Phylum Myxozoa, Class Myxosporea) ----- 1 of 60 affected.

FHM Figure 9.

Branchial epitheliocystis. Epitheliocystis, focal.

A single focus (\sim 20 μ m in diameter) of amorphous basophilic rickettsia-like organisms is present between lamellae of a single filament and is surrounded by small numbers of plump macrophages and lesser numbers of lymphocytes. (Rickettsia-like organism, presumptive) ----- 1 of 60 affected.

FHM Figure 10.

Thymic myxosporidiosis. Myxosporidiosis, thymic, multifocal, low numbers. Within sections of the thymus, there are small multifocal accumulations of multinucleate plasmodia and maturing bi-valved myxosporidian spores surrounded by low numbers of thin and plump macrophages and small accumulations of cellular debris. The spores are approximately 11μm in length and 6-7μm in diameter. (Phylum Myxozoa, Class Myxosporea) ---- 2 of 60 affected.

FHM Figure 11.

FHM 1 -

Morphological diagnosis

1. Myxosporidiosis, intramuscular, focal (Phylum Myxozoa, Class Myxosporea)

FHM 2-

Morphological diagnosis

- 1. Branchitis, multifocal, mild with intra-lesional trematode. (Phylum Platyhelmintheses, Class Trematoda)
- Trematodiasis, endomeningial, semicircular canal, intramuscular, (Phylum Platyhelmintheses, Class Trematoda)

FHM 3 -

Morphological diagnosis

- 1. Branchitis, multifocal, mild.
- 2. Trematodiasis, endomeningial, focal (Phylum Platyhelmintheses, Class Trematoda)

FHM 4 -

Liver – There is mild multifocal accumulation of macrophages and lesser numbers of eosinophilic granular cells within and replacing hepatic parenchyma.

Morphological diagnosis

- 1. Ceolomitis, parasitic, granulomatous, multifocal, mild
- 2. Hepatitis, multifocal, mild

FHM 5 -

- 1. Branchitis, regionally extensive, mild.
- 2. Trematodiasis, intramuscular, solitary.

 Myxosporidiosis, intramuscular, multifocal (Phylum Myxozoa, Class Myxosporea)

FHM 6 -

Morphological diagnosis

- 1. Hepatitis, multifocal to regionally extensive, moderate with intra-lesional nematodes.
- 2. Ceolomitis, multifocal, moderate with intra-lesional nematodes.
- 3. Nematodiasis, intra-hepatic, intra-peritoneal, low numbers (Phylum Nemathelminthes, Class Nematoda)
- 4. Myxosporidiosis, intramuscular, multifocal (Phylum Myxozoa, Class Myxosporea).

FHM 7 -

Morphological diagnosis

- 1. Branchitis, regionally extensive, mild with an intra-lesional trematode. (Phylum Platyhelmintheses, Class Trematoda)
- 2. Trematodiasis, endomeningial, encysted, (Phylum Platyhelmintheses, Class Trematoda).

FHM 8 -

Morphological diagnosis

- 1. Branchitis, multifocal, mild with an intra-lesional trematode. (Phylum Platyhelmintheses, Class Trematoda)
- Trematodiasis, endomeningial, encysted, multifocal (Phylum Platyhelmintheses, Class Trematoda).

FHM 9

Gill – There is regionally extensive accumulations of macrophages, scattered eosinophilic granular cells and increased numbers of rodlet cells filling the space between lamellae. In one section there is a singe protozoan organism within the branchial cavity. The protozoan is not complete but appears to possess a denticular ring and cilia.

Morphological diagnosis

- 1. Trematodiasis, endomeningial, encysted, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- 2. Branchitis, regionally extensive, mild with intra-lesional protozoa (Trichodina: presumptive).
- 3. Hepatitis, multifocal, mild to moderate.
- 4. Granuloma, parasitic, intramuscular, multifocal, small and large.

FHM 10

- 1. Myxosporidiosis, intramuscular, multifocal (Phylum Myxozoa, Class Myxosporea).
- 2. Branchitis, multifocal, mild.

FHM 11 -

Skin – Within a single section of the skin on the ventral surface, there are increased numbers of lymphocytes and macrophages expanding spaces between epidermal cells. Kidney – Within one section of the cranial kidney, there is a single circular accumulation of low numbers of macrophages and lesser numbers of lymphocytes.

Morphological diagnosis

- 1. Trematodiasis, endomeningial, encysted, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- 2. Branchitis, regionally extensive, mild with an intra-lesional protozoa.
- 3. Dermatitis, mild, focal.
- 4. Nephritis, mild focal.

FHM 12 -

Morphological diagnosis

1. Myxosporidiosis, intramuscular, intra-nephric, intravascular, multifocal, moderate numbers (Phylum Myxozoa, Class Myxosporea).

FHM 13 -

Morphological diagnosis

- Trematodiasis, endomeningial, focal (Phylum Platyhelmintheses, Class Trematoda).
- Myxosporidiosis, intramuscular, intravascular, multifocal (Phylum Myxozoa, Class Myxosporea).
- 3. Hepatitis, multifocal, mild to moderate.
- 4. Ceolomitis, focal, mild to moderate.

FHM 14-

Morphological diagnosis

 Trematodiasis, endomeningial, focal (Phylum Platyhelmintheses, Class Trematoda).

FHM 15 -

Morphological diagnosis

- 1. Branchitis, regionally extensive, moderate.
- 2. Trematodiasis, endomeningial and peritoneal (Phylum Platyhelmintheses, Class Trematoda).

FHM 16 -

Morphological diagnosis

- 1. Trematodiasis, endomeningial, solitary (Phylum Platyhelmintheses, Class Trematoda).
- Myxosporidiosis, intramuscular, intravascular, multifocal (Phylum Myxozoa, Class Myxosporea).

FHM 17 -

GI - Within one section of intestine, increased numbers of enterocytes are undergoing degeneration and necrosis associated increased numbers trafficking lymphocytes and macrophages.

Brain – A single empty, parasitic granuloma is present within the rostral endomeninges of the optic tectum.

Morphological diagnosis

- 1. Myxosporidiosis, intramuscular, intravascular, multifocal (Phylum Myxozoa, Class Myxosporea).
- 2. Enteritis, multifocal with intra-lesional enterocyte degeneration and necrosis / unknown intra-cytoplasmic organism.
- 3. Endomeningitis, parasitic, focal.

FHM 18 -

Morphological diagnosis

- 1. Myxosporidiosis, thymic, intramuscular, multifocal (Phylum Myxozoa, Class Myxosporea).
- 2. Trematodiasis, endomeningial, intramuscular, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- 3. Protozoan, branchial arch, multifocal, low numbers (Presumptive Phylum Myxozoa, Class Myxosporea).

FHM 19

Morphological diagnosis

- 1. Branchitis, multifocal, moderate with scattered intra-lesional necrosis, cellular degeneration and trematodes. (Phylum Platyhelmintheses, Class Trematoda)
- 2. Ceolomitis, multifocal mild to moderate with intra-lesional nematodes (Phylum Nemathelminthes, Class Nematoda).
- 3. Myxosporidiosis, intramuscular, multifocal (Phylum Myxozoa, Class Myxosporea).

FHM 20 -

Morphological diagnosis

- 1. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 2. Endomeningitis, parasitic, focal

FHM 21-

Morphological diagnosis

- 1. Myxosporidiosis, intramuscular, nephric, branchial, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 2. Nephritis, interstitial, multifocal with intra-lesional immature multinucleate plasmodium and maturing spores.
- 3. Branchitis, multifocal, mild to moderate intra-lesional immature multinucleate plasmodium and maturing spores.

FHM 22 -

Morphological diagnosis

- 1. Branchitis, multifocal to regionally extensive, mild.
- 2. Granuloma, parasitic, encysted, intramuscular, focal.

FHM 23 -

Morphological diagnosis

- 1. Branchitis, multifocal to regionally extensive, mild.
- 2. Myxosporidiosis, intramuscular, focal. (Phylum Myxozoa, Class Myxosporea).
- 3. Endomeningitis, parasitic, multifocal.

FHM 24 -

Morphological diagnosis

- 1. Branchitis, multifocal, mild.
- 2. Myxosporidiosis, intramuscular, focal. (Phylum Myxozoa, Class Myxosporea).

FHM 25 -

Morphological diagnosis

- 1. Branchitis, multifocal to regionally extensive, mild.
- 2. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 3. Granuloma, parasitic, encysted, intramuscular, focal.
- 4. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).

FHM 26 -

Morphological diagnosis

- 1. Branchitis, regionally extensive to diffuse, mild.
- 2. Granuloma, parasitic, encysted, intramuscular, focal.

FHM 27 -

Morphological diagnosis

- 1. Granuloma, parasitic, encysted, intramuscular, intra-pharyngeal, multifocal.
- 2. Myxosporidiosis, intramuscular, focal. (Phylum Myxozoa, Class Myxosporea).
- 3. Trematodiasis, endomeningial, focal (Phylum Platyhelmintheses, Class Trematoda).

FHM 28 -

Morphological diagnosis

- 1. Granuloma, parasitic, encysted, intramuscular, focal.
- Myxosporidiosis, nephric, interstitial, intravascular multifocal. (Phylum Myxozoa, Class Myxosporea).
- 3. Trematodiasis, endomeningial, focal (Phylum Platyhelmintheses, Class Trematoda).

FHM 29 -

- 1. Granuloma, parasitic, encysted, intramuscular, focal.
- 2. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 3. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- 4. Ceolomitis, multifocal, mild to moderate with intra-lesional nematodes (Phylum Nemathelminthes, Class Nematoda).
- 5. Hepatitis, focally extensive, mild to moderate.
- 6. Branchitis, multifocal, mild.

FHM 30 -

Morphological diagnosis

- 1. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- 2. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 3. Branchitis, multifocal, mild with intra-branchial trematode. (Phylum Platyhelmintheses, Class Trematoda)

FHM 31

Morphological diagnosis

- 1. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 2. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).

FHM 32

GI – There are increased numbers of lymphocytes and lesser numbers of macrophages and eosinophilic granular cells within the lamina propria of one section of gut. There is multifocal enterocyte degeneration along one section of gut.

Muscle – Within one section of a hypaxial muscle bundle of the tail, there are moderate to large numbers of macrophages and lesser numbers of eosinophilic granular expanding space between muscle fibers which in turn surround a center of karyorrhectic debris, degenerative muscle fibers and eosinophilic material.

Morphological diagnosis

- 1. Enteritis, regionally extensive, mild with multifocal enterocyte degeneration.
- 2. Ceolomitis, multifocal, mild with pancreatic degeneration.
- 3. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- 4. Branchitis, regionally extensive, moderate with intra-lesional cellular degeneration and solitary intra-branchial trematode.
- 5. Myositis, histiocytic and granulocytic, focal large.
- 6. Myxosporidiosis, nephric, focal. (Phylum Myxozoa, Class Myxosporea).

FHM 33

- 1. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 2. Branchitis, multifocal, mild.

FHM 34

Morphological diagnosis

- 1. Myositis, histiocytic, multifocal.
- 2. Dermatitis, histiocytic, mild.
- Branchitis, multifocal, mild to moderate with intra-lesional myxosporean (Phylum Myxozoa, Class Myxosporea)

FHM 35 - NSF

FHM 36

Morphological diagnosis

- Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 2. Ceolomitis, multifocal, mild to moderate with intra-lesional nematodes (Phylum Nemathelminthes, Class Nematoda).

FHM 37

Skin – A single cross section of a metazoan organism (monogenetic trematode, presumptive) is associated with sections of the caudal fin.

Morphological diagnosis

- Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- Myositis, histiocytic, focal, mild.
- Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 4. Branchitis, regionally extensive to diffuse, mild to moderate.

FHM 38

Morphological diagnosis

- Epicarditis, focal, mild.
- 2. Myxosporidiosis, intramuscular, focal. (Phylum Myxozoa, Class Myxosporea).
- 3. Branchitis, multifocal, mild with intra-lesional myxosporean (Phylum Myxozoa, Class Myxosporea)

FHM 39

- 1. Branchitis, regionally extensive, moderate
- 2. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- 3. Myositis, histiocytic, focal, moderate.
- Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).

5. Granuloma, parasitic, focal, large.

FHM 40

Morphological diagnosis

- 1. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 2. Ceolomitis, multifocal, mild to moderate with intra-lesional nematodes (Phylum Nemathelminthes, Class Nematoda).
- 3. Hepatitis, multifocal, mild.
- 4. Branchitis, multifocal, mild with intra-lesional trematode. (Phylum Platyhelmintheses, Class Trematoda)

FHM 41

Morphological diagnosis

- 1. Myxosporidiosis, intramuscular, pericardial, intravascular, meningial, perineural, nephric, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 2. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).

FHM 42

Morphological diagnosis

1. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).

FHM 43

Morphological diagnosis

- 1. Trematodiasis, intramuscular, endomeningial,
- 2. Enteritis, lymphocytic, histiocytic, segmental, mild to moderate.

FHM 44

Morphological diagnosis

1. Granuloma, parasitic, focal.

FHM 45

Morphological diagnosis

- 1. Hepatitis, multifocal, mild to moderate.
- 2. Branchitis, multifocal, mild.

FHM 46

Morphological diagnosis

- 1. Myxosporidiosis, intramuscular, nephric, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 2. Granuloma, parasitic, intramuscular, solitary.

FHM 47

1. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).

FHM 48

Morphological diagnosis

- Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 2. Endomeningitis, parasitic, multifocal.
- 3. Epitheliocystis, focal.

FHM 49

Morphological diagnosis

- 1. Hepatitis, focal, mild to moderate with intra-lesional nematode (Phylum Nemathelminthes, Class Nematoda).
- 2. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 4. Nephritis, granulomatous, interstitial, focal.

FHM 50

Morphological diagnosis

 Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).

FHM 51

Morphological diagnosis

 Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).

FHM 52

Morphological diagnosis

- Enteritis, regionally extensive, moderate with enterocyte degeneration and necrosis.
- 2. Branchitis, multifocal, mild to moderate.
- Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).

FHM 53

Morphological diagnosis

- Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 2. Granuloma, parasitic, intramuscular, multifocal
- 3. Branchitis, diffuse, mild.

FHM 54

Morphological diagnosis

- 1. Hepatitis, multifocal, mild
- 2. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 3. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).

FHM 55

Morphological diagnosis

- 1. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 2. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).

FHM 56

Morphological diagnosis

- 1. Trematodiasis, endomeningial, intramuscular, retrobulbar, multifocal (Phylum Platyhelmintheses, Class Trematoda
- 2. Myxosporidiosis, thymic. (Phylum Myxozoa, Class Myxosporea).

FHM 57

Morphological diagnosis

- 1. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda
- 2. Branchitis, multifocal to regionally extensive, mild, with intra-lesional trichodinid protozoan.
- 3. Myxosporidiosis, intramuscular, focal. (Phylum Myxozoa, Class Myxosporea).
- 4. Dermatitis, focal.

FHM 58

Morphological diagnosis

- 1. Endomeningitis, parasitic, multifocal.
- 2. Granuloma, parasitic, intramuscular, multifocal.
- 3. Myxosporidiosis, intramuscular, focal. (Phylum Myxozoa, Class Myxosporea).

FHM 59 - NSF

FHM 60

- 1. Trematodiasis, intramuscular, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- 2. Branchitis, multifocal, mild.
- 3. Myositis, multifocal, mild

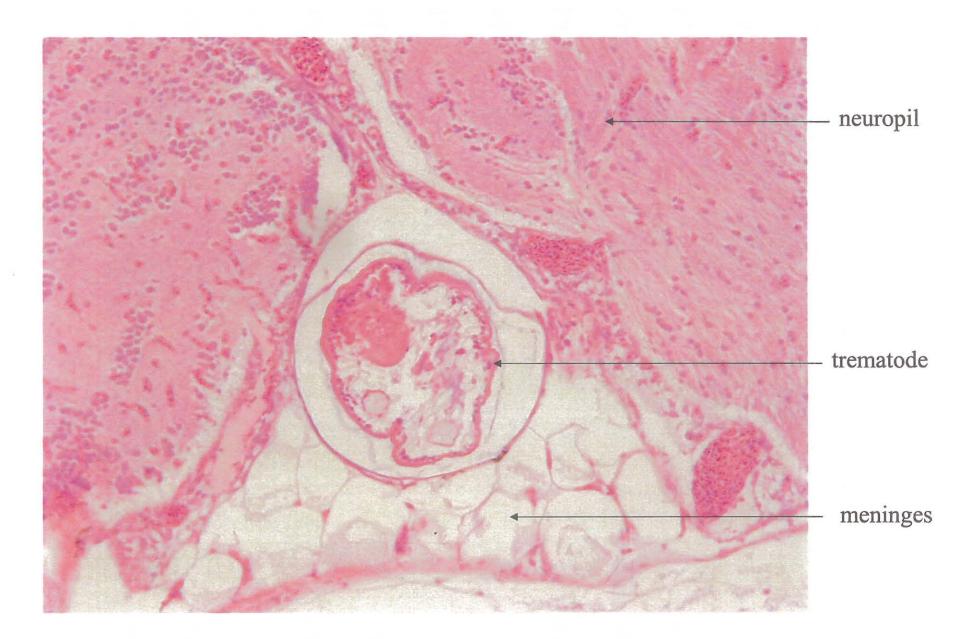
BS Figure 1.

Branchial trichodina - 6/60

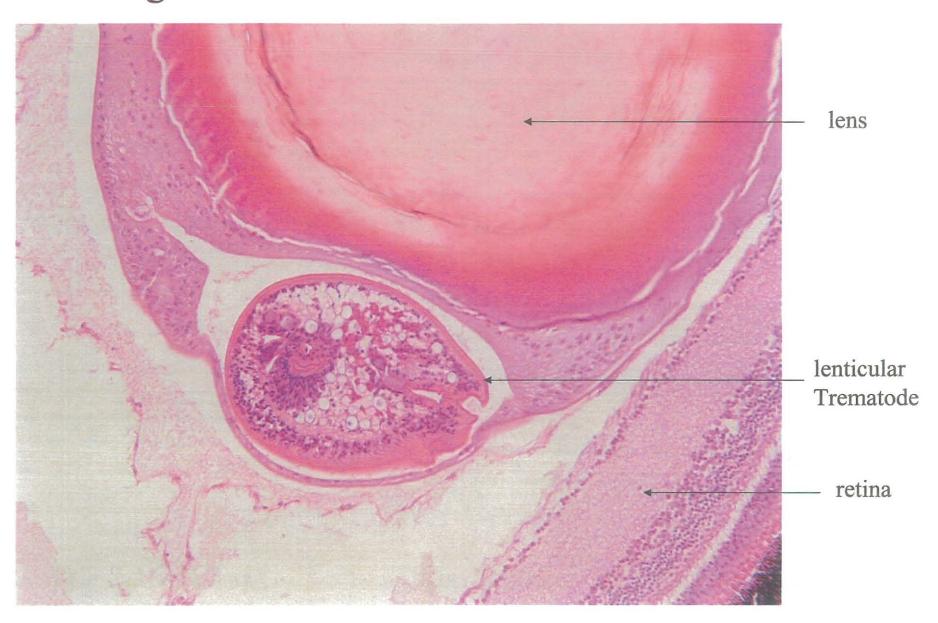


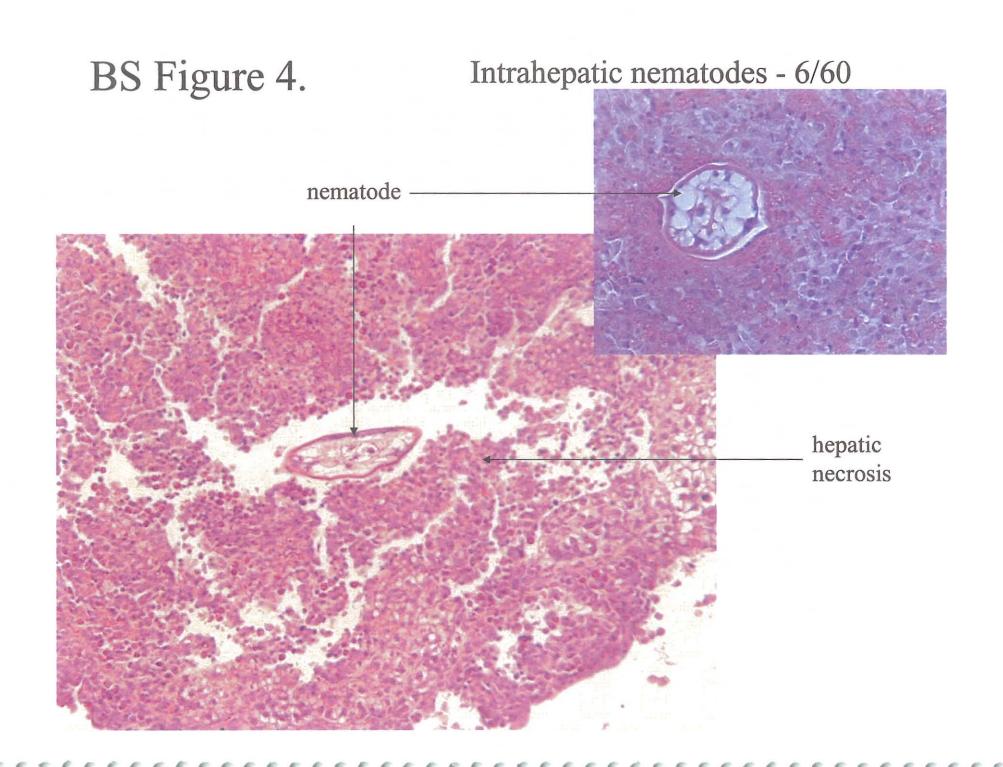
Trichodina sp.

BS Figure 2. Intrameningeal trematodiasis - 2/60

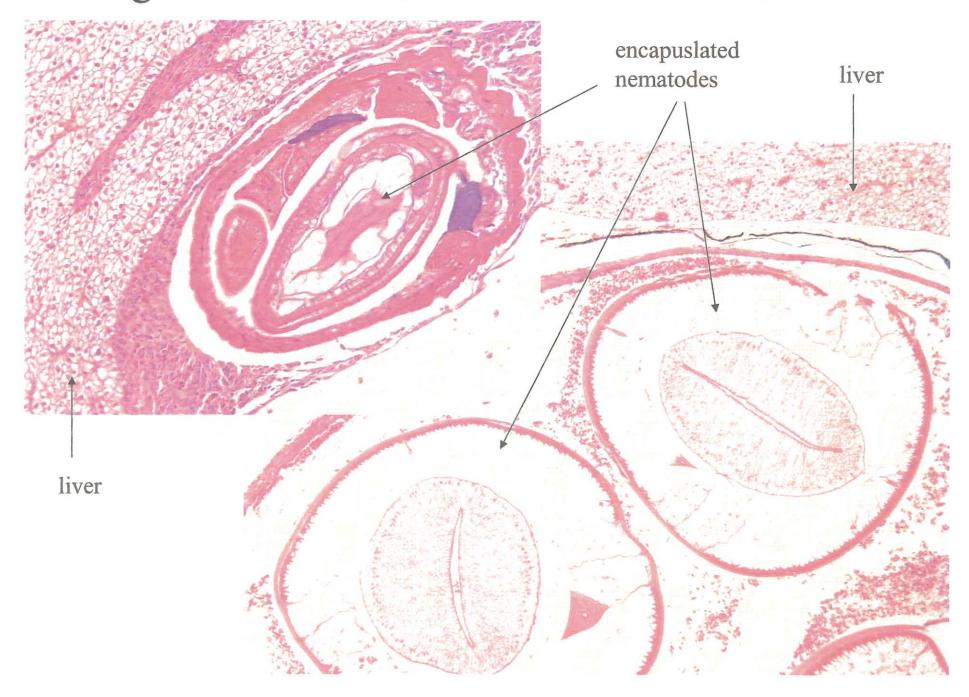


BS Figure 3. Lens trematode - 2/60

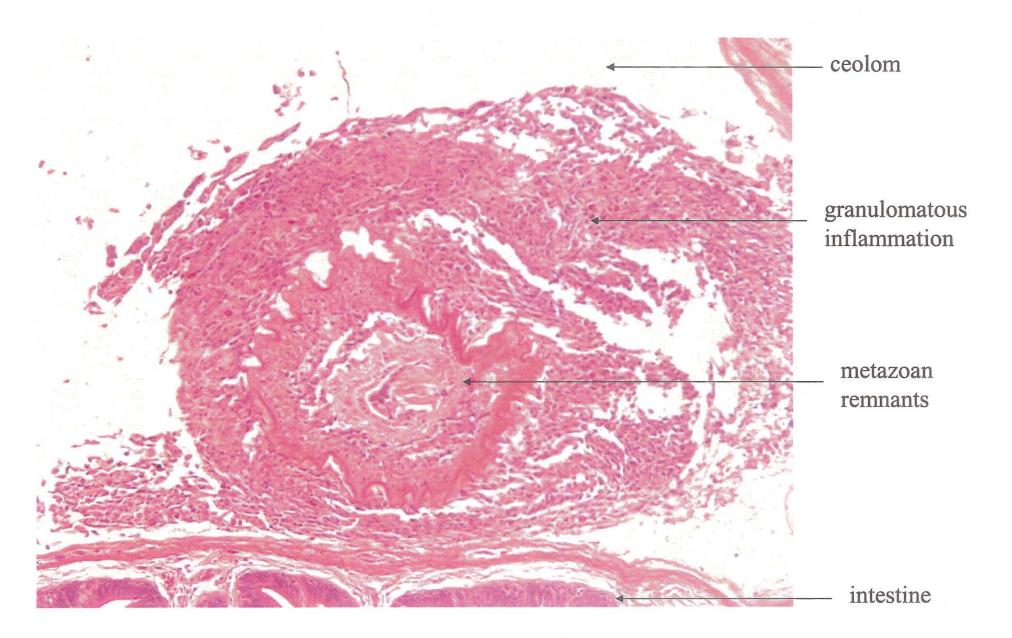




BS Figure 5a. Extrahepatic/mesenteric nematode(s) - 6/60



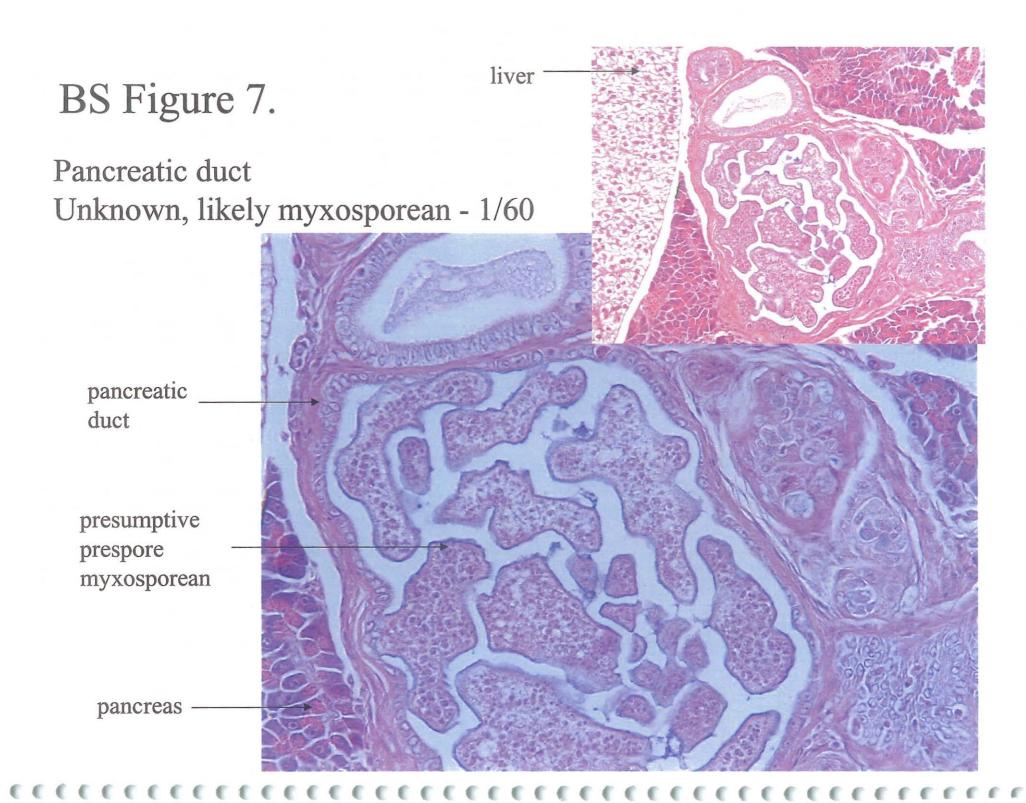
BS Figure 5b. Granulomatous ceolomitis - 6/60



BS Figure 6.

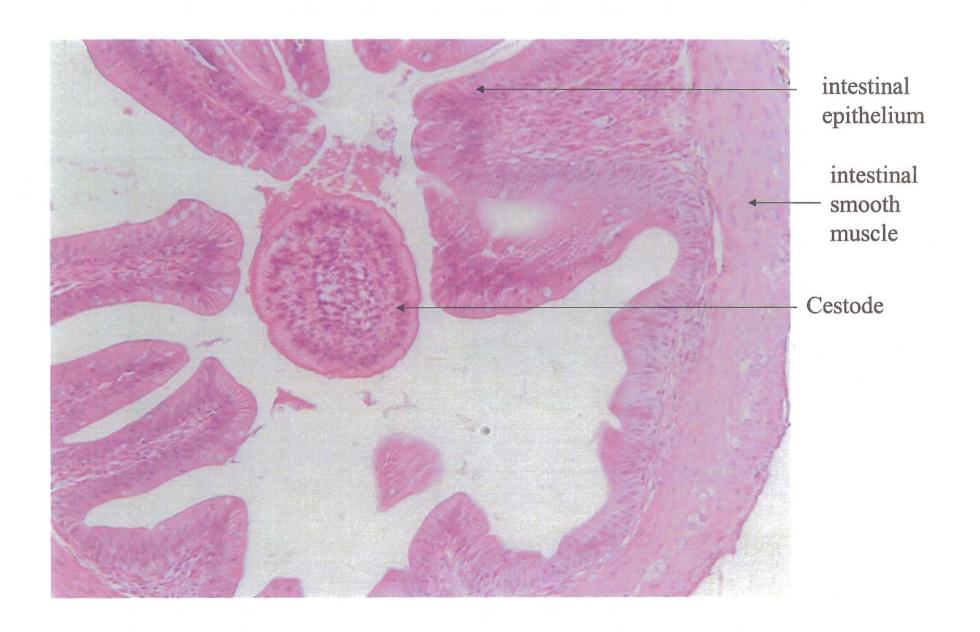
Intraepidermal nematode - 1/60





BS Figure 9.

Intestinal cestode - 2/60



Morphological diagnosis

- 4. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- Branchitis, multifocal, with intra-lesional myxosporeans (Phylum Myxozoa, Class Myxosporea)
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

ES 55

Morphological diagnosis

- Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).
- 3. Granuloma, focal, intramuscular, parasitic.
- 4. Branchitis, mild, multifocal.

ES 56

Morphological diagnosis

- Branchitis, mild, multifocal, intra-lesional myxosporean cysts (Phylum Myxozoa, Class Myxosporea)
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).
- 3. Trematodiasis, multifocal, intramuscular, encysted (Phylum Platyhelminthes, Class Trematoda)

ES 57

Morphological diagnosis

- Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- 2. Granuloma, multifocal, intramuscular, intra-coelimic, parasitic.
- Branchitis, multifocal, with intra-lesional myxosporeans (Phylum Myxozoa, Class Myxosporea)
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

ES 58

Morphological diagnosis

1. Stomatitis, moderate, regionally extensive, with erosion and ulceration.

1. Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

ES 49

Morphological diagnosis

- 1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- 2. Branchitis, multifocal, with intra-lesional myxosporeans (Phylum Myxozoa, Class Myxosporea)

ES 50

Morphological diagnosis

- 1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

ES 51

Morphological diagnosis

- 1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- 2. Branchitis, multifocal, with intra-lesional myxosporeans (Phylum Myxozoa, Class Myxosporea)
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

ES 52

Morphological diagnosis

- 1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- 2. Branchitis, multifocal, with intra-lesional myxosporeans (Phylum Myxozoa, Class Myxosporea)
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

Slide 53

- 1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).
- 3. Branchitis, mild, multifocal.

- 1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- 2. Branchitis, multifocal, with intra-lesional myxosporean cysts (Phylum Myxozoa, Class Myxosporea)
- 3. Granuloma, focal, intramuscular, parasitic.
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

Morphological diagnosis

- Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, Bothriocephalus).
- 2. Branchitis, multifocal, with intra-lesional myxosporeans (Phylum Myxozoa, Class Myxosporea)
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

ES 46

Morphological diagnosis

- 1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- Branchitis, multifocal, with intra-lesional myxosporeans (Phylum Myxozoa, Class Myxosporea)

ES 47

Muscle – Expanding spaces between muscle fibers surrounding the nerve fascicle of the lateral line, there are moderate accumulations of mature myxosporean spores admixed with small to moderate accumulations of macrophages and lesser numbers of lymphocytes. The spores are histomorphologically similar to those previously described in the gill.

Morphological diagnosis

- Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, Bothriocephalus).
- Branchitis, multifocal, with intra-lesional myxosporeans (Phylum Myxozoa, Class Myxosporea)
- 3. Perineuritis and myositis, focal to multifocal, mild to moderate with intra-lesional myxosporeans (Phylum Myxozoa, Class Myxosporea)
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

ES 48

- 2. Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexa (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeridae or Calyptosporidae).
- 3. Branchitis, mild to moderate, focal.

Liver – There is a single small round granuloma within the connective capsule of the liver and compressing subjacent parenchyma. A second similar smaller granuloma is present within the peritoneal space just proximal to the liver.

Morphological diagnosis

1. Granulomas, multifocal, extra hepatic, intra-peritoneal, intramuscular parasitic.

ES 40

Morphological diagnosis

- 1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- 2. Trematodiasis, focal, intramuscular, encysted (Phylum Platyhelminthes, Class Trematoda)
- 3. Myositis, mild to moderate, multifocal, histiocytic

ES 41

Morphological diagnosis

- 1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- 2. Branchitis, multifocal, with intra-lesional myxosporean cysts (Phylum Myxozoa, Class Myxosporea)
- 3. Myositis, mild, focal, histiocytic.

ES 42

Morphological diagnosis

- 1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).
- 3. Cellulitis, focal, periosteal, histiocytic.

ES 43

Morphological diagnosis

- 1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- 2. Branchitis, multifocal, with intra-lesional myxosporean cysts (Phylum Myxozoa, Class Myxosporea)
- 3. Granuloma, focal, intramuscular, parasitic.

ES 44

Morphological diagnosis

- Branchitis, multifocal, with intra-lesional myxosporean cysts (Phylum Myxozoa, Class Myxosporea)
- 2. Granuloma, multifocal, retro bulbar, parasitic.

ES 33

Morphological diagnosis

- 1. Branchitis, multifocal, with intra-lesional myxosporean cysts (Phylum Myxozoa, Class Myxosporea)
- 2. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- 3. Trematodiasis, larval, intramuscular, encysted (Phylum Platyhelminthes, Class Trematoda)

ES 34

Morphological diagnosis

- Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, Bothriocephalus).
- 2. Branchitis, mild to moderate, multifocal intra-lesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 3. Trematodiasis, larval, focal, intramuscular, encysted (Phylum Platyhelminthes, Class Trematoda)

ES 35 - NSF

ES 36

Morphological diagnosis

- Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, Bothriocephalus).
- 2. Trematodiasis, larval, focal, intramuscular, encysted (Phylum Platyhelminthes, Class Trematoda)
- 3. Granuloma, focal, intramuscular, parasitic.

ES 37.

Morphological diagnosis

- Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, Bothriocephalus).
- 2. Trematodiasis, larval, focal, intramuscular, encysted (Phylum Platyhelminthes, Class Trematoda)
- Branchitis, mild to moderate, multifocal intra-lesional trematodes (Phylum Platyhelminthes, Class Trematoda)

ES 38.

Morphological diagnosis

 Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, Bothriocephalus). Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

ES 28

Morphological diagnosis

- 1. Cestodiasis, gastrointestinal, adult (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- 2. Branchitis, multifocal, with intra-lesional myxosporean cysts (Phylum Myxozoa, Class Myxosporea)
- 3. Trematodiasis, larval, intramuscular, encysted (Phylum Platyhelminthes, Class Trematoda)

ES 29

Morphological diagnosis

- 1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- 2. Branchitis, mild, multifocal.

ES 30

Morphological diagnosis

- 1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- 2. Branchitis, multifocal, with intra-lesional myxosporean cysts (Phylum Myxozoa, Class Myxosporea)
- 3. Trematodiasis, larval, intramuscular, encysted (Phylum Platyhelminthes, Class Trematoda)
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

ES 31

Morphological diagnosis

- 1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- 2. Branchitis, multifocal, with intra-lesional myxosporean cysts (Phylum Myxozoa, Class Myxosporea)
- 3. Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

Slide 32

Eye – There are two, small round granulomas expanding connective tissue behind the eye. The granulomas are characterized by a central core of necrotic cellular debris surrounded by plump eosinophilic macrophages and approximately four to seven concentric layers of flattened occasionally highly eosinophilic flattened macrophages.

Eye – Several small myxosporean cysts containing immature and maturing spores (Phylum Myxozoa, Class Myxosporea) are expanding spaces between retro bulbar connective tissue of the eye and a large muscle bundle. The developing spores are similar to those previously described.

Morphological diagnosis

- Branchitis, regionally extensive with intra-lesional myxosporean cysts (Phylum Myxozoa, Class Myxosporea)
- 2. Retro bulbar myxosporean cysts (Phylum Myxozoa, Class Myxosporea)
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

ES 24

Morphological diagnosis

- Stomatitis, oral-pharyngeal, multifocal epithelial hyperplasia, erosion and ulceration.
- Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, Bothriocephalus).
- Branchitis, regionally extensive with intra-lesional myxosporean cysts (Phylum Myxozoa, Class Myxosporea)

ES 25

Morphological diagnosis

- Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, Bothriocephalus).
- 2. Branchitis, mild to moderate, multifocal to regionally extensive, with intralesional remnants of a metazoan organism.
- 3. Trematodiasis, larval, multifocal, intramuscular, encysted (Phylum Platyhelminthes, Class Trematoda)
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

ES 26

Morphological diagnosis

 Branchitis, regionally extensive with intra-lesional myxosporean cysts (Phylum Myxozoa, Class Myxosporea)

ES 27

- Branchitis, regionally extensive with intra-lesional myxosporean cysts (Phylum Myxozoa, Class Myxosporea)
- Branchitis, mild, focal, with intra-lesional remnants of a single metazoan organism.
- Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, Bothriocephalus).

Morphological diagnosis

- 1. Granuloma, small, intramuscular (epaxial), parasitic.
- 2. Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

ES 19

Morphological diagnosis

- 1. Myositis, mild, focal (lateral line), histiocytic.
- 2. Branchitis, mild, multifocal.
- 3. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- 4. Granuloma, intramuscular (tail), metazoan type.
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

ES 20

Morphological diagnosis

- 1. Myositis, mild, focal (lateral line), histiocytic.
- 2. Branchitis, regionally extensive with intra-lesional myxosporean cysts. (Phylum Myxozoa, Class Myxosporea)
- 3. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).

ES 21

 Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

ES 22

Peritoneal – Within one section of abdominal adipose tissue surrounding the GI tract, there is a focal accumulation of small numbers of plump foamy macrophages Morphological diagnosis

- 1. Branchitis, moderate, regionally extensive.
- 2. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).
- 4. Steatitis, focal, histiocytic.

ES 23

- 2. Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).
- 3. Branchitis, mild, multifocal.

Morphological diagnosis

- Branchitis, multifocal with intra-lesional myxosporean cysts (Phylum Myxozoa, Class Myxosporea)
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

ES 15

Morphological diagnosis

- Branchitis, regionally extensive with intra-lesional myxosporeans. (Phylum Myxozoa, Class Myxosporea)
- 2. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

ES 16

Brain – Within the tegumentum of the mesencephalon, there is a single, space occupying, empty parasitic cyst characterized by a thin capsule wall and mild compaction of surrounding neuropil.

Morphological diagnosis

- Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, Bothriocephalus).
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).
- 3. Branchitis, mild, focal.

ES 17

- Dermatitis, locally extensive, ulcerative with mild, multifocal myositis and myodegeneration.
- 2. Branchitis, mild to moderate, multifocal.
- 4. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- 5. Granuloma, small, intramuscular, parasitic.
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

- 2. Myositis, multifocal, mild to moderate with intra-lesional myxosporeans (Phylum Myxozoa, Class Myxosporea)
- 3. Enteritis, mild, multifocal with intra-lesional accumulations of an intracellular protozoan organism (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).
- 4. Granuloma, focal, parasitic

Morphological diagnosis

1. Branchitis, regionally extensive, with few small intra-lesional myxosporean cysts.

ES 10

Morphological diagnosis

- 1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*)).
- 2. Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).
- 3. Branchitis, mild, diffuse with mild postmortem autolysis.

ES 11

Muscle – There is a single metazoan organism migrating through the body wall musculature and penetrating the abdominal wall. The metazoan is characterized by a non-segmented thin tegument supported by row of sub-tegumental cells and loose reticular parenchymatous matrix (Phylum Platyhelminthes, Class Trematoda). There is multifocal degeneration of myofibres surrounding this parasite and small accumulations of macrophages, lymphocytes and remnants of a cyst wall. Within hypaxial muscle bundles of the tail, there is a single granuloma containing degenerate and necrotic components of an encysted metazoan parasite.

Morphological diagnosis

- 1. Branchitis, multifocal with intra-lesional myxosporean cysts (Phylum Myxozoa, Class Myxosporea)
- 2. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- 3. Trematodiasis, intramuscular to peritoneal migration (Phylum Platyhelminthes, Class Trematoda)
- 4. Granuloma, intramuscular, encysted, parasitic.

ES 12 - NAF

ES 13

Morphological diagnosis

1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).

ES 4.

Morphological diagnosis

- Branchitis, multifocal, intra-lesional myxosporean cysts (Phylum Myxozoa, Class Myxosporea)
- 2. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).

ES 5

Morphological diagnosis

- 1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- Enteritis, mild, multifocal with intra-lesional accumulations of an intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).
- 3. Branchitis, mild to moderate, diffuse.

ES 6

Muscle – Two granulomas are present within hypaxial musculature. The large granuloma is characterized by a central core of lightly eosinophilic material bordered by a highly eosinophilic layer of viable and degenerate cells which in turn is surrounded by variable layers concentrically arranged fusiform macrophages. Multiple foci of apoptotic cells and karyorrhectic debris are present within macrophages surrounding the center of eosinophilic material and debris. The small granuloma is characterized by a central core of viable and degenerate macrophages some of which contain a blue amorphous intracytoplasmic substance and or melanin. Variable layers of viable, flattened macrophages surround the central areas of macrophages, cellular debris and melanin accumulations. Morphological diagnosis

- 1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*)).
- 2. Branchitis, mild to moderate, diffuse.
- 3. Myositis, granulomatous, multifocal (Note: lesion similar to those surrounding encysted intra-muscular trematodes)

ES 7

Morphological diagnosis

- 1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*)).
- 2. Branchitis, mild, regionally extensive.
- 3. Myositis, focal, granulomatous (Note: lesion similar to encysted intra-muscular trematodes)

ES8

Morphological diagnosis

 Branchitis, diffuse, with intra-lesional myxosporean cysts. (Phylum Myxozoa, Class Myxosporea) scleritinized hooks and tubular organs embedded within a loose reticular parenchyma (Phylum Platyhelminthes, Class Trematoda). ---- 2 of 60 affected.

ES 1

Oral cavity and GI - Within the mouth and extending into the pharynx, there is mild to moderate, diffuse epithelial hyperplasia and increased numbers of leukocytes. In some areas, there is mild to moderate erosion and ulceration. Sections of a metazoan parasite are present within the proximal pharynx extending distally into gastrointestinal tract. The metazoan possess a scolex with longitudinally elongate bothria, an eosinophilic tegument thrown into regularly spaced folds (proglottids), a lack of digestive tract and numerous round to ovoid, basophilic, calcium bodies (calcareous corpuscles) embedded within a loose paranchymatous matrix (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda). While sections of the head and neck possess calcareous corpuscles, distal segments contain myriad developing eggs. Segments of this metazoan extend into and distend the stomach and upper gastrointestinal tract. Similar sections of the metazoan are present within lower sections of the GI examined. Moderate accumulations of arthropods were present within the lower GI exhibiting characteristic segmented chitonous exoskeleton and striated muscle (these are assumed to be dietary).

Muscle – Within a single muscle fascicle muscle surrounding the branchial cavity, there is a single large myxosporean cyst consisting of both immature multinucleate sporoplasm and maturing spores. The spores are ellipsoid to oval (valvular view) in shape with two pyriform polar capsules.

Morphological diagnosis

- 1. Stomatitis, oral-pharyngeal, mild to moderate with epithelial hyperplasia, erosion, and ulceration.
- 2. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).
- 3. Branchitis, multifocal to regionally extensive, with intra-lesional myxosporean cysts.
- 4. Myxosporidiosis, intra-muscular, focal (Phylum Myxozoa, Class Myxosporea)

ES 2

Peritoneal cavity – There is a single large granuloma embedded within mesenteric fat surrounding the intestine. The granuloma is characterized by central core of eosinophilic cellular debris surrounded by multiple layers of loosely packed macrophages and lesser numbers of leukocytes.

Morphological diagnosis

- 1. Branchitis, regionally extensive, with intra-lesional myxosporean cysts. (Phylum Myxozoa, Class Myxosporea)
- 2. Stomatitis, regionally extensive epithelial hyperplasia with erosion, ulceration and filamentous bacteria.
- 3. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*)).

ES 3- NAF

Intestinal apicomplexans - Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans.

Multiple developmental stages of an intracellular protozoan parasite are present within small numbers of epithelial cells lining sections of the gastrointestinal tract. In most foci there is no associated host reaction however in some sections there are small numbers of trafficking lymphocytes and smaller number of macrophages surrounding the parasite in various stages of development. The developmental stages (unsporulated oocysts, sporulated oocysts, and macrogametes) observed are similar to those of a coccidian apicomplexan (Phylum Apicomplexa, Subclass Coccidia, Family Eimeriidae or Calyptosporidae, presumptive). In still other areas there is erosion of the epithelial lining within areas of inflammation and accumulation of sporulated oocysts. ---- 28 of 30 affected.

See ES Figure 3.

Intramuscular trematodes – Trematodiasis, intramuscular, encysted or Granuloma, intramuscular, parasitic.

There is a single metazoan organism migrating through the body wall musculature and penetrating the abdominal wall. The metazoan is characterized by a non-segmented thin tegument supported by row of sub-tegumental cells and loose reticular parenchymatous matrix (Phylum Platyhelminthes, Class Trematoda). There is multifocal degeneration of myofibres surrounding this parasite and small accumulations of macrophages, lymphocytes and remnants of a cyst wall. Similar lesions (i.e. granulomas – see more detailed description for ES 6 below) with and without intra-lesional trematodes are present in other sections examined. ---- 24 of 60 affected.

See ES Figure 4.

Perineural and muscular myxosporeans – Perineuritis and myositis, focal to multifocal, mild to moderate with intra-lesional myxosporeans.

Expanding spaces between muscle fibers and extending into the perineural space surrounding the nerve fascicle of the lateral line, there are moderate accumulations of mature myxosporean spores admixed with small to moderate accumulations of macrophages and lesser numbers of lymphocytes. The spores are histomorphologically similar to those previously described in the gill. (Phylum Myxozoa, Class Myxosporea) --- 4 of 60 affected. Note: fish with histomorphologically identical inflammatory lesions surrounding the lateral line nerve but without myxosporean parasites were included in the number of fish affected.

See ES Figure 5.

Branchial trematodes - - Branchitis, multifocal, mild to moderate, intra-lesional trematodes

Between lamellae of filaments there are moderate accumulations of macrophages and lymphocytes expanding lamellae surrounding tubular metazoan organism which possess

Emerald Shiner (ES - Notropis atherinoides)

The sections consists of multiple sections of whole fish including skin, gill, heart, liver, spleen, pancreas, head kidney, caudal kidney, gastrointestinal tract (stomach and intestine), thymus, swim bladder, brain, spinal cord, eye and ovary/testis. All tissues are not present on each slide.

The notable lesions and/or organisms are described in detail and pictures of these have been provided in most cases. Less notable alterations are occasionally included in the summary of lesions for each individual fish (see later).

The majority of the organisms are identified to the level of class with some exceptions, notably for the emerald shiner. Further work with available materials could narrow this further for the other species and for additional organisms in emerald shiner. Due to time constraints this work will not be included in the interim report for all species. For example, myxosporeans could be identified to genus in many cases by more detailed light microscopy including a broader range of special stains and electron microscopy of micro/myxosporeans would better identify these agents. Further sectioning of some of the metazoans would likely enable better anatomical detail and allow more precise classification. All measurements are approximations based on comparison with normal anatomic structures.

Branchial myxosporeans - Branchitis, multifocal to diffuse, with intra-lesional myxosporean cysts

Within various sections of gill, there are solitary to multiple myxosporean cysts between lamellae and surrounded by multifocal to regionally extensive epithelial hyperplasia admixed within increased numbers of leukocytes, rodlet cells and karyorrhectic debris. The cysts contain both immature multinucleate sporoplasm and maturing spores. The spores are ellipsoid to oval (valvular view) in shape with two pyriform polar capsules and approximately 14µm in length. There is multifocal to diffuse accumulations of macrophages, lymphocytes, and eosinophilic granular cells surrounding the cysts. (Phylum Myxozoa, Class Myxosporea) ---- 32 of 60 affected.

See ES Figure 1.

Intestinal cestodes – Cestodiasis, intra-luminal, intestinal

Sections of a cestode parasite are present within multiple sites along the digestive tract (i.e. the proximal pharynx extending distally into distal intestine). The **metazoan** possess a scolex with longitudinally elongate bothria, an eosinophilic tegument thrown into regularly spaced folds (proglottids), a lack of digestive tract and numerous round to ovoid, basophilic, calcium bodies (calcareous corpuscles) embedded within a loose paranchymatous matrix (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda). While sections of the head and neck possess calcareous corpuscles, distal segments contain myriad developing eggs (for a more detailed description – see ES 1). ----44 of 60 affected.

See ES Figure 2.

2. Branchitis, multifocal, with intra-lesional myxosporeans (Phylum Myxozoa, Class Myxosporea)

3. Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

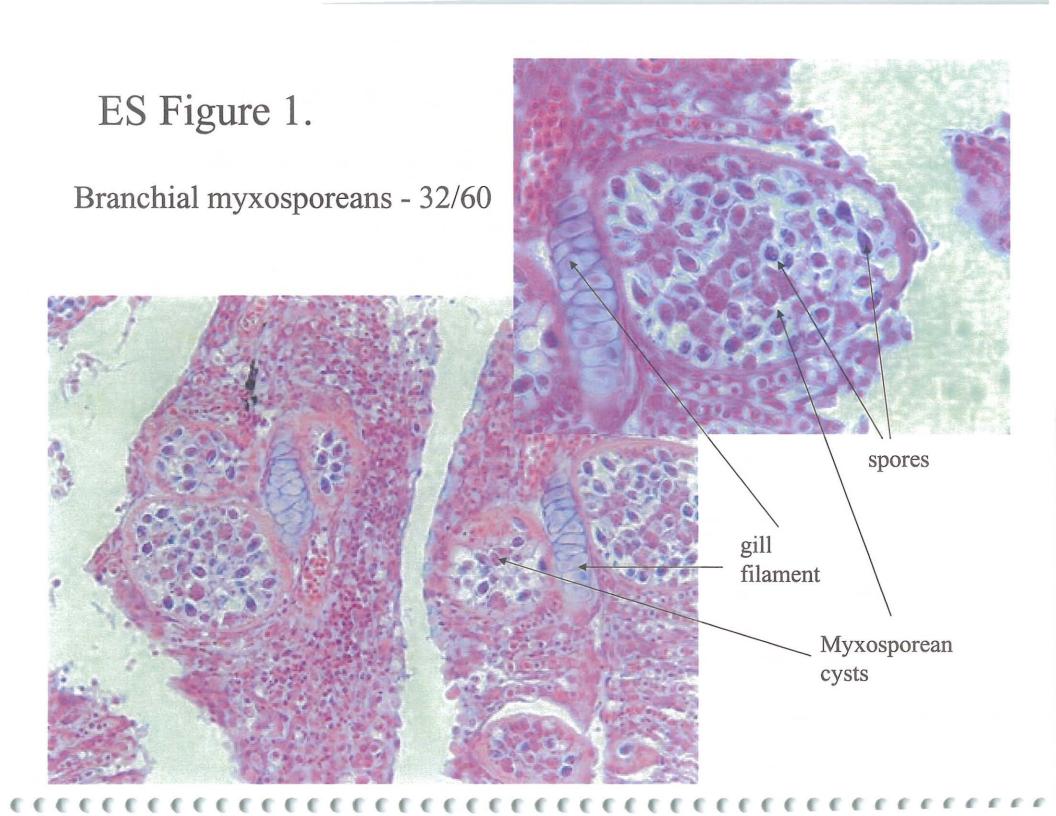
ES 59

Morphological diagnosis.

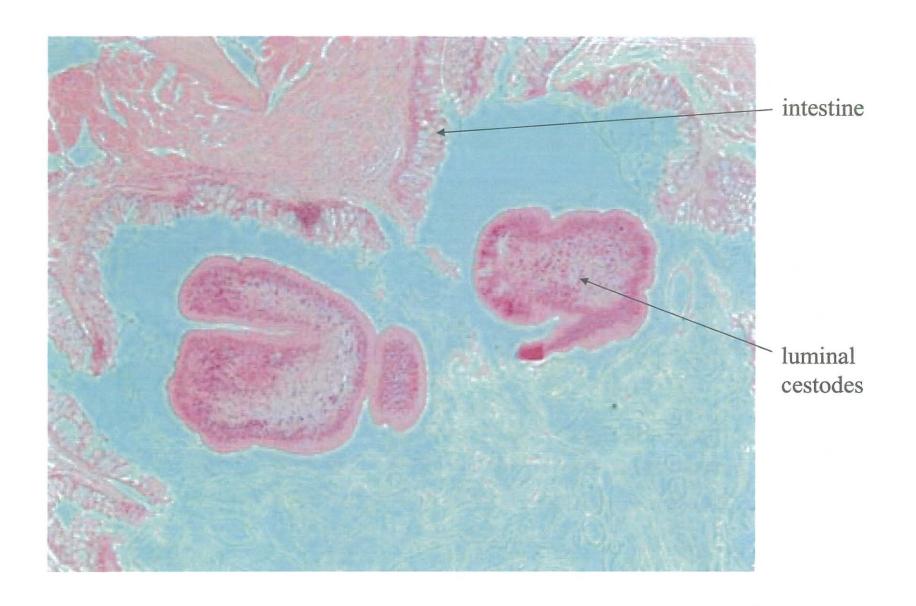
- 1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).
- 3. Trematodiasis, multifocal, intramuscular, encysted (Phylum Platyhelminthes, Class Trematoda)

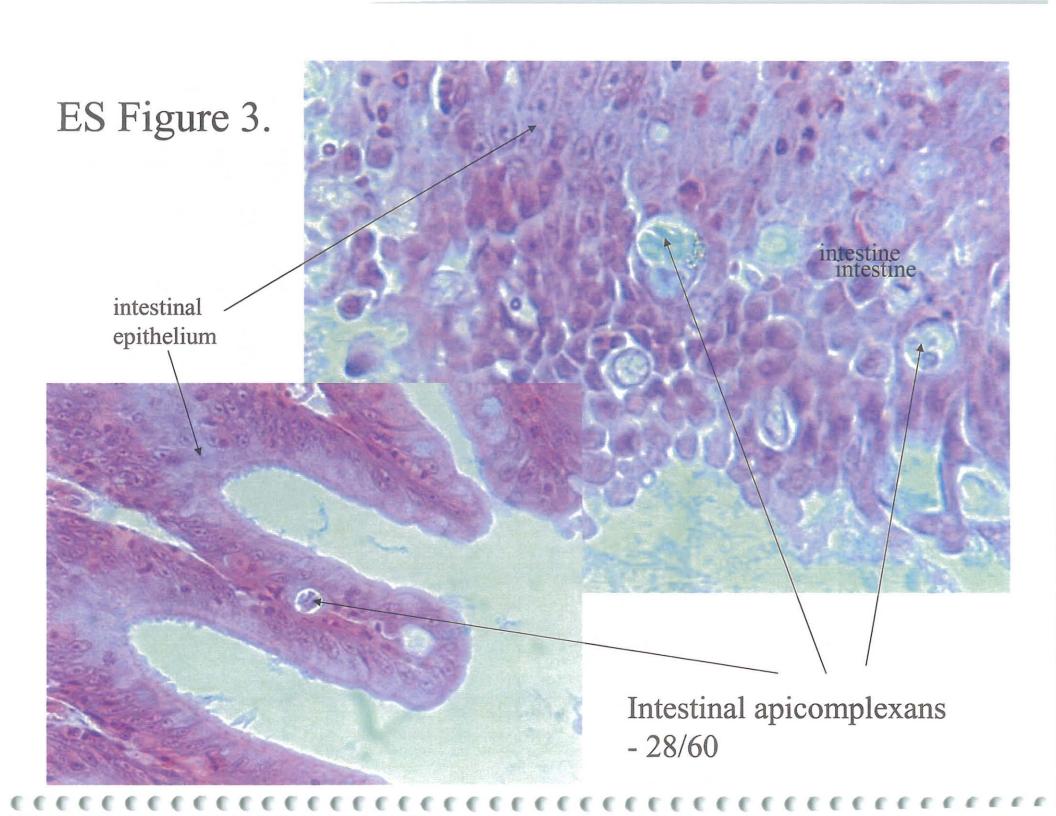
ES 60

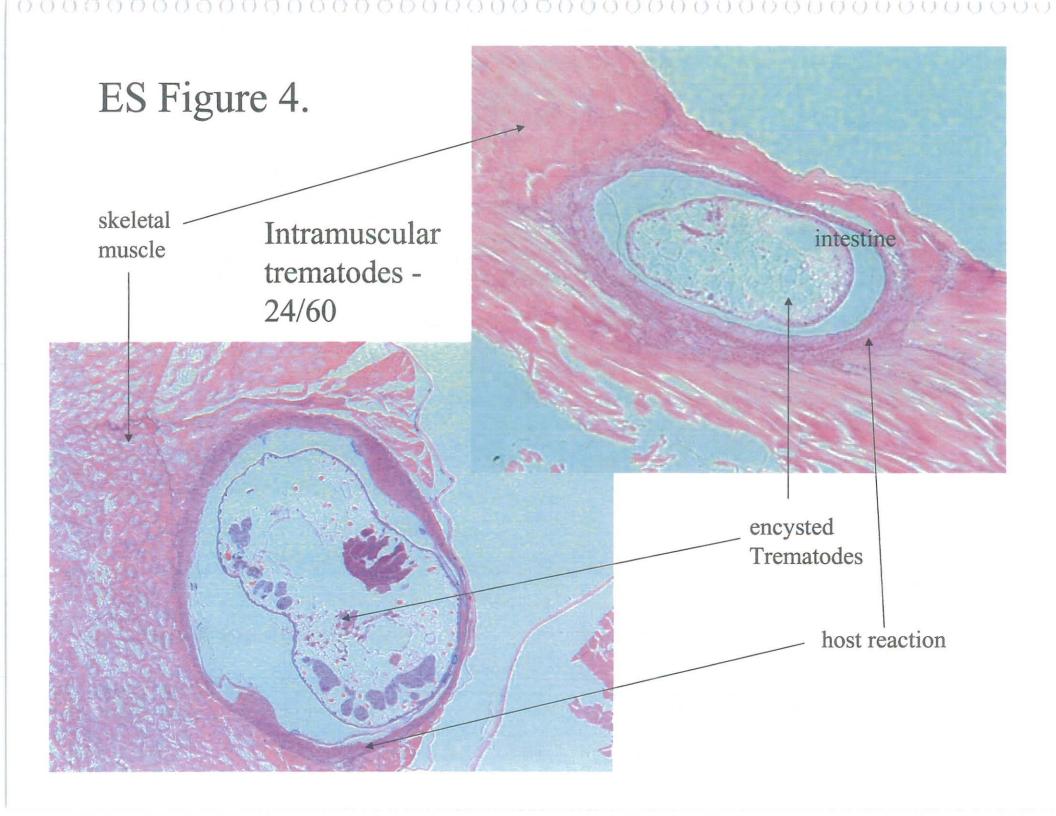
- 1. Cestodiasis, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda, Family Bothriocephalidae, *Bothriocephalus*).
- 2. Branchitis, mild, multifocal.
- 3. Enteritis, mild, multifocal with intra-lesional, intracellular apicomplexans (Phylum Apicomplexa, Subclass, Coccidia, Family Eimeriidae or Calyptosporidae).

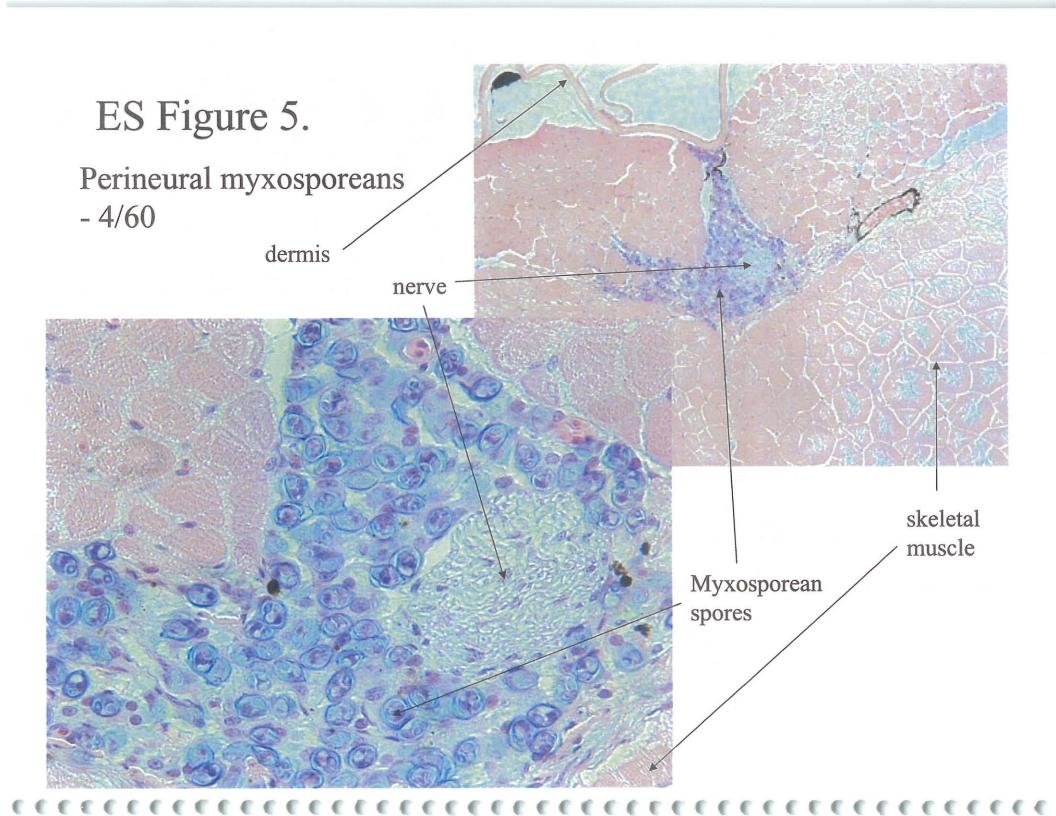


ES Figure 2. Intestinal cestodes - 44/60









Fathead minnow (FHM - Pimephales promelas)

Each slide consists of multiple sections of whole fish including skin, gill, heart, liver, spleen, pancreas, head kidney, caudal kidney, gastrointestinal tract (stomach and intestine), thymus, swim bladder, brain, spinal cord, eye and ovary/testis. All tissues are not present on each slide.

The notable lesions and/or organisms are described in detail and pictures of these have been provided in most cases. Less notable alterations are occasionally included in the summary for lesions of each individual fish (see later).

The majority of the organisms are identified to the level of class with some exceptions, notably for the emerald shiner. Further work with available materials could narrow this further for the other species. Due to time constraints this work will not be included in the interim report for all species. For example, myxosporeans could be identified to genus in many cases by more detailed light microscopy including a broader range of special stains and electron microscopy of micro/myxosporeans would better identify these agents. Further sectioning of some of the metazoans would likely enable better anatomical detail and allow more precise classification. All measurements are approximations based on comparison with normal anatomic structures.

Intra-muscular myxosporidiosis. Myxosporidiosis, intra-muscular, focal to multifocal. Replacing sections of skeletal myofibres, there are single to several myxosporean cysts (ranging is size from 100μm to 200μm) consisting of both immature multinucleate sporoplasts and maturing spores (approx 7x15μm). The spores are ellipsoid to oval (valvular view) in shape with two pyriform polar capsules. There is only limited or no inflammatory reaction in most lesions. (Phylum Myxozoa, Class Myxosporea) ----- 38 of 60 affected.

FHM Figure 1.

Branchial trematodiasis: Branchitis, multifocal to regionally extensive, mild to moderate, with intra-lesional trematodes, likely a monogenetic trematode. Within several fish, cross sections of trematodes within the branchial cavity are associated mild to moderate, multifocal to regionally extensive accumulations of macrophages and lesser numbers of eosinophils. The trematodes, ranging is size from 100μm to 175μm in diameter, are characterized by a non-segmented thin tegument supported by a row of sub-tegumental cells and loose reticular parenchymatous matrix. At least one of these trematodes has a haptor visible. At least one fish also had a section with some characteristics of a monogenetic trematode on the skin (Phylum Platyhelminthes, Class Trematoda). ----- 9 0f 60 affected.

FHM Figure 2.

Endomeningial trematodiasis: Trematodiasis, endomeningial, encysted, focal and multifocal.

Cross sections of trematode organisms ranging in size from 50 µm to 200µm in diameter are expanding endomeningial spaces surrounding multiple sections of the brain. The trematodes are encysted by single to few layers of flattened macrophages and characterized by a thin eosinophilic tegument and a parenchymatous matrix with no calcareous corpuscles. (Phylum Platyhelmintheses, Class Trematoda) ----- 31 of 60 affected. Note: Four fish that possessed identical endomeningial lesions but lacked cross sections of a trematode were included in the number of fish affected because the lesions are histomorphologically identical but the trematode was missed during sectioning. Similar lesions with intra-lesional trematodes were present within the semicircular canal of small numbers of fish.

FHM Figure 3a.

Intramuscular trematodiasis: Myositis, multifocal, small and large with intra-lesional trematodes.

Single to multiple cross sections (200µm to 500µm) of a trematode parasite are encysted within various sections of epaxial and hypaxial skeletal myofibers. The trematodes are characterized by a thin eosinophilic tegument surrounding a parenchymatous matrix with the absence of calcareous corpuscles. In some sections, a digestive tract is present and in still others, reproductive organs are also present. The trematodes are surrounded by multiple layers of plump and attenuated macrophages (cyst wall), and variable number of melanin-laden macrophages. (Phylum Platyhelminthes, Class Trematoda) ----- 15 of 60 affected. Note 12 of 15 affected fish possess histomorphologically similar parasitic granulomas without cross sections of the trematode parasite but due to the similarity of the lesion they are included in the number of fish affected with this condition.

FHM Figure 3b.

Hepatic and extra-hepatic nematodiasis. Hepatitis, multifocal to regionally extensive, mild to moderate, with intra-lesional nematodes and/or; Ceolomitis, multifocal to regionally extensive, mild to moderate, with intra-lesional trematodes. Multiple sections of histomorphologically dissimilar nematodes are embedded within hepatic tissue or are free within the peritoneal cavity. In the liver, there is mild to moderate, multifocal to regionally extensive accumulations of plump amphophilic macrophages and lesser numbers of eosinophils surrounding one to several spherical cross sections (ranging in size from 75 um to 120 um in diameter) of a nematode parasite admixed with multifocal accumulations of degenerative and necrotic cellular debris. Within the peritoneum, the nematodes, which are larger, (~100 to 200um) are associated within mild to moderate accumulations of macrophages and mild to moderate accumulations of cellular debris. The nematodes have a highly eosinophilic cuticle possessing lateral alae attached internally to lateral chords and a psuedocoelom containing a digestive tract and occasional reproductive organs. (Phylum Nemathelminthes, Class Nematoda) ---- 12 of 60 affected. Note: All affected fish do not possess sections of nematodes within the lesions. However the lesions are histomorphologically similar to those with nematodes and are therefore included in the number of fish affected with the condition of nematodiasis.

FHM Figure 4 + 5.

Renal myxosporidiosis. Myxosporidiosis, multifocal, mild.

Within the interstitium, there is a small myxosporean cyst (70µm to 95µm in diameter) consisting of both immature multinucleate sporoplasm and maturing spores. The spores are ellipsoid to oval (valvular view) in shape and are histomorphologically similar to those described in the muscle. No or few numbers of inflammatory macrophages, eosinophilic granular cells, lymphocytes or plasma cells surround the myxosporidian cysts. 5 of 60 affected.

FHM Figure 6.

Myocardial and Renal intravascular myxosporidiosis. Myxosporidiosis, multifocal, myocardial, nephric, inter-vascular.

Heart - Attached and abutted against sections of the endocardium, there are small to moderate numbers of multinucleate plasmodium (15μm to 40μm in diameter). Within some sections the plasmodia are surrounded by moderate numbers of active, hypertrophied endocardial cells. Kidney – Small to moderate numbers of multinucleate plasmodium are scattered throughout large renal vascular channels transecting sections of the kidney. (Phylum Myxozoa, Class Myxosporea; suspect) ----- 6 of 60 affected (5 of 60 with myocardial plasmodia and 2 of 60 with renal vascular plasmodia).

FHM Figure 7a + 7b.

Branchial myxosporidiosis. Branchitis, multifocal, mild to moderate with intra-lesional myxsporeans.

Between lamellae on scattered filaments, there are multifocal myxosporean cysts (60μm to 100μm in diameter) consisting of both immature multinucleate sporoplasm and maturing spores. Small numbers of macrophages, lymphocytes and lesser numbers of eosinophilic granular cells surround these cysts. In some sections, small multinucleate plasmodia are within interstitial spaces of the filaments and hypodermis of the branchial arch. (Phylum Myxozoa, Class Myxosporea) ----- 5 of 60 affected.

FHM Figure 8.

Pericardial myxosporidiosis. Myxosporidiosis, regionally extensive, pericardial, large numbers.

Large numbers of mostly mature myxosporean spores are filling spaces between connective tissue of the pericardium. The spores are ellipsoid to pyriform in shape and approximately $14\mu m$ in length and $7\mu m$ at the widest diameter. Little to no inflammatory cells surrounds the accumulation of spores. (Phylum Myxozoa, Class Myxosporea) ----- 1 of 60 affected.

FHM Figure 9.

Branchial epitheliocystis. Epitheliocystis, focal.

A single focus ($\sim 20 \mu m$ in diameter) of amorphous basophilic rickettsia-like organisms is present between lamellae of a single filament and is surrounded by small numbers of plump macrophages and lesser numbers of lymphocytes. (Rickettsia-like organism, presumptive) ----- 1 of 60 affected.

FHM Figure 10.

Thymic myxosporidiosis. Myxosporidiosis, thymic, multifocal, low numbers.

Within sections of the thymus, there are small multifocal accumulations of multinucleate plasmodia and maturing bi-valved myxosporidian spores surrounded by low numbers of thin and plump macrophages and small accumulations of cellular debris. The spores are approximately 11µm in length and 6-7µm in diameter. (Phylum Myxozoa, Class Myxosporea) ---- 2 of 60 affected.

FHM Figure 11.

FHM 1 -

Morphological diagnosis

1. Myxosporidiosis, intramuscular, focal (Phylum Myxozoa, Class Myxosporea)

FHM 2-

Morphological diagnosis

- 1. Branchitis, multifocal, mild with intra-lesional trematode. (Phylum Platyhelmintheses, Class Trematoda)
- Trematodiasis, endomeningial, semicircular canal, intramuscular, (Phylum Platyhelmintheses, Class Trematoda)

FHM 3 -

Morphological diagnosis

- 1. Branchitis, multifocal, mild.
- 2. Trematodiasis, endomeningial, focal (Phylum Platyhelmintheses, Class Trematoda)

FHM 4-

Liver – There is mild multifocal accumulation of macrophages and lesser numbers of eosinophilic granular cells within and replacing hepatic parenchyma.

Morphological diagnosis

- 1. Ceolomitis, parasitic, granulomatous, multifocal, mild
- 2. Hepatitis, multifocal, mild

FHM 5 -

- 1. Branchitis, regionally extensive, mild.
- 2. Trematodiasis, intramuscular, solitary.

3. Myxosporidiosis, intramuscular, multifocal (Phylum Myxozoa, Class Myxosporea)

FHM 6-

Morphological diagnosis

- Hepatitis, multifocal to regionally extensive, moderate with intra-lesional nematodes.
- 2. Ceolomitis, multifocal, moderate with intra-lesional nematodes.
- 3. Nematodiasis, intra-hepatic, intra-peritoneal, low numbers (Phylum Nemathelminthes, Class Nematoda)
- Myxosporidiosis, intramuscular, multifocal (Phylum Myxozoa, Class Myxosporea).

FHM 7 -

Morphological diagnosis

- Branchitis, regionally extensive, mild with an intra-lesional trematode. (Phylum Platyhelmintheses, Class Trematoda)
- Trematodiasis, endomeningial, encysted, (Phylum Platyhelmintheses, Class Trematoda).

FHM 8 -

Morphological diagnosis

- 1. Branchitis, multifocal, mild with an intra-lesional trematode. (Phylum Platyhelmintheses, Class Trematoda)
- Trematodiasis, endomeningial, encysted, multifocal (Phylum Platyhelmintheses, Class Trematoda).

FHM9

Gill – There is regionally extensive accumulations of macrophages, scattered eosinophilic granular cells and increased numbers of rodlet cells filling the space between lamellae. In one section there is a singe protozoan organism within the branchial cavity. The protozoan is not complete but appears to possess a denticular ring and cilia.

Morphological diagnosis

- 1. Trematodiasis, endomeningial, encysted, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- Branchitis, regionally extensive, mild with intra-lesional protozoa (Trichodina: presumptive).
- 3. Hepatitis, multifocal, mild to moderate.
- 4. Granuloma, parasitic, intramuscular, multifocal, small and large.

FHM 10

- 1. Myxosporidiosis, intramuscular, multifocal (Phylum Myxozoa, Class Myxosporea).
- 2. Branchitis, multifocal, mild.

FHM 11 -

Skin – Within a single section of the skin on the ventral surface, there are increased numbers of lymphocytes and macrophages expanding spaces between epidermal cells. Kidney – Within one section of the cranial kidney, there is a single circular accumulation of low numbers of macrophages and lesser numbers of lymphocytes.

Morphological diagnosis

- 1. Trematodiasis, endomeningial, encysted, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- 2. Branchitis, regionally extensive, mild with an intra-lesional protozoa.
- 3. Dermatitis, mild, focal.
- 4. Nephritis, mild focal.

FHM 12 -

Morphological diagnosis

1. Myxosporidiosis, intramuscular, intra-nephric, intravascular, multifocal, moderate numbers (Phylum Myxozoa, Class Myxosporea).

FHM 13 -

Morphological diagnosis

- 1. Trematodiasis, endomeningial, focal (Phylum Platyhelmintheses, Class Trematoda).
- Myxosporidiosis, intramuscular, intravascular, multifocal (Phylum Myxozoa, Class Myxosporea).
- 3. Hepatitis, multifocal, mild to moderate.
- 4. Ceolomitis, focal, mild to moderate.

FHM 14-

Morphological diagnosis

1. Trematodiasis, endomeningial, focal (Phylum Platyhelmintheses, Class Trematoda).

FHM 15 -

Morphological diagnosis

- 1. Branchitis, regionally extensive, moderate.
- 2. Trematodiasis, endomeningial and peritoneal (Phylum Platyhelmintheses, Class Trematoda).

FHM 16 -

Morphological diagnosis

- 1. Trematodiasis, endomeningial, solitary (Phylum Platyhelmintheses, Class Trematoda).
- 2. Myxosporidiosis, intramuscular, intravascular, multifocal (Phylum Myxozoa, Class Myxosporea).

FHM 17 -

GI - Within one section of intestine, increased numbers of enterocytes are undergoing degeneration and necrosis associated increased numbers trafficking lymphocytes and macrophages.

Brain – A single empty, parasitic granuloma is present within the rostral endomeninges of the optic tectum.

Morphological diagnosis

- Myxosporidiosis, intramuscular, intravascular, multifocal (Phylum Myxozoa, Class Myxosporea).
- Enteritis, multifocal with intra-lesional enterocyte degeneration and necrosis / unknown intra-cytoplasmic organism.
- 3. Endomeningitis, parasitic, focal.

FHM 18 -

Morphological diagnosis

- Myxosporidiosis, thymic, intramuscular, multifocal (Phylum Myxozoa, Class Myxosporea).
- Trematodiasis, endomeningial, intramuscular, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- 3. Protozoan, branchial arch, multifocal, low numbers (Presumptive Phylum Myxozoa, Class Myxosporea).

FHM 19

Morphological diagnosis

- Branchitis, multifocal, moderate with scattered intra-lesional necrosis, cellular degeneration and trematodes. (Phylum Platyhelmintheses, Class Trematoda)
- Ceolomitis, multifocal mild to moderate with intra-lesional nematodes (Phylum Nemathelminthes, Class Nematoda).
- Myxosporidiosis, intramuscular, multifocal (Phylum Myxozoa, Class Myxosporea).

FHM 20 -

Morphological diagnosis

- Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 2. Endomeningitis, parasitic, focal

FHM 21-

Morphological diagnosis

- Myxosporidiosis, intramuscular, nephric, branchial, multifocal. (Phylum Myxozoa, Class Myxosporea).
- Nephritis, interstitial, multifocal with intra-lesional immature multinucleate plasmodium and maturing spores.
- Branchitis, multifocal, mild to moderate intra-lesional immature multinucleate plasmodium and maturing spores.

FHM 22 -

Morphological diagnosis

- 1. Branchitis, multifocal to regionally extensive, mild.
- 2. Granuloma, parasitic, encysted, intramuscular, focal.

FHM 23 -

Morphological diagnosis

- 1. Branchitis, multifocal to regionally extensive, mild.
- 2. Myxosporidiosis, intramuscular, focal. (Phylum Myxozoa, Class Myxosporea).
- 3. Endomeningitis, parasitic, multifocal.

FHM 24 -

Morphological diagnosis

- 1. Branchitis, multifocal, mild.
- 2. Myxosporidiosis, intramuscular, focal. (Phylum Myxozoa, Class Myxosporea).

FHM 25 -

Morphological diagnosis

- 1. Branchitis, multifocal to regionally extensive, mild.
- 2. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 3. Granuloma, parasitic, encysted, intramuscular, focal.
- 4. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).

FHM 26 -

Morphological diagnosis

- 1. Branchitis, regionally extensive to diffuse, mild.
- 2. Granuloma, parasitic, encysted, intramuscular, focal.

FHM 27 -

Morphological diagnosis

- 1. Granuloma, parasitic, encysted, intramuscular, intra-pharyngeal, multifocal.
- 2. Myxosporidiosis, intramuscular, focal. (Phylum Myxozoa, Class Myxosporea).
- 3. Trematodiasis, endomeningial, focal (Phylum Platyhelmintheses, Class Trematoda).

FHM 28 -

Morphological diagnosis

- 1. Granuloma, parasitic, encysted, intramuscular, focal.
- 2. Myxosporidiosis, nephric, interstitial, intravascular multifocal. (Phylum Myxozoa, Class Myxosporea).
- 3. Trematodiasis, endomeningial, focal (Phylum Platyhelmintheses, Class Trematoda).

FHM 29 -

- 1. Granuloma, parasitic, encysted, intramuscular, focal.
- Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- 4. Ceolomitis, multifocal, mild to moderate with intra-lesional nematodes (Phylum Nemathelminthes, Class Nematoda).
- 5. Hepatitis, focally extensive, mild to moderate.
- 6. Branchitis, multifocal, mild.

FHM 30 -

Morphological diagnosis

- Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 3. Branchitis, multifocal, mild with intra-branchial trematode. (Phylum Platyhelmintheses, Class Trematoda)

FHM 31

Morphological diagnosis

- Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 2. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).

FHM 32

GI – There are increased numbers of lymphocytes and lesser numbers of macrophages and eosinophilic granular cells within the lamina propria of one section of gut. There is multifocal enterocyte degeneration along one section of gut.

Muscle – Within one section of a hypaxial muscle bundle of the tail, there are moderate to large numbers of macrophages and lesser numbers of eosinophilic granular expanding space between muscle fibers which in turn surround a center of karyorrhectic debris, degenerative muscle fibers and eosinophilic material.

Morphological diagnosis

- 1. Enteritis, regionally extensive, mild with multifocal enterocyte degeneration.
- 2. Ceolomitis, multifocal, mild with pancreatic degeneration.
- 3. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- 4. Branchitis, regionally extensive, moderate with intra-lesional cellular degeneration and solitary intra-branchial trematode.
- 5. Myositis, histiocytic and granulocytic, focal large.
- 6. Myxosporidiosis, nephric, focal. (Phylum Myxozoa, Class Myxosporea).

FHM 33

- 1. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 2. Branchitis, multifocal, mild.

FHM 34

Morphological diagnosis

- 1. Myositis, histiocytic, multifocal.
- 2. Dermatitis, histiocytic, mild.
- 3. Branchitis, multifocal, mild to moderate with intra-lesional myxosporean (Phylum Myxozoa, Class Myxosporea)

FHM 35 - NSF

FHM 36

Morphological diagnosis

- 1. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 2. Ceolomitis, multifocal, mild to moderate with intra-lesional nematodes (Phylum Nemathelminthes, Class Nematoda).

FHM 37

Skin – A single cross section of a metazoan organism (monogenetic trematode, presumptive) is associated with sections of the caudal fin.

Morphological diagnosis

- 1. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- 2. Myositis, histiocytic, focal, mild.
- 3. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 4. Branchitis, regionally extensive to diffuse, mild to moderate.

FHM 38

Morphological diagnosis

- 1. Epicarditis, focal, mild.
- 2. Myxosporidiosis, intramuscular, focal. (Phylum Myxozoa, Class Myxosporea).
- 3. Branchitis, multifocal, mild with intra-lesional myxosporean (Phylum Myxozoa, Class Myxosporea)

FHM 39

- 1. Branchitis, regionally extensive, moderate
- 2. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- 3. Myositis, histiocytic, focal, moderate.
- 4. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).

5. Granuloma, parasitic, focal, large.

FHM 40

Morphological diagnosis

- Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- Ceolomitis, multifocal, mild to moderate with intra-lesional nematodes (Phylum Nemathelminthes, Class Nematoda).
- 3. Hepatitis, multifocal, mild.
- 4. Branchitis, multifocal, mild with intra-lesional trematode. (Phylum Platyhelmintheses, Class Trematoda)

FHM 41

Morphological diagnosis

- 1. Myxosporidiosis, intramuscular, pericardial, intravascular, meningial, perineural, nephric, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 2. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).

FHM 42

Morphological diagnosis

 Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).

FHM 43

Morphological diagnosis

- 1. Trematodiasis, intramuscular, endomeningial,
- 2. Enteritis, lymphocytic, histiocytic, segmental, mild to moderate.

FHM 44

Morphological diagnosis

1. Granuloma, parasitic, focal.

FHM 45

Morphological diagnosis

- 1. Hepatitis, multifocal, mild to moderate.
- 2. Branchitis, multifocal, mild.

FHM 46

Morphological diagnosis

- Myxosporidiosis, intramuscular, nephric, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 2. Granuloma, parasitic, intramuscular, solitary.

FHM 47

1. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).

FHM 48

Morphological diagnosis

- 1. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 2. Endomeningitis, parasitic, multifocal.
- 3. Epitheliocystis, focal.

FHM 49

Morphological diagnosis

- 1. Hepatitis, focal, mild to moderate with intra-lesional nematode (Phylum Nemathelminthes, Class Nematoda).
- 2. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- 3. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 4. Nephritis, granulomatous, interstitial, focal.

FHM 50

Morphological diagnosis

1. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).

FHM 51

Morphological diagnosis

 Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).

FHM 52

Morphological diagnosis

- 1. Enteritis, regionally extensive, moderate with enterocyte degeneration and necrosis.
- 2. Branchitis, multifocal, mild to moderate.
- 3. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).

FHM 53

Morphological diagnosis

- 1. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 2. Granuloma, parasitic, intramuscular, multifocal
- 3. Branchitis, diffuse, mild.

FHM 54

Morphological diagnosis

- 1. Hepatitis, multifocal, mild
- Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).

FHM 55

Morphological diagnosis

- 1. Myxosporidiosis, intramuscular, multifocal. (Phylum Myxozoa, Class Myxosporea).
- 2. Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).

FHM 56

Morphological diagnosis

- Trematodiasis, endomeningial, intramuscular, retrobulbar, multifocal (Phylum Platyhelmintheses, Class Trematoda
- 2. Myxosporidiosis, thymic. (Phylum Myxozoa, Class Myxosporea).

FHM 57

Morphological diagnosis

- Trematodiasis, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda
- 2. Branchitis, multifocal to regionally extensive, mild, with intra-lesional trichodinid protozoan.
- 3. Myxosporidiosis, intramuscular, focal. (Phylum Myxozoa, Class Myxosporea).
- 4. Dermatitis, focal.

FHM 58

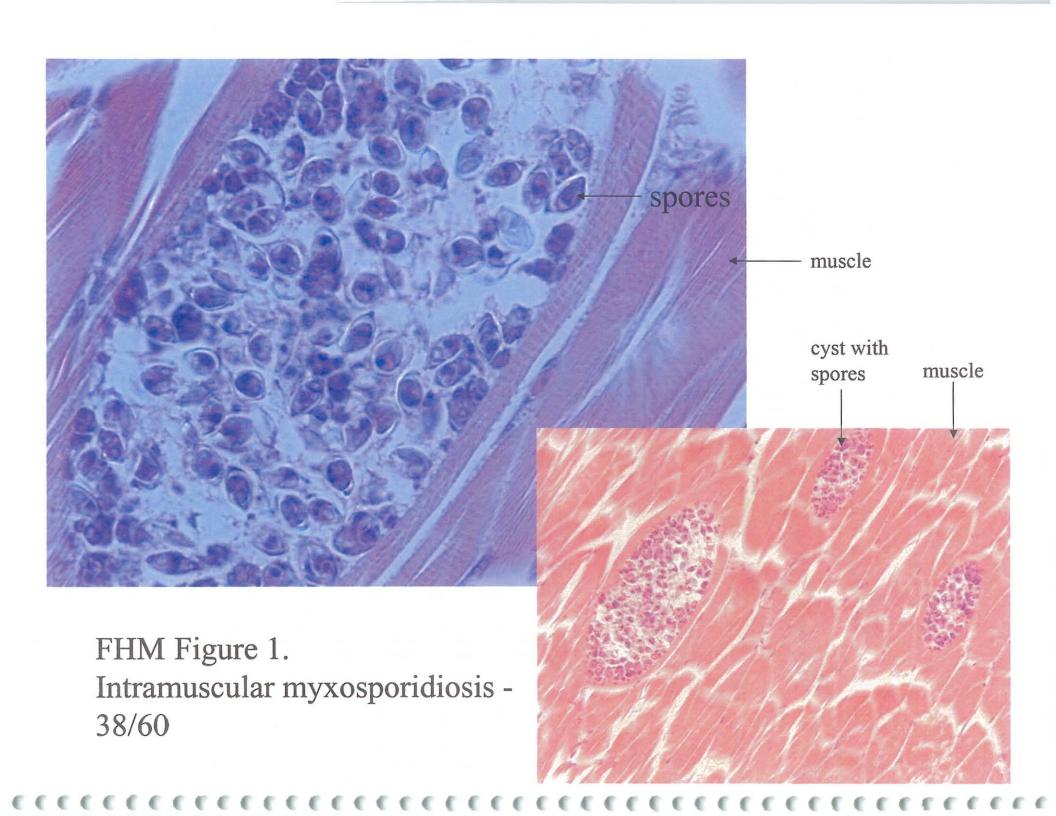
Morphological diagnosis

- 1. Endomeningitis, parasitic, multifocal.
- 2. Granuloma, parasitic, intramuscular, multifocal.
- 3. Myxosporidiosis, intramuscular, focal. (Phylum Myxozoa, Class Myxosporea).

FHM 59 - NSF

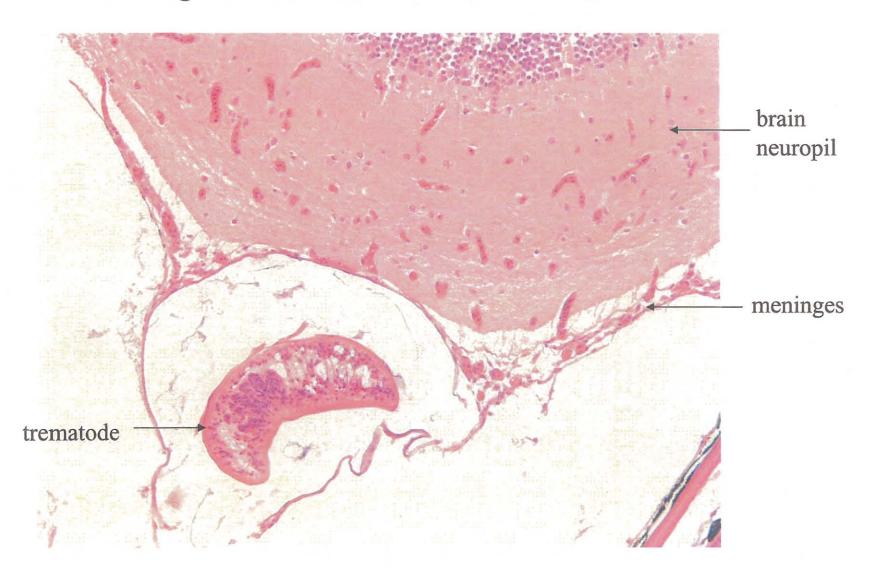
FHM 60

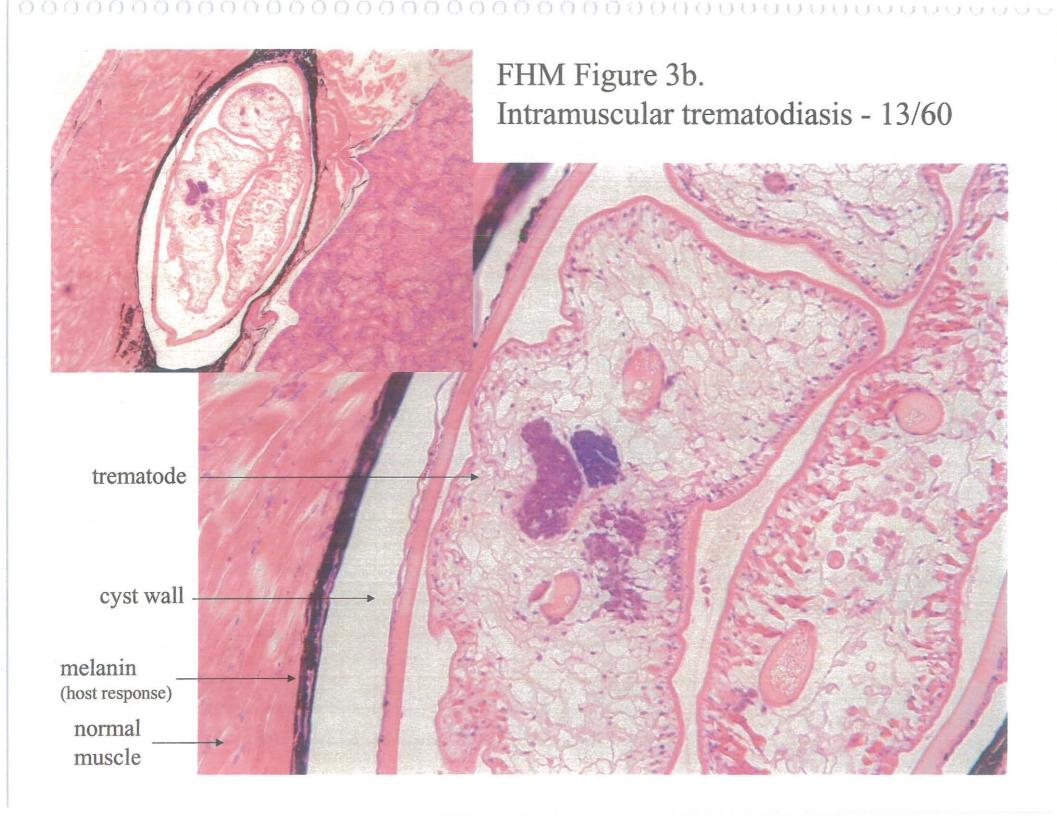
- 1. Trematodiasis, intramuscular, endomeningial, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- 2. Branchitis, multifocal, mild.
- Myositis, multifocal, mild

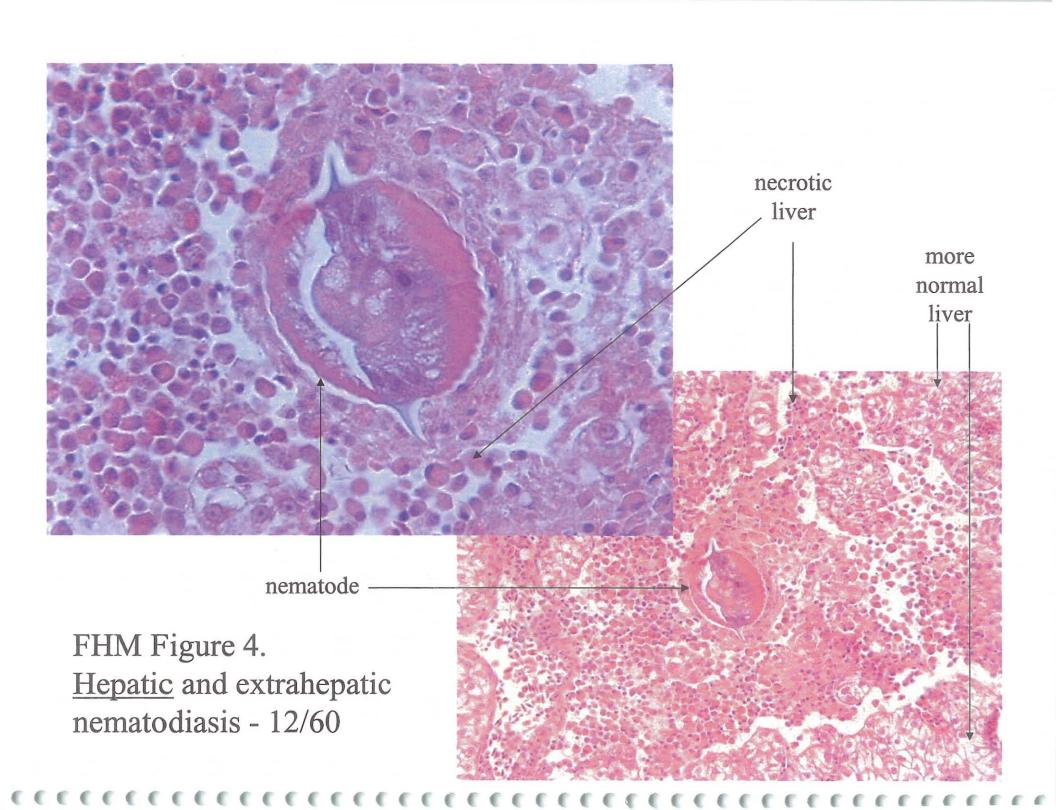


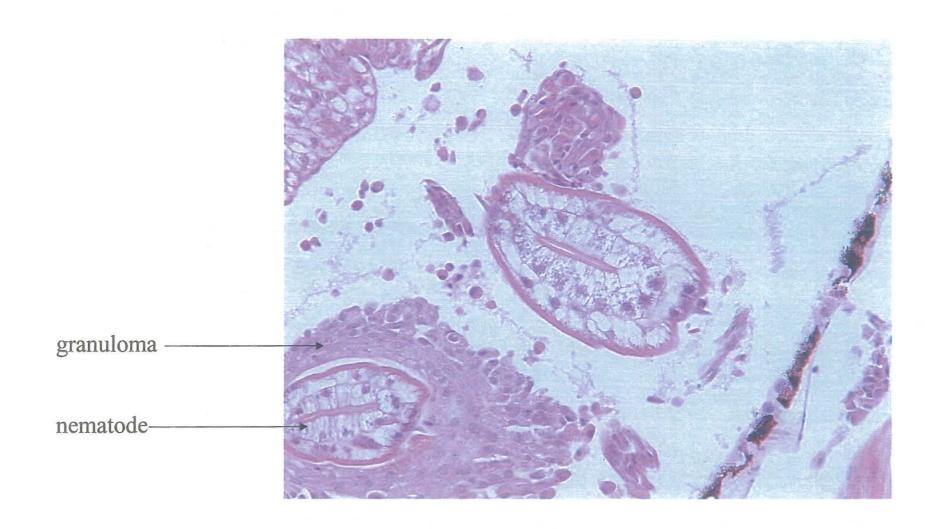
FHM Figure 2. filament Branchial trematodiasis - 6/60 cartilage filament trematode lamella

FHM Figure 3a. Endomeningeal trematodes - 31/60



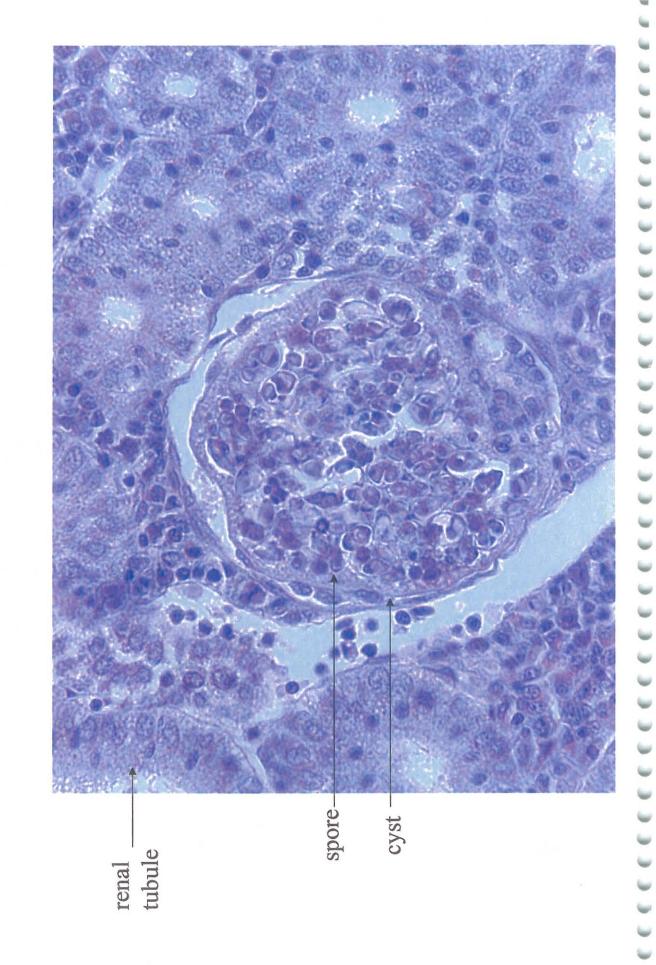


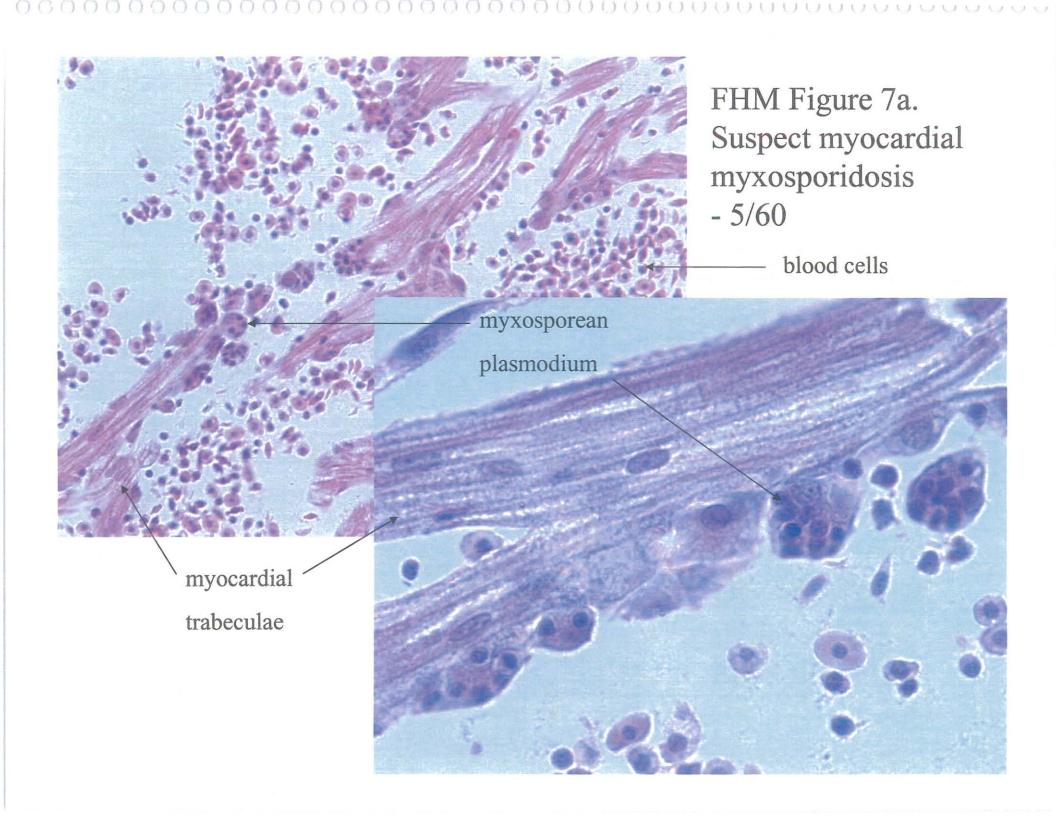


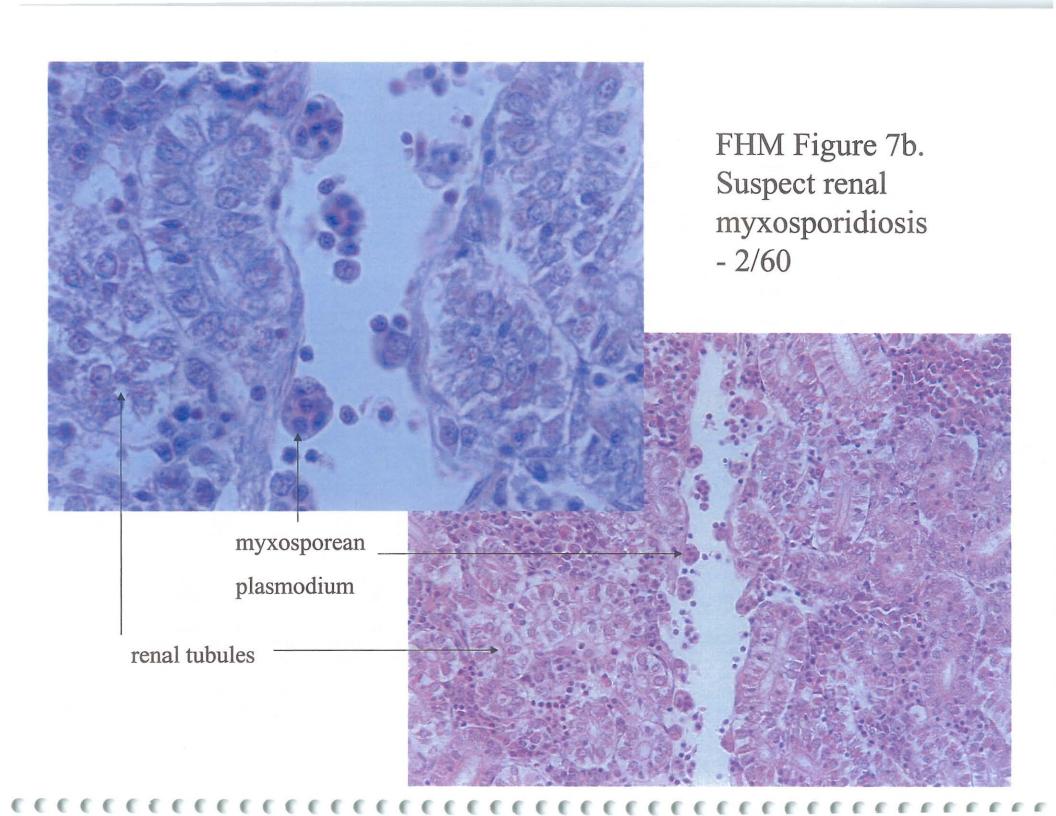


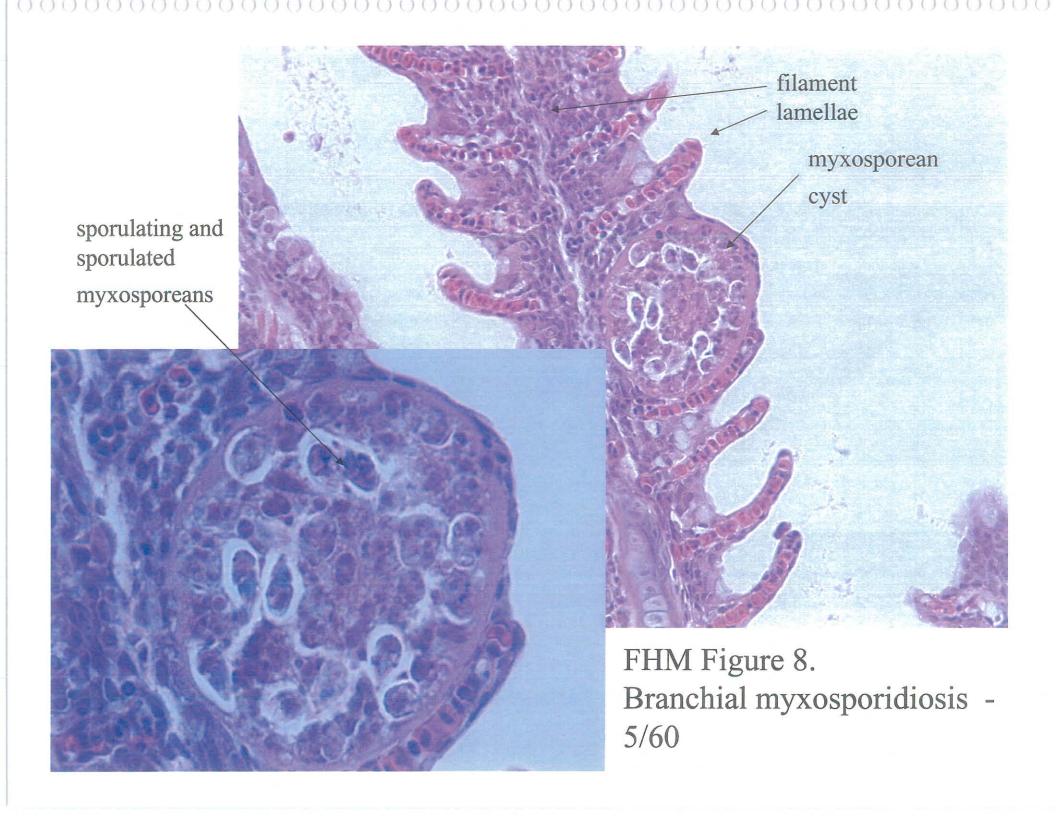
FHM Figure 5. Extrahepatic nematodiasis

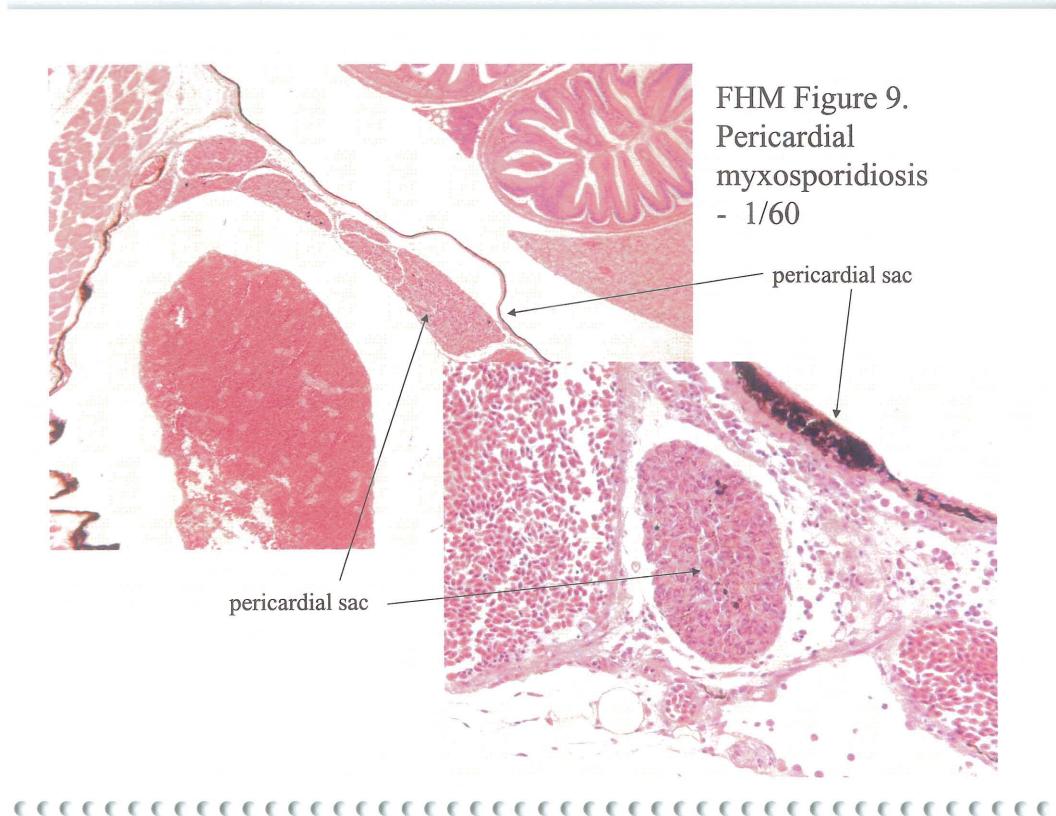
FHM Figure 6. Renal mxyosporidiosis - 5/60



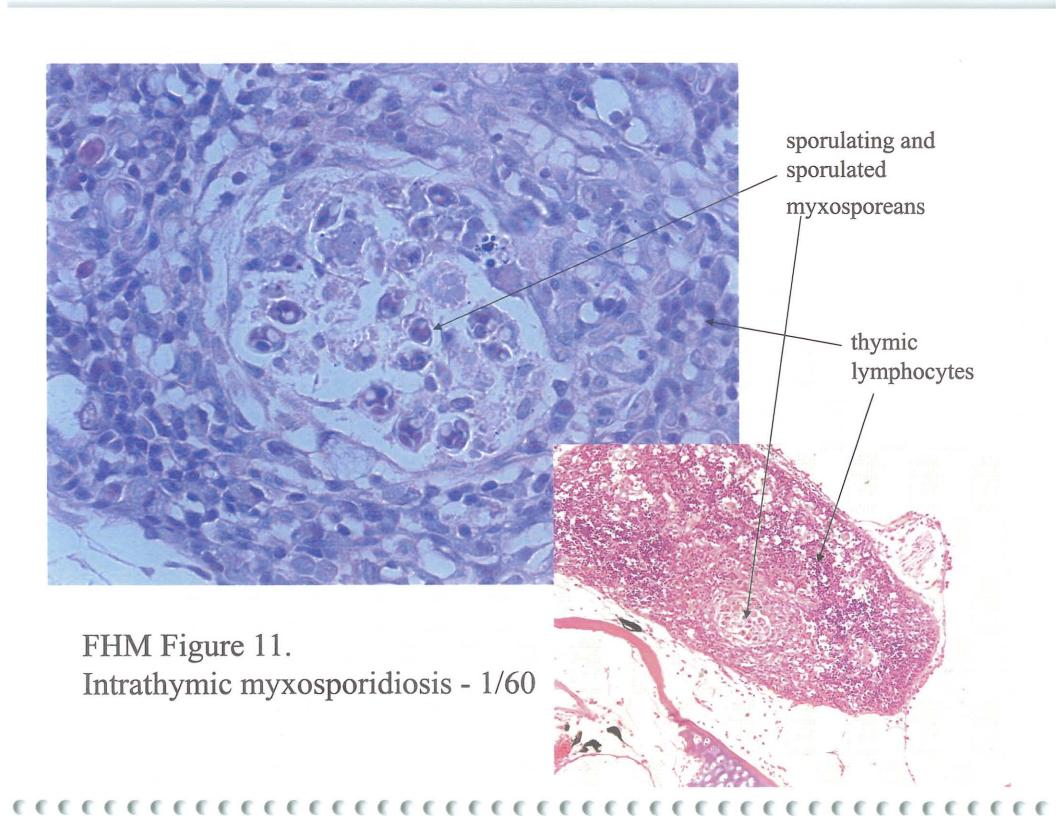








FHM Figure 10. filament Branchial epitheliocystis - 1/60 lamellae bacterial microcolony



Yellow Perch (YP - Perca flavescens)

The sections consists of multiple cross sections whole fish including skin, gill, heart, liver, spleen, pancreas, head kidney, caudal kidney, gastrointestinal tract (stomach and intestines), thymus, swim bladder, brain, spinal cord, eye and ovary/testis. All tissues are not present on each slide.

The notable lesions and/or organisms are described in detail and pictures of these have been provided in most cases. Less notable alterations are occasionally included in the summary for lesions of each individual fish (see later).

The majority of the organisms are identified to the level of class with some exceptions, notably for the emerald shiner. Further work with available materials could narrow this further for the other species. Due to time constraints this work will not be included in the interim report for all species. For example, myxosporeans could be identified to genus in many cases by more detailed light microscopy including a broader range of special stains and electron microscopy of micro/myxosporeans would better identify these agents. Further sectioning of some of the metazoans would likely enable better anatomical detail and allow more precise classification. All measurements are approximations based on comparison with normal anatomic structures.

Branchial Epitheliocystis - Epitheliocystis, multifocal.

Along scattered filaments, there is mild to moderate filling of intra-lamellar spaces by moderate accumulations of hyperplastic epithelial cells and small numbers of lymphocytes and macrophages. Within several sections, the cytoplasm of individual epithelial cells contains large accumulations of a uniform granular basophilic substance (Rickettsia-like organism, presumptive). ---- 4 of 60 affected.

YP Figure 1

Renal myxosporidiosis - Myxosporidiosis, renal.

Within the lumen of both ureters of the caudal kidney, there are low to moderate numbers of multinucleated plasmodium-like protozoan (approximately 15µm to 25µm in diameter) organisms. There are increased numbers of mononuclear cells and lesser numbers of eosinophilic granular cells trafficking between urethral epithelial cells. In some sections there are multiple foci of cellular necrosis and increased numbers of rodlet cells. In still other areas, there are accumulations of lymphocytes and lesser numbers of eosinophilic granular cells expanding spaces between tubules. (Phylum Myxozoa, Class Myxosporea) ---- 6 of 60 affected.

YP Figure 2

Hepatic myxosporidiosis - Myxosporidiosis, hepatic, multifocal.

There are multiple small and large accumulations of pyriform myxosporean spores (approximately $10\mu m$ in length) embedded within hepatic parenchyma and around portal triads. The spores are surrounded by several layers of thin macrophages admixed with lesser numbers of eosinophils. There is mild compression of hepatic parenchyma surrounding these cysts. In some areas the cyst are within and replacing hepatopancreas

tissue surrounding blood vessels. (Phylum Myxozoa, Class Myxosporea) ---- 3 of 60 affected.

YP Figure 3

Intestinal encysted nematodes – Nematodiasis, intestinal wall, encysted. Encysted within longitudinal muscle of the gut, there focal and multifocal cross-section of a metazoan parasite characterized by the presence of a digestive tract, psuedocoelom and cuticle (Phylum Nemathelminthes, Class Nematoda). Variable numbers of thin and plump macrophages surround the metazoan organism. (Phylum Nemathelminthes, Class Nematoda) ---- 2 of 60 affected.

YP Figure 4

Dermal Trichodina - Trichodinosis, dermal.

Along sections of the skin and within the branchial chamber, there are multiple sections of a Trichodinid protozoan organism characterized by aboral denticular ring and a ring of oral cilia at the anterior pole. Little to no host reaction is associated with these protozoa. (Phylum Ciliophora, Class Litostomatea, Family Trichodina) ---- 5 of 60 affected.

YP Figure 5

Cranial Chondritis - Perichondritis, multifocal, mild.

Within sections of the capitus, there is multifocal chondrocyte degeneration associated with small numbers of macrophages and lesser numbers of lymphocytes and granulocytic cells. Increased numbers of macrophages, lymphocytes and lesser numbers of eosinophilic granular cells are trafficking through the connective tissue lining the perichondrium. ---- 1 of 60 affected.

YP Figure 6

Intramuscular granuloma / encysted cestodes — Myositis, granulomatous, multifocal, mild to moderate with occasional sections of an intra-lesional cestode. In several sections, there are large accumulations of plump amphophilic macrophages replacing hypaxial muscle fibers just ventral to the lateral nerve fascicle. Small numbers of macrophages and lymphocytes are trafficking between surrounding myofibers. Several layers of compact macrophages admixed with degenerate myofibers encircle a central core of viable, plump macrophages and karyorrhectic debris. In one section, a cestode characterized by an eosinophilic tegument, a lack of digestive tract and numerous, round to ovoid, basophilic, calcium bodies (calcareous corpuscles) embedded within a loose parenchymatous matrix, is present within the center of the granuloma. ---- 3 of 60 affected.

YP Figure 7

Peritoneal nematodes – Nematodiasis, peritoneal, encysted.

Embedded within the connective tissue underlying the swim bladder, there are multifocal encysted metazoan organisms. The metazoans are characterized by a thin eosinophilic cuticle, psuedocoelom and a muscular tri-radiate pharynx. Several layers of plump amphophilic macrophages and low numbers of eosinophilic granular cells surround both metazoans. (Phylum Nemathelminthes, Class Nematoda) ---- 4 of 60 affected. (no photo)

Intramuscular and peritoneal encysted trematodes – Trematodiasis, intramuscular and peritoneal, encysted.

Encysted by few to several layers of thin attenuated macrophages there are solitary to multifocal sections of a trematode parasite within skeletal myofibers of the body wall and within connective tissue between intestinal organs. The trematodes are characterized by a thin tegument surrounding a layer of sub-tegumental cells and a loose parenchyma. In some sections, scleritinized hooks are present. (Phylum Platyhelmintheses, Class Trematoda) ---- 3 of 60 affected. (no photo)

Hepatic trematodes - Trematodiasis, hepatic, multifocal

There are multiple parasitic granuloma scattered throughout the parenchyma. Within in one granuloma, there are two cross-sections of a metazoan parasite characterized by a thin eosinophilic tegument, lack of body cavity and a parenchymatous body matrix. In one section a muscular oral sucker is present. (Phylum Platyhelmintheses, Class Trematoda) ---- 1 of 60 affected.

YP Figure 8

Spinal column myxosporean cyst - Myxosporidiosis, intra-spinal, solitary. A single, approximately 100µm in diameter, myxosporean cysts containing both immature multinucleate plasmodium and mature spores is encysted within the neuropil of the spinal cord. (Phylum Myxozoa, Class Myxosporea) ---- 1 0f 60 affected.

YP Figure 9

Intestinal cestodes - Cestodes, intra-luminal, intestinal

There are multiple sections of a metazoan organism filling the lumen of the gastrointestinal tract of numerous fish. The metazoan is characterized by an eosinophilic tegument thrown into regularly spaced folds (proglottids), a lack of digestive tract and numerous round to ovoid, basophilic, calcium bodies (calcareous corpuscles) embedded within a loose parenchymatous matrix (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda). No scolex is present within sections. Distal segments of the metazoan contain myriad developing eggs. ---- 17 of 60 affected.

YP Figure 10

Intestinal Apicomplexans - Enteritis, multifocal, mild with intra-lesional accumulations of an intracellular protozoan organism (Phylum Apicomplexa). Along sections of the intestine, multiple developmental stages of an intracellular protozoan parasite is present within epithelial cells lining sections of the gastrointestinal

tract. In most foci there is no associated host reaction however in some sections there are small numbers of trafficking lymphocytes and smaller number of macrophages surrounding the parasite in various stages of development. ---- 1 of 60 affected. (no photo)

Branchial protozoan - Protozoa (*Caprinia* species), branchial, multifocal. There are small numbers of protozoan organisms attached to lamellae of scattered filaments. The shape of the protozoan is variable but more it is often elongate (45μm – 90μm) or saclike with a centrally located nucleus and numerous tentacles projecting from cytoplasm on the opposite side of attachment. In still other sections there is mild multifocal epithelial hyperplasia between lamellae. (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*) ---- 12 of 60 affected.

YP Figure 11

YP 1-

Muscle – There is a single large accumulation of plump amphophilic macrophages replacing hypaxial muscle fibers just ventral to the lateral nerve fascicle. Small numbers of macrophages and lymphocytes are trafficking between surrounding myofibers. Several layers of compact macrophages admixed with degenerate myofibers encircle a central core of viable, plump macrophages and karyorrhectic debris. No organisms are observed.

Morphological diagnosis

- 1. Cestodiasis, enteric, adult (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).
- 2. Myositis, granulomatous, , focal.
- 3. Trichodinosis, dermal, small numbers (Phylum Ciliophora, Class Litostomatea, Family Trichodina).

YP2-

Morphological diagnosis

1. Epitheliocystis, multifocal, mild. (Rickettsia-like organism, presumptive)

YP 3 – No fish/slide

YP 4 - No fish/slide

YP 5 -

Morphological diagnosis

1. Cestodes, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).

YP 6-

Morphological diagnosis

1. Cestodes, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).

 Protozoa (Caprinia species, formerly Trichophyra species), branchial, multifocal, small numbers (Phylum Ciliophora, Subclass Suctoria, Caprinia sp (formerly Trichophyra)

YP 7 -

Morpological diagnosis

- 1. Myositis, granulomatous, multifocal, mild with intra-lesional cestode (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, mild, multifocal

YP 8 -

Morphological diagnosis

- 1. Protozoa (*Caprinia* species), branchial, multifocal, small numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*).
- Branchitis, moderate, multifocal with epithelial hyperplasia and multifocal single cell necrosis.
- 3. Trichodinosis, dermal, low numbers. (Phylum Ciliophora, Class Litostomatea, Family Trichodina)

YP 9 -

Morphological diagnosis

1. Perichondritis, mild, multifocal.

YP 10 - NSF

YP 11 -

Gill – There is regionally extensive filling of inter-lamellar spaces by large numbers of hyperplastic epithelial cells, lymphocytes, macrophages and lesser numbers of eosinophilic granular cells. Within these areas of inflammation there are foci of cellular necrosis and karyorrhectic debris.

Morphological diagnosis

1. Branchitis, regionally extensive, moderate with necrotic cellular debris.

YP 12 -

Morphological diagnosis

1. Branchitis, multifocal, mild.

YP 13 -

Morphological diagnosis

- Cestodes, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).
- 2. Granulomas, multifocal, peritoneal, parasitic.
- 3. Branchitis, multifocal, mild to moderate.

YP 14-

Morphological diagnosis

- 1. Branchitis, regionally extensive, mild to moderate, histiocytic and eosinophilic with multifocal epithelial hyperplasia and single cell necrosis.
- 2. Myxosporidiosis, hepatic, multifocal, large numbers (Phylum Myxozoa, Class Myxosporea)

YP 15 -

Morphological diagnosis

- 1. Branchitis, multifocal, mononuclear and granulocytic, moderate numbers.
- 2. Protozoa (*Caprinia* species), branchial, multifocal, small numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*).
- 3. Cestodes, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).
- 4. Nematodiasis, intestinal wall, encysted (Phylum Nemathelminthes, Class Nematoda).
- 5. Myxosporidiosis, renal, small numbers (Phylum Myxozoa, Class Myxosporea).
- 6. Enteritis, multifocal, mild with intra-lesional accumulations of an intracellular protozoan organism (Phylum Apicomplexa).
- 7. Epicarditis, lymphocytic, focal, mild.

YP 16 -

Morphological diagnosis

- 1. Epitheliocystis, focal. (Rickettsia-like organism, presumptive)
- 2. Protozoa (*Caprinia* species), branchial, multifocal, small numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*).
- 3. Cestodes, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).

YP 17 -

Spleen – Multiple cells undergoing necrosis within several splenic ellipsoids characterized by karyorrhectic debris and increased numbers of leukocytes trafficking through affected ellipsoids.

Morphological diagnosis

- 1. Enteritis, multifocal, mild with intra-lesional enterocyte degeneration.
- 2. Splenitis, multifocal, mild.
- 3. Trichodinosis, branchial, solitary (Phylum Ciliophora, Class Litostomatea, Family Trichodina).

YP 18 -

Morphological diagnosis

1. Branchitis, lymphocytic and granulocytic multifocal, moderate, with cellular necrosis and degeneration.

YP 19

Morphological diagnosis

1. Cestodes, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).

2. Protozoa (*Caprinia* species), branchial, multifocal, small numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*).

YP 20 -

Morphological diagnosis

 Myxosporidiosis, multifocal, hepatic, large numbers - (Phylum Myxozoa, Class Myxosporea)

YP 21 -

Morphological diagnosis

- 1. Myxosporidiosis, renal, small numbers (Phylum Myxozoa, Class Myxosporea).
- Cestodes, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).

YP 22 -

Morphological diagnosis

- 1. Branchitis, multifocal, mild.
- 2. Cestodes, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).

YP 23 -

Morphological diagnosis

1. Protozoa (*Caprinia* species), branchial, multifocal, small numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*)

YP 24 -

Morphological diagnosis

- 1. Branchitis, multifocal, inter-lamellar, mild with scattered lamellar synechae.
- Cestodes, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).

YP 25 -

Morphological diagnosis

1. Protozoa (*Caprinia* species), branchial, multifocal, small numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*)

YP 26 -

Morphological diagnosis

- 1. Branchitis, multifocal, mild.
- 2. Protozoa (*Caprinia* species), branchial, multifocal, small numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*)

YP 27 -

Morphological diagnosis

1. Cestodes, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).

YP 28 -

Morphological diagnosis

- 1. Branchitis, multifocal, mild to moderate.
- 2. Protozoa (*Caprinia* species), branchial, multifocal, small numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*).
- 3. Myxosporidiosis, renal, small numbers (Phylum Myxozoa, Class Myxosporea).

YP 29 -

Morphological diagnosis

1. Branchitis, multifocal, mild.

YP 30 -

Morphological diagnosis

- 1. Cestodes, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).
- 2. Nematodiasis, intestinal wall, encysted (Phylum Nemathelminthes, Class Nematoda).

YP 31

Muscle – There are multifocal accumulations of leukocytes between muscle fibers of the caudal trunk. In some areas, there is myocyte degeneration.

Morphological diagnosis

- 1. Epitheliocystis, multifocal. (Rickettsia-like organism, presumptive)
- 2. Myositis, multifocal, mild with occasional myocyte degeneration.

YP 32

Morphological diagnosis

- Cestodes, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).
- 2. Protozoa (*Caprinia* species), branchial, multifocal, small numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*).
- 3. Nematodiasis, peritoneal, encysted (Phylum Nemathelminthes, Class Nematoda).

YP 33

Morphological diagnosis

- 1. Trematodiasis, hepatic, multifocal (Phylum Platyhelmintheses, Class Trematoda).
- 2. Myxosporidiosis, renal, moderate numbers (Phylum Myxozoa, Class Myxosporea).
- 3. Branchitis, multifocal mild.
- 4. Protozoa (*Caprinia* species), branchial, multifocal, small numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*).

YP 34

Morphological diagnosis

1. Protozoa (*Caprinia* species), branchial, multifocal, small numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*).

YP 35

Morphological diagnosis

- 1. Trematodiasis, intramuscular, solitary. (Phylum Platyhelmintheses, Class Trematoda)
- 2. Protozoa (*Caprinia* species), branchial, multifocal, small numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*).

YP 36

Morphological diagnosis

- 1. Protozoa (*Caprinia* species), branchial, multifocal, small numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*)
- 2. Myxosporidiosis, renal, moderate numbers (Phylum Myxozoa, Class Myxosporea).

YP 37

Morphological diagnosis

1. Protozoa (*Caprinia* species), branchial, multifocal, small numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*).

YP 38

Morphological diagnosis

- 1. Myxosporidiosis, intra-spinal, solitary (Phylum Myxozoa, Class Myxosporea).
- 2. Branchitis, multifocal, mild.

YP 39

Morphological diagnosis

- Cestodes, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).
- Nematodiasis, intestinal wall, encysted (Phylum Nemathelminthes, Class Nematoda).
- 3. Myxosporidiosis, renal, moderate numbers (Phylum Myxozoa, Class Myxosporea).
- 4. Branchitis, multifocal, mild.

YP 40

Morphological diagnosis

- 1. Branchitis, multifocal mild with occasional intra-lesional foci of necrosis
- 2. Trichodinosis, dermal, small numbers (Phylum Ciliophora, Class Litostomatea, Family Trichodina).

YP 41

Morphological diagnosis

1. Protozoa (*Caprinia* species), branchial, multifocal, moderate numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*).

YP 42

Morphological diagnosis

 Nematodiasis, peritoneal, encysted, multifocal. (Phylum Nemathelminthes, Class Nematoda)

YP 43

Morphological diagnosis

- 1. Protozoa (*Caprinia* species), branchial, multifocal, moderate numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*).
- 2. Branchitis,, multifocal mild with occasional intra-lesional foci of necrosis.
- 3. Epicarditis, lymphocytic, focal, mild.

Slide 44

Morphological diagnosis

- 1. Nematodiasis, peritoneal, encysted, multifocal. (Phylum Nemathelminthes, Class Nematoda)
- 2. Splenitis, histiocytic, multifocal, mild.

YP 45

Morphological diagnosis

- 1. Trichodina, solitary, oral cavity
- 2. Protozoa (*Caprinia* species), branchial, multifocal, small numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*).
- 3. Cestodes, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).

YP 46

Morphological diagnosis

- 1. Cestodes, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).
- 2. Protozoa (*Caprinia* species), branchial, multifocal, small numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*).

YP 47

Morphological diagnosis

1. Branchitis, multifocal with moderate to marked inter-lamellar filling and intralesional foci of necrosis.

YP 48

Skin – Two regionally extensive sections of skin are covered by a variably thick acelluar, glycoproteinaceous mucous matrix. In thicker sections of this mucous matrix there are moderate numbers of small developing, multi-cellular, metazoan, organisms (embryos

presumptive). Underlying the mucus coat, there is multifocal epidermal loss (erosion) and cellular degeneration and necrosis.

Morphological diagnosis

1. Dermatitis, regionally extensive, erosive, mild with an overlying variably thick mucous layer containing numerous developing metazoan organisms (embryos)

YP 49

Morphological diagnosis

1. Cestodes, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).

YP 50 - NSF

YP 51

Morphological diagnosis

- 1. Protozoa (*Caprinia* species), branchial, multifocal, small numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*).
- 2. Branchitis, multifocal, mild.
- 3. Nematodiasis, peritoneal, encysted, multifocal. (Phylum Nemathelminthes, Class Nematoda)

YP 52

Morphological diagnosis

- Myxosporidiosis, renal, moderate numbers (Phylum Myxozoa, Class Myxosporea).
- 2. Protozoa (*Caprinia* species), branchial, multifocal, small numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*).
- 3. Trichodinosis, small numbers (Phylum Ciliophora, Class Litostomatea, Family Trichodina).
- 4. Branchitis, multifocal, mild.
- Cestodes, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).

YP 53

Morphological diagnosis

- 1. Protozoa (*Caprinia* species), branchial, multifocal, small numbers (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*).
- 2. Branchitis, multifocal, mild.

YP 54

Morphological diagnosis

- 1. Branchitis, multifocal, mild.
- 2. Nematodiasis, peritoneal, encysted, multifocal. (Phylum Nemathelminthes, Class Nematoda)

YP 55

Morphological diagnosis

- 1. Nematodiasis, peritoneal, encysted, solitary. (Phylum Nemathelminthes, Class Nematoda)
- 2. Epitheliocystis, multifocal. (Rickettsia-like organism, presumptive)
- 3. Branchitis, multifocal, mild.
- 4. Myxosporidiosis, hepatic, multifocal. (Phylum Myxozoa, Class Myxosporea)
- 5. Myxosporidiosis, renal, multifocal.

YP 56

Morphological diagnosis

- 1. Branchitis, regionally extensive, mild.
- 2. Myxosporidiosis, renal, multifocal. (Phylum Myxozoa, Class Myxosporea)
- 3. Myositis, histiocytic, focal with intra-lesional myocyte degeneration.
- 4. Granuloma, parasitic, enteric, encysted.
- 5. Trematodiasis, intra-muscular, encysted. (Phylum Platyhelmintheses, Class Trematoda)

YP 57

Morphological diagnosis

- 1. Myxosporidiosis, renal, multifocal. (Phylum Myxozoa, Class Myxosporea)
- 2. Trematodiasis, intramuscular, encysted. (Phylum Platyhelmintheses, Class Trematoda)
- 3. Trichodinosis, branchial, low numbers. (Phylum Ciliophora, Class Litostomatea, Family Trichodina)

YP 58

Morphological diagnosis

- 1. Branchitis, multifocal, mild.
- 2. Trematodiasis, peritoneal, encysted. (Phylum Platyhelmintheses, Class Trematoda)
- 3. Cestodiasis, enteric (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).
- 4. Myositis, histiocytic, multifocal, mild with intra-lesional myocyte degeneration.
- 5. Splenitis, histiocytic, multifocal, mild with intra-lesional foci of cellular degeneration and necrosis.

YP 59

Morphological diagnosis.

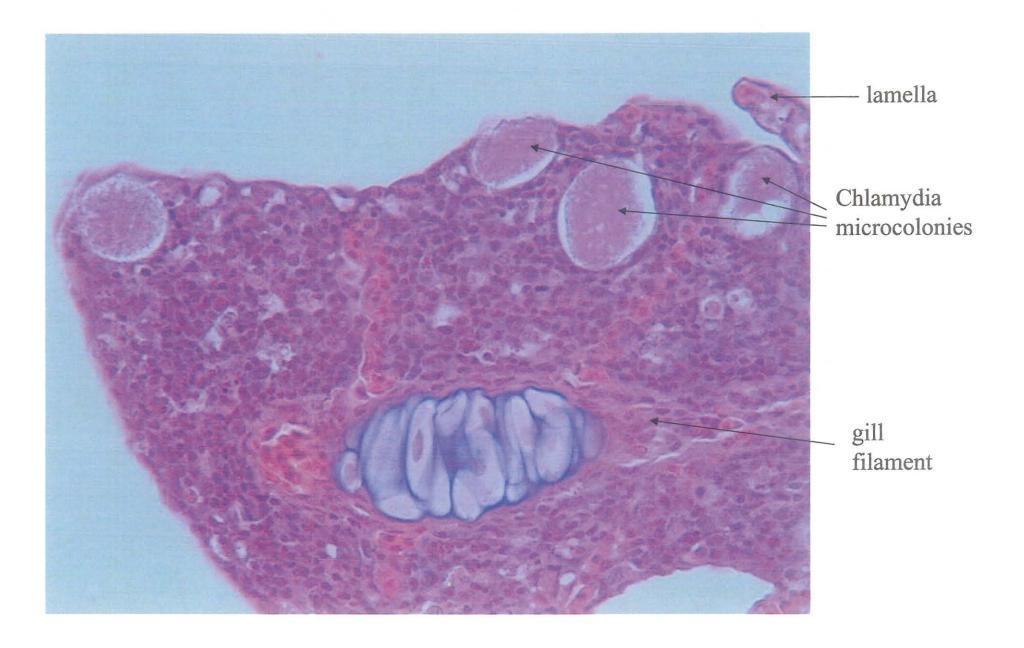
1. Cestodes, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).

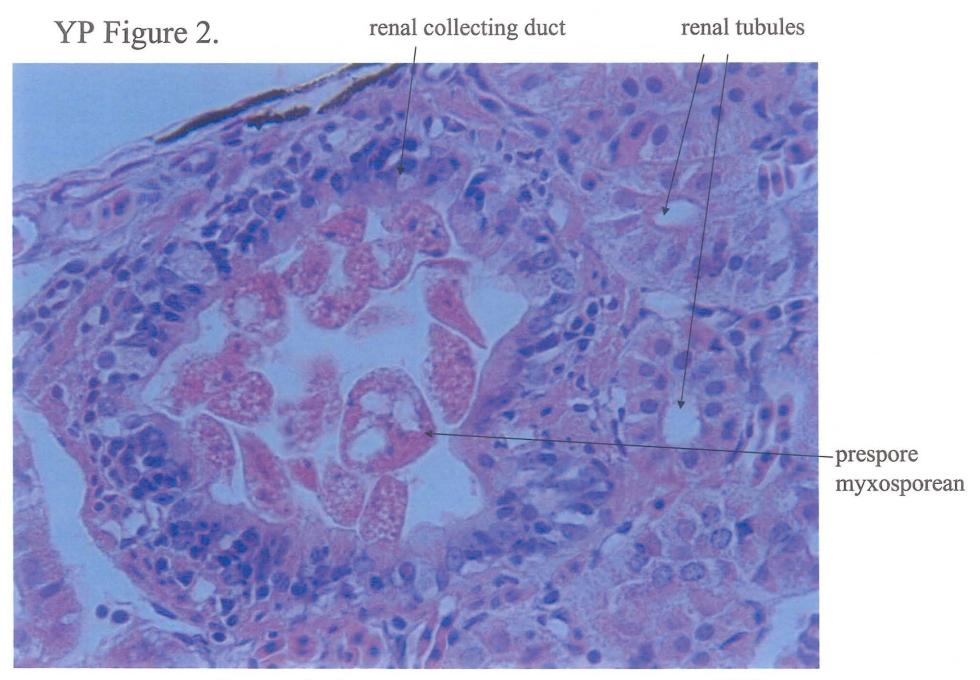
YP 60

Morphological diagnosis.

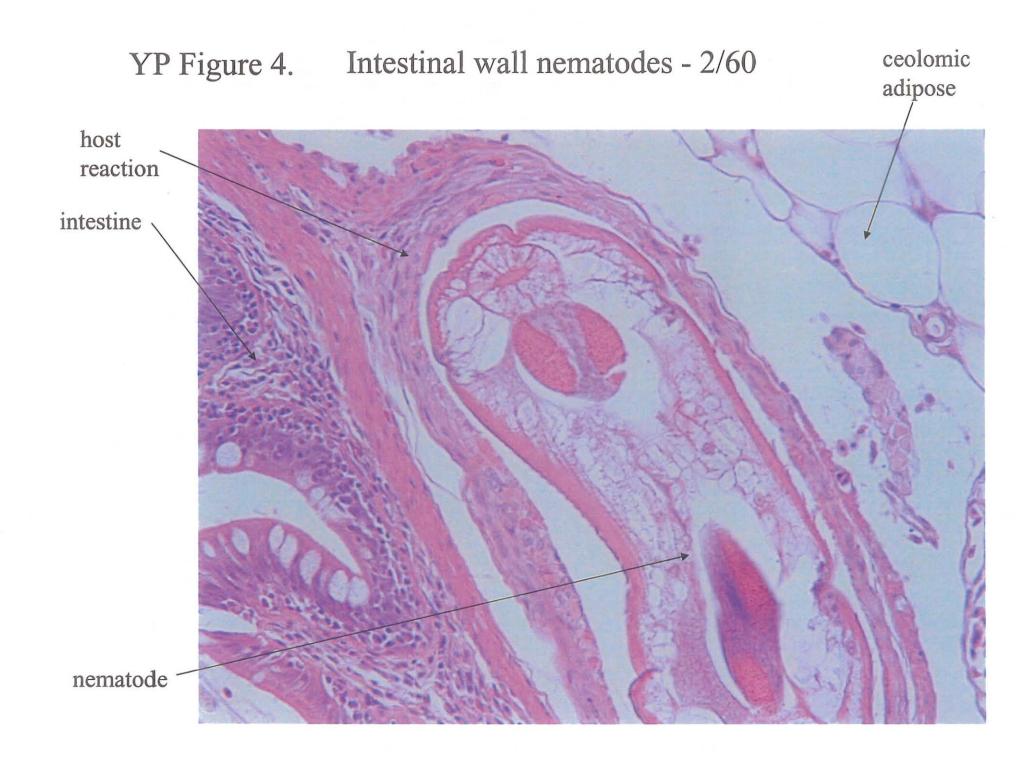
 Cestodes, intra-luminal, intestinal (Phylum Platyhelminthes, Class Cestoidea, Subclass Eucestoda).

YP Figure 1. Branchial chlamydia - 4/60





Intratubular prespore myxosporeans - 6/60

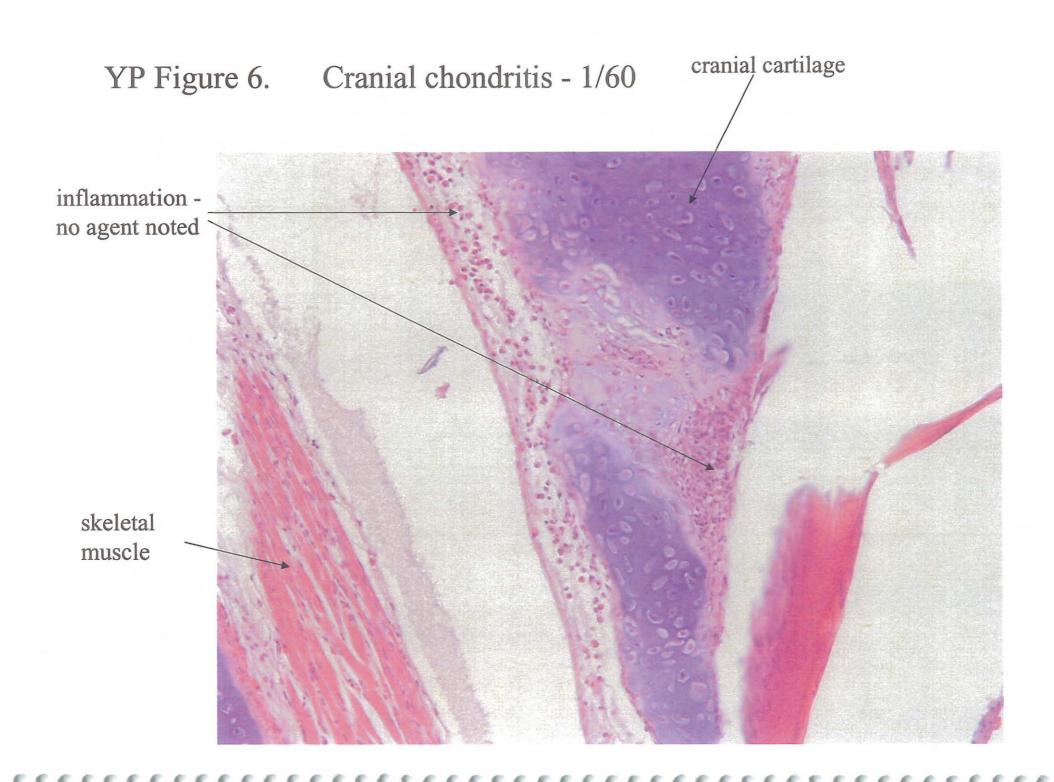


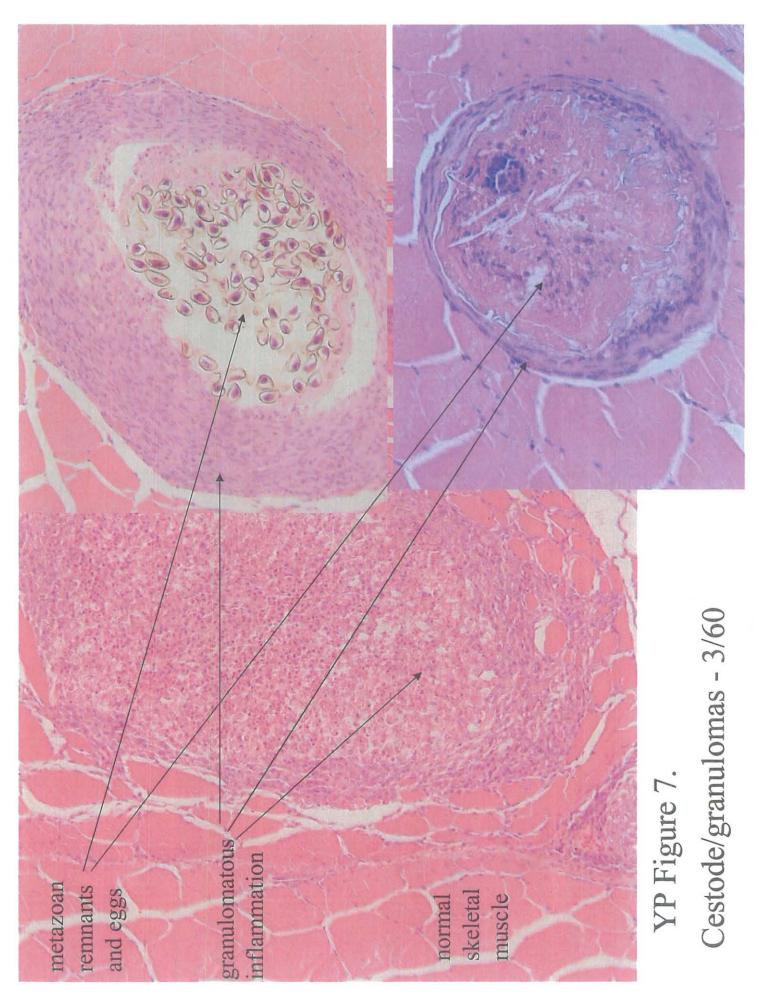
YP Figure 5.

Dermal trichodina - 5/60

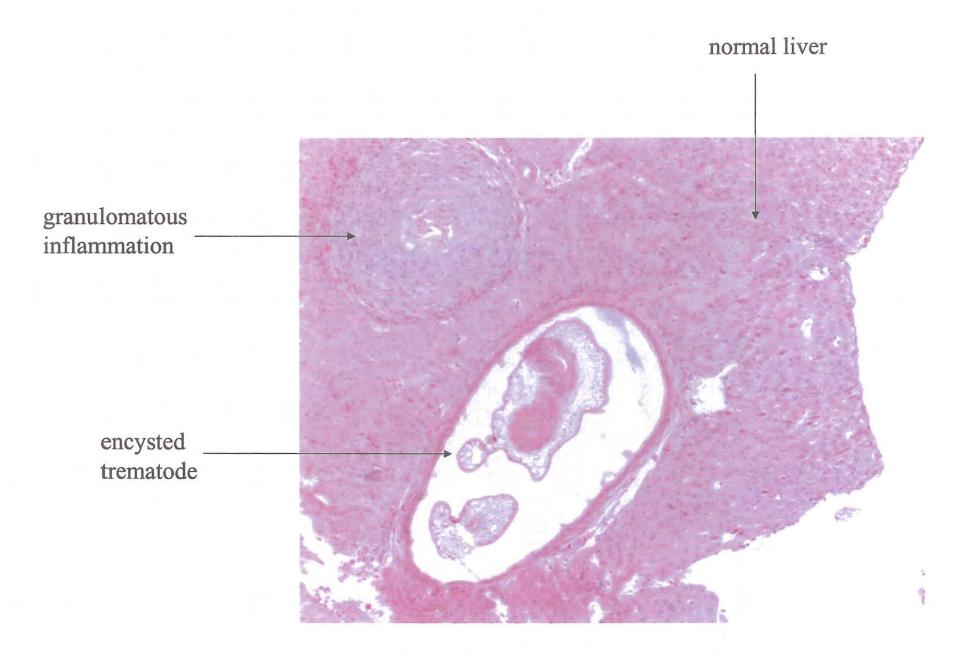


Trichodina sp.





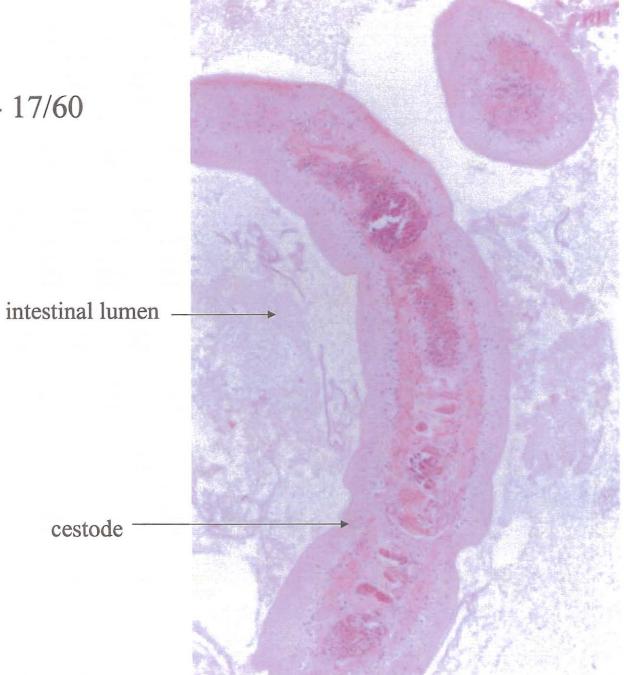
YP Figure 8. Hepatic trematodes - 1/60



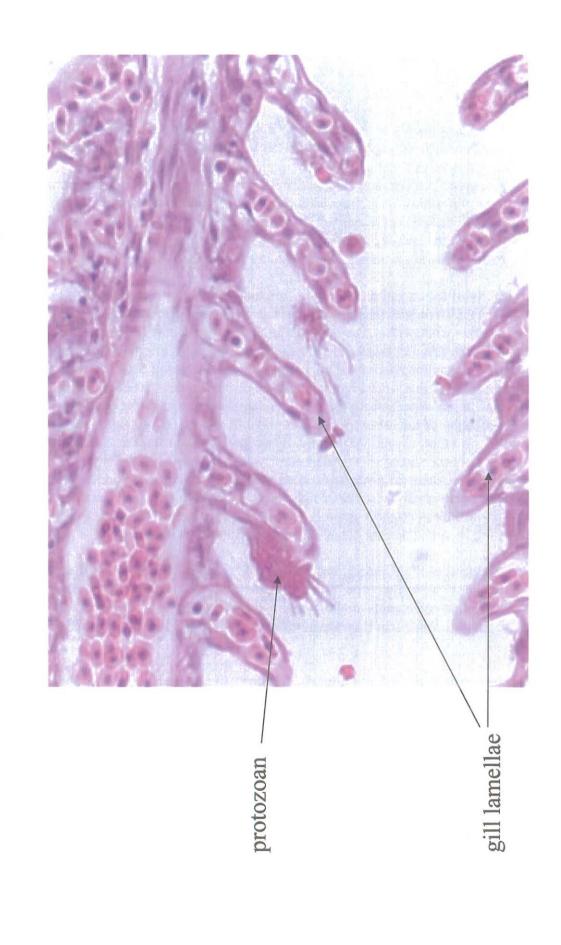
YP Figure 9.

Spinal cord myxosporean cyst - 1/60 spinal cord myxosporean cyst skeletal muscle YP Figure 10.

Intestinal cestodes - 17/60



YP Figure 11. Branchial protozoans - 12/60



Goldeye (Hiodon allosoides)

Fish were predominantly oriented as transverse sections through the musculature and body cavity, with longitudinal sections through the head and as individual tissues for ceolomic organs. Tissues routinely examined included skeletal muscle, skin, spinal cord, brain, kidney, liver, intestine, stomach, exocrine pancreas, heart, gills, swim bladder, and spleen. Spleen was the organ most often missing from the sections examined. Tissues less commonly or only occasionally in section included the endocrine pancreas, thyroid, inter-renal and chromafin tissues and corpuscles of Stannius, as well as the pseudobranch and thymus.

The notable lesions and/or organisms are described in detail and pictures of these have been provided in most cases. Less notable alterations are occasionally included in the summary for lesions of each individual fish (see later).

The majority of the organisms are identified to the level of class with some exceptions, notably for the emerald shiner. Further work with available materials could narrow this further for the other species. Due to time constraints this work will not be included in the interim report for all species. For example, myxosporeans could be identified to genus in many cases by more detailed light microscopy including a broader range of special stains and electron microscopy of micro/myxosporeans would better identify these agents. Further sectioning of some of the metazoans would likely enable better anatomical detail and allow more precise classification. All measurements are approximations based on comparison with normal anatomic structures. Relative to many of the other species examined in this survey goldeye has relatively few notable lesions. A

common lesion that was not systematically enumerated was a mild to moderate lymphohistic tholangiohepatitis/pancreatitis (inflammatory cells primarily oriented around either bile ducts or exocrine pancreas). There were no organisms noted associated with this lesion. This is a common finding in wild and also occurs occasionally in cultured fish and is often of little significance. Fish with 'NAF' may have had this lesion and/or the eosinophilic granular cell rich branchitis but since these were not systematically rated for severity and recorded are not listed with the individual fish below, unless the lesion was exceptional for some reason.

An unusual feature of this species of fish was the numerous rodlet cells that were found in many tissues. Unusually, these were also present in moderate numbers scattered in the neuropil of the brain. The general consensus is that rodlet cells are host cells of unknown function. Some have postulated that they are parasitic (largely discounted) or that they are involved in the inflammatory response (likely in some cases) (See G Figure 1).

Capsulitis/ceolomitis, splenic/hepatic, proliferative — The majority of fish (some of those not included in the tally likely have very mild lesions) had a focally extensive to diffuse proliferative capsulitis that was most apparent on the surface of the spleen but also to a lesser degree and in fewer fish on the capsular surface of the liver. Even less commonly a similar reaction was notable in and around other ceolomic organs and in ceolomic fat. The predominant alteration is the presence of mesothelial proliferative tags that also sometimes contain substantial fibroplasia, few macrophages and other inflammatory cells and notably, varied numbers of rodlet cells. The adjacent subcapsular splenic parenchyma often protruded and was densely filled with red blood cells (resembled an aneurysm). There were no organisms visible that were associated with these lesions. They likely represent a chronic reaction to ceolomic metazoans or other agent. —— 21 of 60 affected.

G Figure 2.

Intestinal cestodes – Cestode, luminal intestinal. These have calcareous corpuscles, are \sim 150-200 μ m wide, and with regular folding (proglottids) sometimes apparent, and have a loose parenchymatous matrix and

eosinophilic tegument. No gastrointestinal structures are visible. (Phylum Platyhelminthes, Class Cestoidea) ---- 26 of 60 affected.

G Figure 3.

Intestinal trematodes – Trematode, luminal intestinal. There are typically few visible in any one section. They are ~150-250µm wide, have an oral sucker and a thin tegument underlined by basophilic subtegumental cells with a sparse parenchymatous matrix with paired cecae, reproductive tissue and eggs. (Phylum Platyhelmintheses, Class Trematoda) ---- 5 of 60 affected.

G Figure 4.

Gastric nematodes - Nematodes, few to moderate, gastric, luminal.

There were few to moderate numbers of thin ~70-90um nematodes with a chitinous exoskeleton, thick muscular esophagus/intestine and reproductive tissue. No host reaction was associated with the presence of these nematodes. (Phylum Nemathelminthes, Class Nematoda) ---- 3 of 60 affected.

G Figure 5. G17/53B

Branchitis – This is common to majority of fish. The interlamellar and to a lesser extent, lamellar epithelium has varied but often moderate numbers of eosinophilic granular cells present. Mild multifocal epithelial hyperplasia may also be present. In the vast majority of cases there are no organisms present on the gills of these fish. (no photo).

Trichodina sp., few, lamellar.

There were very small numbers of $\sim 20 \mu m$ protozoans that were dorsoventrally flattened, had cilia and a denticular ring. The tissue reaction (branchitis described above) noted for these fish did not seem to be associated with the presence of these organisms. (Phylum Ciliophora, Class Litostomatea, Family Trichodina) (no photo) ---- 2 of 60 affected.

Myo/epicarditis, mild, focal, lymphohistiocytic.

Few fish had focal accumulations of small to moderate numbers of lymphocytes with fewer macrophages, either within the myocardium (less common) or within and expanding the epicardium. No organisms were associated with these lesions. Small, histiocyte rich foci were also present occasionally in the pericardial fat. ---- 7 of 60 affected (no photo).

G 1-

Morphological diagnosis

- 1. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Trematode, luminal intestinal, few (Phylum Platyhelmintheses, Class Trematoda)

G 2-

Morphological diagnosis

1. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

G 3-

Morphological diagnosis

1. Cestode, luminal intestinal, numerous (Phylum Platyhelminthes, Class Cestoidea)

G4-NAF

G 5-

There is a small focus of macrophages within ceolomic connective tissue. There are no organisms present. Morphological diagnosis

- 1. Trichodina sp., few, lamellar (Phylum Ciliophora, Class Litostomatea, Family Trichodina)
- 2. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 3. Ceolomitis, focal, histiocytic.

G6-NAF

G 7-

Gills – There is a moderate branchitis with large numbers of eosinophilic granular cells. There is a single section of what might be a monogenetic trematode (no photo and not seen in any other fish). Morphological diagnosis

1. Branchitis, eosinophilic granular cell rich, with lamellar monogenetic trematode

G8-NAF

G 9-

Morphological diagnosis

- 1. Cestode, luminal intestinal, numerous (Phylum Platyhelminthes, Class Cestoidea)
- 2. Capsulitis/ceolomitis, splenic/hepatic, mild to moderate, proliferative

G 10 - NAF

G 11 - NAF

G 12 - NAF

G 13-

Kidney - There is a mild degree of thickening of glomerular basement membranes.

Morphological diagnosis

- 1. Cestode, luminal intestinal, numerous with intestinal dilation (Phylum Platyhelminthes, Class Cestoidea)
- 2. Capsulitis/ceolomitis, splenic/hepatic, mild to moderate, proliferative
- 3. Glomeruli, diffuse, basement membrane thickening.

G 14-

Morphological diagnosis

- 1. Trematode, luminal intestinal, few (Phylum Platyhelmintheses, Class Trematoda)
- 2. Capsulitis/ceolomitis, splenic/hepatic, mild to moderate, proliferative

G 15 - NAF

G 16-

Morphological diagnosis

1. Capsulitis/ceolomitis, splenic/hepatic, mild to moderate, proliferative

G 17-

Morphological diagnosis

1. Nematodes, few to moderate, gastric, luminal (Phylum Nemathelminthes, Class Nematoda)

G 18-

Liver – There is a single phenotypically distinct \sim 150 μ m, basophilic focus.

Morphological diagnosis

- 1. Capsulitis/ceolomitis, splenic/hepatic, proliferative
- 2. Hepatic altered focus

G 19-

Morphological diagnosis

1. Myo/epicarditis, mild, focal, lymphohistiocytic

G 20-

Morphological diagnosis

- 1. Cestode, luminal intestinal, moderate (Phylum Platyhelminthes, Class Cestoidea)
- 2. Myo/epicarditis, mild, focal, lymphohistiocytic

G 21-

Morphological diagnosis

1. Cestode, luminal intestinal, numerous (Phylum Platyhelminthes, Class Cestoidea)

G 22-

Morphological diagnosis

1. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

G 23-

Morphological diagnosis

1. Capsulitis/ceolomitis, splenic/hepatic, mild, proliferative

G 24-

Morphological diagnosis

1. Capsulitis/ceolomitis, splenic/hepatic, mild, proliferative

G 25-

Morphological diagnosis

- 1. Capsulitis/ceolomitis, splenic/hepatic, mild, proliferative
- 2. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

G 26 - NAF

G 27-

Morphological diagnosis

1. Trematode, luminal intestinal, moderate (Phylum Platyhelmintheses, Class Trematoda)

G 28 - NAF

G 29-

Morphological diagnosis

1. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

G 30-

Morphological diagnosis

- 1. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Capsulitis/ceolomitis, splenic/hepatic, moderate, proliferative

G 31-

Morphological diagnosis

1. Myo/epicarditis, mild, focal, lymphohistiocytic

G 32-

Morphological diagnosis

1. Cestode, luminal intestinal, numerous (Phylum Platyhelminthes, Class Cestoidea)

G 33-

Morphological diagnosis

- 1. Trematode, luminal intestinal, moderate (Phylum Platyhelmintheses, Class Trematoda)
- 2. Capsulitis/ceolomitis, splenic/hepatic, moderate, proliferative

G 34-

Morphological diagnosis

- 1. Capsulitis/ceolomitis, splenic/hepatic, mild, proliferative
- 2. Cestode, luminal intestinal, few to moderate (Phylum Platyhelminthes, Class Cestoidea)

G 35-

Morphological diagnosis

- 1. Capsulitis/ceolomitis, splenic/hepatic, mild, proliferative
- 2. Myo/epicarditis, mild, focal, lymphohistiocytic

G 36-

Liver – There is a single phenotypically distinct $\sim 150 \mu m$, roughly round, eosinophilic focus (liver on G37 – mislabeled).

- 1. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Capsulitis/ceolomitis, splenic/hepatic, mild to moderate, proliferative
- 3. Hepatic altered focus

G 37 - NAF

G 38 – NAF

G 39-

Morphological diagnosis

- 1. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Capsulitis/ceolomitis, splenic/hepatic, mild, proliferative

G 40-

Morphological diagnosis

- 1. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Capsulitis/ceolomitis, splenic/hepatic, mild, proliferative

G 41-

Morphological diagnosis

- 1. Capsulitis/ceolomitis, splenic/hepatic, moderate, proliferative
- 2. Cestode, luminal intestinal, moderate (Phylum Platyhelminthes, Class Cestoidea)

G 42-

Morphological diagnosis

- 1. Trichodina sp., few, lamellar (Phylum Ciliophora, Class Litostomatea, Family Trichodina)
- 2. Cestode, luminal intestinal, moderate (Phylum Platyhelminthes, Class Cestoidea)

G 43-

Morphological diagnosis

1. Capsulitis/ceolomitis, splenic/hepatic, mild, proliferative

G 44-

Gills – There are several branchial metazoans that resemble crustaceans with jointed appendages, striated muscle, reproductive tissue and a chitinous exoskeletion. These are very poorly preserved and there is no inflammatory reaction. These are likely regurgitated food items.

Morphological diagnosis

1. Cestode, luminal intestinal, moderate (Phylum Platyhelminthes, Class Cestoidea)

G 45-

Morphological diagnosis

1. Capsulitis/ceolomitis, splenic/hepatic, moderate, proliferative

G 46-

Morphological diagnosis

1. Cestode, luminal intestinal, numerous (Phylum Platyhelminthes, Class Cestoidea)

G 47-

1. Cestode, luminal intestinal, few to moderate (Phylum Platyhelminthes, Class Cestoidea)

G 48-

Morphological diagnosis

1. Trematode, luminal intestinal, few (Phylum Platyhelmintheses, Class Trematoda)

G 49-

Morphological diagnosis

1. Capsulitis/ceolomitis, splenic/hepatic, mild, proliferative

G50 - NAF

G51 - NAF

G 52-

Morphological diagnosis

1. Capsulitis/ceolomitis, splenic/hepatic, mild, proliferative

G 53-

Morphological diagnosis

- 1. Capsulitis/ceolomitis, splenic/hepatic, mild, proliferative
- 2. Nematodes, few to moderate, gastric, luminal (Phylum Nemathelminthes, Class Nematoda)

G 54-

Morphological diagnosis

- 1. Cestode, luminal intestinal, numerous (Phylum Platyhelminthes, Class Cestoidea)
- 2. Nematodes, few to moderate, gastric, luminal (Phylum Nemathelminthes, Class Nematoda)

G 55-

Morphological diagnosis

1. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

G 56-

Morphological diagnosis

1. Myo/epicarditis, mild, focal, lymphohistiocytic

G 57 - NAF

G 58-

Morphological diagnosis

- 1. Myo/epicarditis, mild, focal, lymphohistiocytic
- 2. Capsulitis/ceolomitis, splenic/hepatic, moderate to severe, proliferative
- 3. Cestode, luminal intestinal, few to moderate (Phylum Platyhelminthes, Class Cestoidea)

G 59-

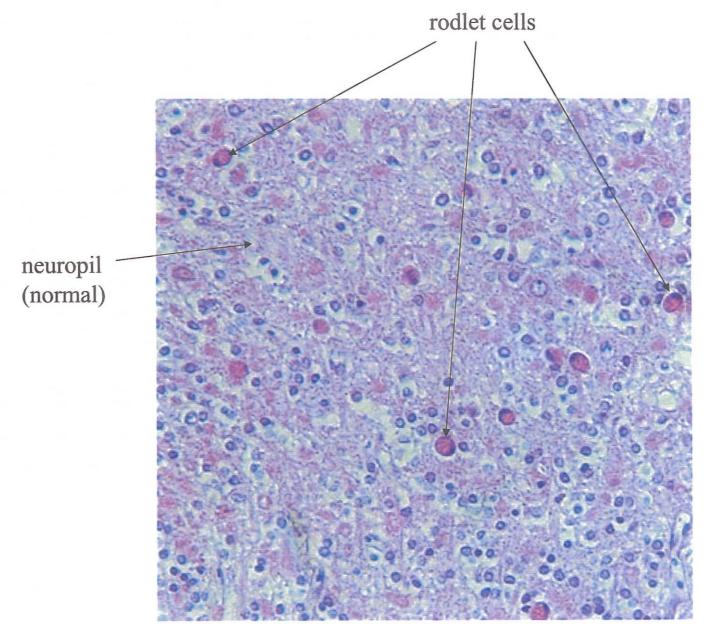
1. Cestode, luminal intestinal, moderate (Phylum Platyhelminthes, Class Cestoidea)

G 60-

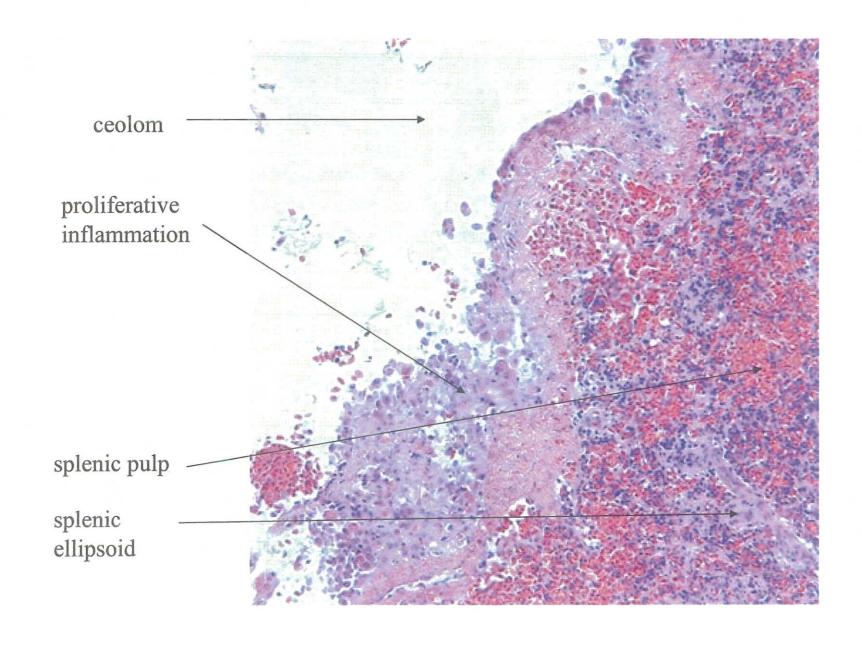
Morphological diagnosis

1. Myo/epicarditis, mild, focal, lymphohistiocytic

G Figure 1. Rodlet cells in many tissues including brain



G Figure 2. Splenic/hepatic capsulitis/ceolomitis - 21/60

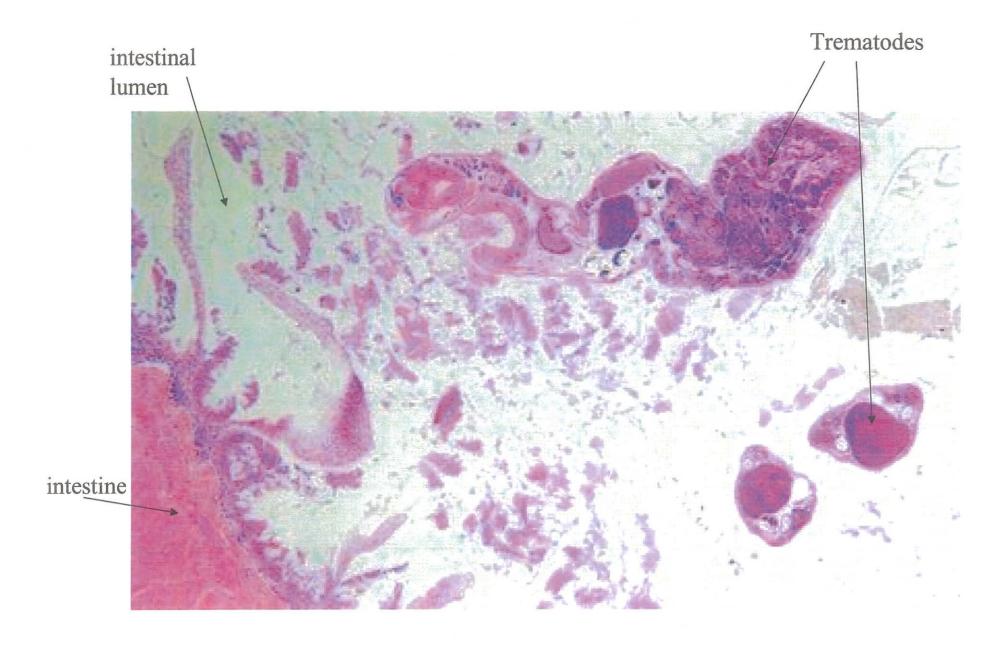


Cestodes intestinal lumen

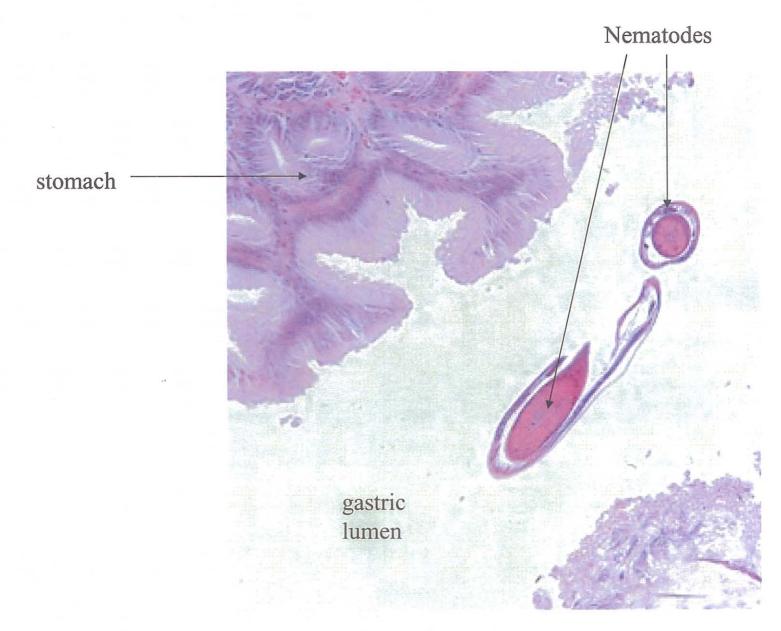
G Figure 3.

Intestinal cestodiasis - 26/60

G Figure 4. Intestinal trematodiasis - 5/60



G Figure 5. Gastric nematodiasis - 3/60



Northern pike (NP - Esox lucius)

The sections consist of dissected tissues including skin, muscle, gill, heart, liver, spleen, pancreas, kidney, gastrointestinal tract (stomach and intestines), swim bladder, eye and ovary/testis.

The notable lesions and/or organisms are described in detail and pictures of these have been provided in two cases. Less notable alterations are occasionally included in the summary for lesions of each individual fish (see later).

Occasionally these fish have heart lesions ranging from focal splitting with outpouching of the compact layer (resembling an aneurysm), to equivocal to mild multifocal myocardial hypereosinophilia often with pyknotic myo- and endocardial nuclei (mild myocardial degeneration/necrosis), or focal areas with mild multifocal endocardial hypertrophy, or mild focal accumulations of lymphocytes in the myocardium but more often the epicardium; however these lesions were not systematically evaluated. No organisms were noted in the hearts of any fish.

Multifocal cholangiohepatitis. This lesion was very common and was not enumerated in a systematic fashion. The majority of lesions were mild to moderate but occasionally were more severe (NP 56). No organisms were seen associated with these lesions.

Typically, these were collars of inflammatory cells consisting of lymphocytes with fewer macrophages, plasma cells and small leukocytes (neutrophils). Occasionally the neutrophils were predominant. Also occasionally in these areas there was a mild to moderate degree of focal hepatocellular necrosis. Inflammatory foci were most often present surrounding exocrine pancreatic tissue or biliary ducts but were occasionally apparently present randomly between and separating hepatocytes (which were occasionally necrotic). Less commonly, the degree of inflammation was more severe. In these cases, there were foci of tightly-packed epitheliod macrophages, with rare multinucleate cells. Less commonly, some of these fish also had a fibrinoid vasculitis, affecting venules more severely and these often had eosinophilic granular cells adjacent to the necrotic endothelium. These lesions could be due to metazoan migration but vasculitis also is commonly associated with viral agents and toxins.

Intestinal cestodes - Cestodes, intra-luminal, intestinal

There are multiple sections of a metazoan organism in the lumen of the gastrointestinal tract of numerous fish. The metazoan is characterized by an eosinophilic tegument, a lack of digestive tract and numerous round to ovoid, basophilic, calcium bodies (calcareous corpuscles) embedded within a loose parenchymatous matrix (Phylum Platyhelminthes, Class Cestoidea). No scolex is present within sections. ---- 19 of 60 affected.

NP Figure 1.

Branchial trematodes - Branchitis, multifocal, mild to moderate with intra-lesional monogenetic trematodes Between lamellae of filaments there is occasionally focal epithelial hyperplasia that expands lamellae. Adjacent to affected lamellae there is often a metazoan organism that can occasionally be seen to possess scleritinized hooks and a loose reticular parenchyma and thin tegument (Phylum Platyhelminthes, Class Trematoda). (no photo) ---- 7 of 7 affected.

Branchial protozoan – Protozoa (Trichodina sp.), few, multifocal.

There were very small numbers of ~20µm protozoans that were dorsoventrally flattened, had cilia and a denticular ring. (Phylum Ciliophora, Class Litostomatea, Family Trichodina) (no photo) ---- 2 of 60 affected.

Branchial protozoan - Protozoa (Caprinia sp.), branchial, multifocal.

There are small numbers of protozoan organisms attached to lamellae of scattered filaments. The shape of the protozoan is variable but more it is often elongate $(45\mu m - 90\mu m)$ or saclike with a centrally located nucleus and numerous tentacles projecting from cytoplasm on the opposite side of attachment. (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp. (formerly *Trichophyra*) ---- 4 of 60 affected. These protozoans are morphologically similar to those seen in white bass (WB Figure 5).

Intratubular renal myxosporidiosis – Myxosporidiosis, renal intratubular.

Within the lumen of renal tubules, that are occasionally mildly dilated, in the caudal kidney, there are low to moderate numbers of multinucleated plasmodium-like protozoan (~ 15µm in diameter) organisms. (Phylum Myxozoa, Class Myxosporea) ---- 10 of 60 affected.

NP Figure 2.

Renal myxosporidiosis. Myxosporidiosis, multifocal, renal interstitial

Within the interstitium, there are cyst-like structures (70 to 150µm in diameter) with a ~5-10µm thick non-descript wall and that contain numerous multinucleated plasmodium-like protozoans. These are tightly packed and are ~7-10µm in diameter. No inflammatory reaction is present. (Phylum Myxozoa, Class Myxosporea) ----7 of 60 affected.

NP Figure 3.

Intestinal/gastric nematodes – Nematodes, intra-luminal, intestinal

There are a few cross sections of a metazoan organism that is ~200μm in diameter, with a thick eosinophilic cuticle, lateral alae and chords, an intestine and reproductive organs. In one fish (NP 30), the nematodes contain numerous ~25x35μm eggs. (Phylum Nemathelminthes, Class Nematoda) ---- 3 of 60 affected.

NP 1 - NAF

NP2 -

Morphological diagnosis

1. Cestodes, intra-luminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

NP 3 - NAF

NP4-

Morphological diagnosis

1. Myxosporidiosis, renal intratubular (Phylum Myxozoa, Class Myxosporea)

NP 5 -

Morphological diagnosis

1. Cestodes, intra-luminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

NP 6 - NAF

NP 7 -

1. Cestodes, intra-luminal, intestinal, moderate (Phylum Platyhelminthes, Class Cestoidea)

NP 8 -

Morphological diagnosis

1. Cestodes, intra-luminal, intestinal, moderate (Phylum Platyhelminthes, Class Cestoidea)

NP 9-

Morphological diagnosis

1. Cestodes, intra-luminal, intestinal, moderate (Phylum Platyhelminthes, Class Cestoidea)

NP 10- NAF

NP 11- NAF

NP 12 - NAF

NP 13 -

Morphological diagnosis

- 1. Cestodes, intra-luminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Myxosporidiosis, renal intratubular (Phylum Myxozoa, Class Myxosporea)

NP 14-

Stomach – There is a focal, transmural necrotizing lesion, approximately 200µm wide (serosal surface) with cavitation of the outer muscular layer and mild serosal mesothelial proliferation and fibrosis, subtended by a hypercellular inner muscle layer with satellite cell proliferation (regeneration), then by hypercosinophilic and disorganized collagen intermixed with proliferating fibroblasts and few macrophages which surrounds a thrombosed artery and finally a largely normal mucosa that has mild glandular epithelial degeneration and necrosis. No organisms are present. This resembles a penetrating wound.

Morphological diagnosis

1. Myxosporidiosis, renal intratubular (Phylum Myxozoa, Class Myxosporea)

NP 15 - NAF

NP 16 - NAF

NP 17 - NAF

NP 18 -

Morphological diagnosis

1. Cestodes, intra-luminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

NP 19 -

- 1. Cestodes, intra-luminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Nematodes, intra-luminal, intestinal, few (Phylum Nemathelminthes, Class Nematoda)

NP 20 -

Morphological diagnosis

1. Branchial Trichodina sp., few, lamellar

NP 21 -

Morphological diagnosis

1. Cestodes, intra-luminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

NP 22 - NAF

NP23 - NAF

NP 24 -

Morphological diagnosis

1. Cestodes, intra-luminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

NP 25 -

Morphological diagnosis

1. Branchitis, multifocal, mild, with intra-lesional monogenetic trematodes

NP 26 - NAF

NP 27 - NAF

NP 28 -

Morphological diagnosis

- 1. Cestodes, intra-luminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Myxosporidiosis, renal intratubular (Phylum Myxozoa, Class Myxosporea)

NP 29-

No discernible anatomic location – There is a large $\sim 300 \times 500 \mu m$ cyst-like structure with a wall composed of compressed macrophages/fibroblasts, that is subtended by foamy cells with an eosinophilic cytoplasm and that is filled mostly with degenerating leukocytes but also contains clumps of amorphous basophilic material.

Morphological diagnosis

1. Myxosporidiosis, multifocal, few, renal interstitial (Phylum Myxozoa, Class Myxosporea)

NP 30 -

Morphological diagnosis

1. Nematodes, intra-luminal, intestinal (Phylum Nemathelminthes, Class Nematoda)

NP 31 -

There is moderate diffuse expansion of blood vessels and the submucosa of the intestine by clear spaces (edema).

Morphological diagnosis

1. Submucosal edema, Gastric/intestinal, moderate, diffuse.

NP 32 -

Morphological diagnosis

- 1. Protozoa (Caprinia sp.), branchial, moderate, (Phylum Ciliophora, Subclass Suctoria, Caprinia sp.)
- 2. Myxosporidiosis, multifocal, few, renal interstitial (Phylum Myxozoa, Class Myxosporea)

NP 33 - NAF

NP 34 -

Morphological diagnosis

- 1. Protozoa (Caprinia sp.), branchial, mild (Phylum Ciliophora, Subclass Suctoria, Caprinia sp.)
- 2. Myxosporidiosis, multifocal, few, renal interstitial (Phylum Myxozoa, Class Myxosporea)

NP 35 - NAF

NP 36 - NAF

NP 37 - NAF

NP 38 -

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Morphological diagnosis

1. Protozoa (Caprinia sp.), branchial, mild (Phylum Ciliophora, Subclass Suctoria, Caprinia sp.)

NP 39 -

Morphological diagnosis

1. Branchial Trichodina sp., few, lamellar

No NP 40

NP 41 - NAF

NP 42 - NAF

NP 43 - NAF

NP 44 -

Morphological diagnosis

1. Cestodes, intra-luminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

NP 45 - NAF

NP 46 -

Morphological diagnosis

1. Myxosporidiosis, renal intratubular (Phylum Myxozoa, Class Myxosporea)

NP 47 -

- 1. Cestodes, intra-luminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Myxosporidiosis, renal intratubular (Phylum Myxozoa, Class Myxosporea)

NP48 -

Morphological diagnosis

1. Myxosporidiosis, multifocal, moderate, renal interstitial (Phylum Myxozoa, Class Myxosporea)

NP49 -NAF

NP50 -

Morphological diagnosis

- 1. Cestodes, intra-luminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, multifocal, mild to moderate with intra-lesional monogenetic trematodes

NP 51 -

Morphological diagnosis

- 1. Branchitis, multifocal, mild, with intra-lesional monogenetic trematodes
- 2. Myxosporidiosis, renal intratubular (Phylum Myxozoa, Class Myxosporea)

NP 52 -

Morphological diagnosis

- 1. Cestodes, intra-luminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, multifocal, mild to moderate with intra-lesional monogenetic trematodes

NP 53 -

Morphological diagnosis

- 1. Branchitis, multifocal, mild to moderate with intra-lesional monogenetic trematodes
- 2. Myxosporidiosis, multifocal, moderate, renal interstitial (Phylum Myxozoa, Class Myxosporea)

NP 54 -

Morphological diagnosis

1. Cestodes, intra-luminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

NP 55 -

Morphological diagnosis

1. Branchitis, multifocal, mild to moderate with intra-lesional monogenetic trematodes

NP 56a -

Morphological diagnosis

1. Nematode, intra-luminal, gastric, single (Phylum Nemathelminthes, Class Nematoda). This fish also had a mild focal histiocytic-rich gastritis.

NP 56b - NAF

NP 57 -

- 1. Myxosporidiosis, renal intratubular (Phylum Myxozoa, Class Myxosporea)
- 2. Myxosporidiosis, multifocal, few, renal interstitial (Phylum Myxozoa, Class Myxosporea)

NP 58 -

Morphological diagnosis

1. Cestodes, intra-luminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

NP59 -

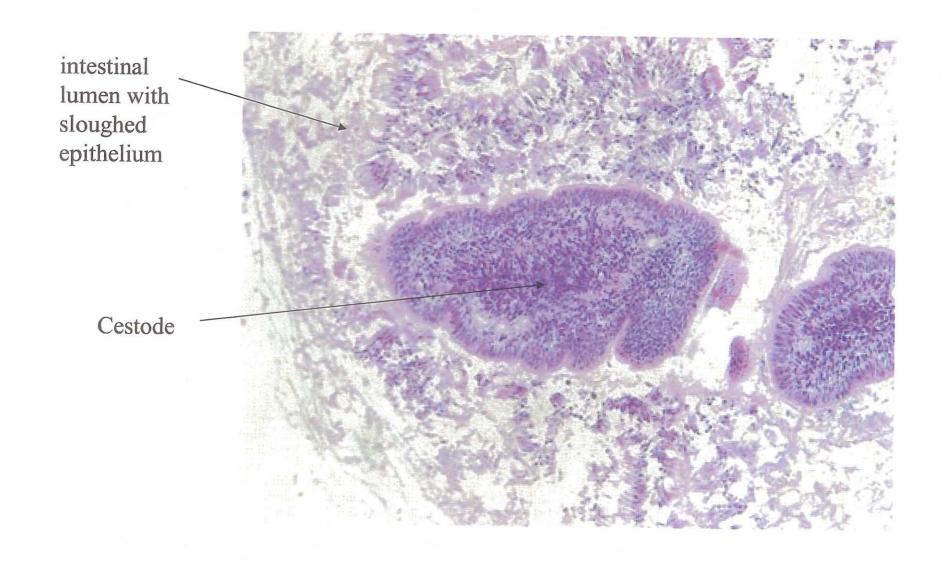
Morphological diagnosis

- 1. Branchitis, multifocal, mild, with intra-lesional monogenetic trematodes
- 2. Myxosporidiosis, renal intratubular (Phylum Myxozoa, Class Myxosporea)
- 3. Myxosporidiosis, multifocal, moderate, renal interstitial (Phylum Myxozoa, Class Myxosporea)

MP 60 -

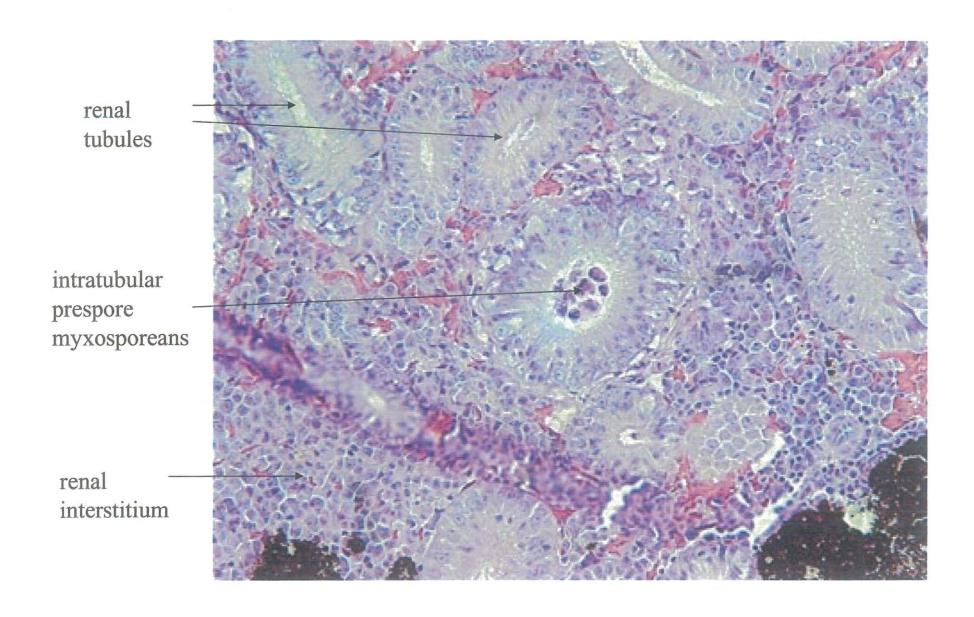
- 1. Cestodes, intra-luminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Myxosporidiosis, renal intratubular (Phylum Myxozoa, Class Myxosporea)

NP Figure 1. Intestinal cestodes - 19/60



NP Figure 2.

Renal intratubular prespore myxosporeans - 10/60



NP Figure 3. renal tubules Presumptive myxosporean cysts Renal interstitial prespore myxosporeans - 7/60

White Bass (WB - Morone chrysops)

Fish were predominantly oriented as transverse sections with occasional longitudinal sections and individual tissues. Tissues routinely examined included skeletal muscle, skin, spinal cord, brain kidney (caudal and less commonly cranial), liver, intestine, stomach, exocrine pancreas, heart, gills, swim bladder, and spleen. Spleen was the organ most often missing from the sections examined. Tissues less commonly or only occasionally in section included the endocrine pancreas, thyroid, inter-renal and chromafin tissues and corpuscles of Stannius, as well as the pseudobranch and thymus.

Many of the fish had varying numbers of epithelial apoptotic bodies in the mucosa. There were also mild infliltrates of mixed inflammatory cells (lymphocytes and macrophages most commonly) in the submucosa and lamina propria. No organisms were associated with these infiltrates. These were not evaluated systematically.

Intestinal cestodes - Cestodes, intraluminal, intestinal

There are single to very numerous large ~300-400um in width metazoans with a thick (~40um) eosinophilic tegument and a parenchymatous matrix with calcareous corpuscles and lacking in digestive tubes (Cestode). Occasionally, there is segmentation and numerous eggs contained within ovaries and less often free in the intestinal lumen. Tissue reaction is minimal but occasionally there is a scolex visible in the section with clamped mucosa undergoing necrosis (see Figure 1a). Very heavily infested sections of intestine are dilated with minimally thinned mucosa. (Phylum Platyhelminthes, Class Cestoidea) ---- 35 of 56 affected Intrahepatic (biliary) cestodes – Cholangiohepatitis, focally extensive, with intraluminal cestodes and with hepatic atrophy

These are morphologically similar to those described in the intestine that lack segmentation and eggs. They appear to be contained within extremely dilated bile ducts that have thinned or ulcerated epithelium surrounded by condensed hepatocellular parenchyma, minimal fibrosis and small to moderate numbers of macrophages, lymphocytes and small leukocytes (Figure 1b). (Phylum Platyhelminthes, Class Cestoidea) ---- 2 of 56 affected

WB Figure 1a and 1b.

Branchial epitheliocystis – Branchitis, focal to multifocal or regionally extensive, with filamental interepithelial bacterial microcolonies

There are single to several closely spaced ~70-150um tightly-packed basophilic foci with a thin ~5um eosinophilic limiting membrane that delineates each microcolony. The microcolony is filled with ~1um (but almost not distinctly visible) basophilic bacteria. These microcolonies efface the filament epithelium and often produce distortion of the filament tip, including the cartilage. Immediately surrounding these microcolonies there is typically a limited increase in inflammatory cells (macrophages, lymphocytes, smaller leukocytes and eosinophilic granular cells) with limited degeneration and necrosis of epithelium and leukocytes. Less commonly there is moderate peripheral necrosis and in one case there were no microcolonies remaining but there was substantial focal necrosis (eruption event?). (Rickettsia-like organism, presumptive) ---- 23 of 56 affected

WB Figure 2.

Branchial trematodes - Branchitis, multifocal with intralesional monogenetic trematode (not buried) (This metazoan appears to be distinct in location and in morphology of the lesion produced from the monogene described next that has its haptor buried in the terminal filament)

There is a single to few cross-section(s) of an $\sim 70 \times 100 \mu m$ metazoan characterized by a non-segmented thin tegument supported by a row of sub-tegumental cells and loose reticular parenchymal matrix. A terminal scleritinized haptor is sometimes visible in contact with the interlamellar epithelium. Mild focal epithelial necrosis can be seen. These are present randomly on the lamellar surface (Phylum Platyhelminthes, Class Trematoda). ---- 2 or 3 of 56 affected

WB Figure 3.

Branchial trematodes - Branchitis, distal, focally extensive, hyperplastic, with (or without) intralesional monogenetic trematodes (this monogenetic trematode is distinct from the previous one)

Numerous filaments have regionally extensive epithelial hyperplasia that is particularly severe at the distal portion. There is a moderate infiltrate of morphologically mixed inflammatory cells and mild necrosis surrounding a ~200x500µm metazoan with a scleritinized haptor buried within the filament. This metazoan has a tegument and granular basophilic contents and no reproductive system or intestines were noted (Phylum Platyhelminthes, Class Trematoda). The lesion is very characteristic and was included even if the metazoan was not actually visible in the section. ---- 51 of 56 affected

WB Figure 4.

Branchial protozoans - Branchitis, diffuse, with lamellar protozoans

There are single to very numerous (100's) of ~20x50um slightly dorsoventrally-flattened protozoans with a basophilic nucleus and thin ~2um multiple tentacles (these are not uniformly arranged and are opposite from the surface in contact with the epithelium) present on the lamellar epithelial surface. (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*).

Usually present in small numbers are similarly shaped and sized organism without stalks that appear to be holotrichous. Chilodonella or similar, presumptive (Phylum Ciliophora).

There are also small to numerous $\sim 25 \mu m$ dorsoventrally flattened protozoans with a chitinous endoskeleton (denticular ring) and ring of cilia. *Trichodina* sp. (Phylum Ciliophora, Class Litostomatea, Family Trichodina) Since these are common and numerous it was not practical to differentiate which fish had each of these protozoans. ---- 36 of 56 affected

WB Figure 5.

Branchial microsporidean - Branchitis, multifocal, with few microsporidian cysts

There are single to several round to oval $\sim 40\text{-}50\mu\text{m}$ structures buried within the interlamellar epithelium, that have a granular basophilic content made up of $\sim 2\mu\text{m}$ spores and a thin $\sim 3\mu\text{m}$ hyaline eosinophilic cyst wall (Phylum Microspora, Class Microsporea). There is no or limited tissue reaction but these cysts minimally distort the adjacent lamellae. ---- 2 of 56 affected

WB Figure 6.

Intramuscular trematode – Trematode, encysted intramuscular, skeletal.

There is a single ~200µm metazoan within a fibrous encapsulated wall (likely within a myocyte with compressed parenchyma). There is no host reaction. The metazoan has a thin eosinophilic tegument and a parenchymatous matrix with no calcareous corpuscles (Phylum Platyhelminthes, Class Trematoda). ---- 5 of 56

WB Figure 7.

Multifocal cholangiohepatitis. This lesion was very common and was not enumerated in a systematic fashion. Most lesions were mild to moderate. No organisms were seen associated with these lesions.

Typically, these were collars of inflammatory cells consisting of lymphocytes with fewer macrophages, plasma cells and small leukocytes and often enlarged macrophages containing small amounts of golden-brown pigment. These foci were most often present surrounding exocrine pancreatic tissue or biliary ducts but were occasionally apparently present randomly between and separating hepatocytes. In some occasions, such as that pictured, the pancreatic tissue appears to be effaced to some degree.

WB Figure 8.

WB 1 -

Kidney – There are mild amounts of basophilic material in tubules and mild distortion of the tubular epithelium. Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, moderate, distal, focally extensive, hyperplastic, with moderate intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 3. Branchitis, diffuse, with numerous lamellar protozoans
- 4. Intratubular mineralization, caudal kidney, mild (nephrocaclcinosis)

WB 2 -

Morphological diagnosis

- 1. Branchitis, focal to multifocal or regionally extensive, with filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)
- 2. Branchitis, diffuse, with moderate lamellar protozoans

No WB 3.

WB 4 -

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, distal, focally extensive, hyperplastic, with moderate intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)

WB 5 -

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, focal to multifocal or regionally extensive, with moderate filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)
- 3. Trematode, encysted intramuscular, skeletal (Phylum Platyhelminthes, Class Trematoda)

WB 6 -

Morphological diagnosis

1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

2. Branchitis, mild, distal, focally extensive, hyperplastic, with intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)

WB 7 -

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, moderate, distal, focally extensive, hyperplastic, with moderate intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)

WB 8 -

Morphological diagnosis

- 1. Branchitis, mild, distal, focally extensive, hyperplastic, with moderate intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 2. Branchitis, diffuse, with few lamellar protozoans

WB 9 -

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, moderate, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 3. Branchitis, diffuse, with numerous lamellar protozoans

WB 10 -

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, focal to multifocal or regionally extensive, with single filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)
- 3. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 4. Trematode, encysted intramuscular, skeletal, few (Phylum Platyhelminthes, Class Trematoda)

WB 11 -

Morphological diagnosis

- 1. Branchitis, focal to multifocal or regionally extensive, with numerous filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)
- 2. Branchitis, moderate, distal, focally extensive, hyperplastic, with numerous intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)

WB 12 -

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, mild, distal, focally extensive, hyperplastic, with moderate intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 3. Branchitis, diffuse, with few lamellar protozoans

WB 13 -

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, moderate, distal, focally extensive, hyperplastic, with moderate intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 3. Branchitis, diffuse, with moderate lamellar protozoans

WB 14 -

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)

WB 15 -

Intestine - Several sections of intestine have submucosal edema and loosely arranged histiocytic foci. There are also fewer lymphocytes and eosinophilic granular cells however no organisms are present.

Morphological diagnosis

- 1. Branchitis, focal to multifocal or regionally extensive, with numerous filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)
- 2. Branchitis, moderate, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 3. Trematode, encysted intramuscular, skeletal (Phylum Platyhelminthes, Class Trematoda)

WB 19 -

Morphological diagnosis

- 1. Branchitis, moderate, distal, focally extensive, hyperplastic, with intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 2. Branchitis, diffuse, with moderate lamellar protozoans
- 3. Trematode, encysted intramuscular, skeletal (Phylum Platyhelminthes, Class Trematoda)

WB 20 -

Intestine - Several areas in which the mucosal folds are thickened – edema is prominent as well as few lymphocytes, macrophages and eosinophilic granular cells, no organisms are present.

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)
- Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 4. Branchitis, diffuse, with moderate lamellar protozoans
- 5. Branchitis, multifocal, with few microsporidian cysts (Phylum Microspora, Class Microsporea)

WB 21 -

There is a single small, metazoan-type granuloma near the thymus. Contents are amorphous. Morphological diagnosis

1. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)

2. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)

WB 22 -

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)
- 3. Branchitis, moderate, distal, focally extensive, hyperplastic

Histiocytic focus, skeletal muscle, single.

- 4. Branchitis, diffuse, with numerous lamellar protozoans
- 5. Branchitis, multifocal, with few microsporidian cysts (Phylum Microspora, Class Microsporea)

WB 23 -

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)
- 3. Branchitis, moderate to severe, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 4. Branchitis, diffuse, with moderate lamellar protozoans
- 5. Granuloma, skeletal muscle, single focal.

WB 24 -

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 3. Branchitis, diffuse, with moderate lamellar protozoans

WB 25 -

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda) and Dermatitis (branchial cavity wall) focal, hyperplastic with intralesional trematode (same as for gills)
- 3. Branchitis, diffuse, with moderate lamellar protozoans
- 4. Granuloma, skeletal muscle, single focal

WB 26 -

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)
- 3. Branchitis, mild to moderate, distal, focally extensive, hyperplastic
- 4. Branchitis, diffuse, with few lamellar protozoans

WB 27 -

Morphological diagnosis

1. Branchitis, mild to moderate, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)

WB 28 -

Skeletal muscle, dermis and epidermis - There is a focally extensive area of myonecrosis with macrophage infiltration and early fibrosis and regeneration. There is also extensive edema and the overlying dermis also necrotic with loss of epidermis and scales. (similar in appearance to a previous penetrating wound).

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, moderate, distal, focally extensive, hyperplastic
- 3. Branchitis, diffuse, with numerous lamellar protozoans

WB 29 -

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, moderate, distal, focally extensive, hyperplastic, with numerous intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 3. Branchitis, diffuse, with few lamellar protozoans

WB 30 -

Morphological diagnosis

- 1. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 2. Branchitis, diffuse, with few lamellar protozoans

WB 31 -

Morphological diagnosis

- 1. Branchitis, mild, distal, focally extensive, hyperplastic
- 2. Branchitis, diffuse, with few lamellar protozoans

WB 32 -

Morphological diagnosis

- 1. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)
- 2. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 3. Branchitis, diffuse, with few lamellar protozoans

WB 33 -

Intestine – There is a submucosal zone of edema and with inflammatory cells exiting from a blood vessel. No organisms are present.

Morphological diagnosis

- 1. Branchitis, focal to multifocal or regionally extensive, with moderate filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)
- 2. Branchitis, mild to moderate, distal, focally extensive, hyperplastic
- 3. Trematode, encysted, cranial connective tissue (Phylum Platyhelminthes, Class Trematoda)

WB 34 -

Morphological diagnosis

- 1. Branchitis, focal to multifocal or regionally extensive, with moderate filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)
- 2. Branchitis, mild to moderate, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 3. Branchitis, diffuse, with moderate lamellar protozoans

WB 35 -

Liver – There is a single focus of nodular hyperplasia.

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, diffuse, with numerous lamellar protozoans

WB 36 -

Skin – There is a focal lymphocytic dermatitis and the overlying epidermis is eroded but not ulcerated and there is an intense local infiltrate of lymphocytes and plasma cells. There are no organisms present.

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, moderate (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, moderate, distal, focally extensive, hyperplastic, with moderate intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 3. Branchitis, diffuse, with very numerous lamellar protozoans

WB 37 -

Morphological diagnosis

1. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)

WB 38 -

Mesentery – There is an encysted metazoan however the structure is indistinct.

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, numerous (Phylum Platyhelminthes, Class Cestoidea)
- 2. Cholangiohepatitis, focally extensive, with intraluminal cestodes and with hepatic atrophy (Phylum Platyhelminthes, Class Cestoidea)
- 3. Branchitis, mild, distal, focally extensive, hyperplastic

WB 39 -

Morphological diagnosis

1. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)

2. Lymphohistiocytic focus, skeletal muscle, single.

WB 40 -

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, moderate, distal, focally extensive, hyperplastic
- 3. Branchitis, diffuse, with few lamellar protozoans

WB 41 -

0

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)
- 3. Branchitis, moderate, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 4. Branchitis, diffuse, with few lamellar protozoans

WB 42 -

Mesentery – There is a single $\sim 100 \mu m$ granuloma invested with golden-brown pigment and that contains no visible organisms.

Morphological diagnosis

- 1. Branchitis, moderate, distal, focally extensive, hyperplastic, with moderate intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 2. Branchitis, diffuse, with few lamellar protozoans
- 3. Histiocytic focus, skeletal muscle, few.

WB 43 -

Skeletal muscle - There is a large encapsulated metazoan (remnants) with 30-40 cell thick collar of inflammation with macrophages, lymphocytes and few melanomacrophages.

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, diffuse, with few lamellar protozoans

WB 44 -

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)
- 3. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes
- 4. Branchitis, diffuse, with few lamellar protozoans

WB 45 -

Morphological diagnosis

1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

WB 46 -

Morphological diagnosis

- 1. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies Branchitis, diffuse, with few lamellar protozoans
- 2. Branchitis, moderate, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 3. Branchitis, diffuse, with few lamellar protozoans

WB 47 -

Skin – There is a mild focal dermatitis with increased dermal lymphocytes and macrophages. There is also a regionally extensive histiocytic myositis and cellulitis. No organisms are present.

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, multifocal with intralesional monogenetic trematode (not buried) (Phylum Platyhelminthes, Class Trematoda)
- 3. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 4. Branchitis, diffuse, with numerous lamellar protozoans

WB 48 -

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, focal to multifocal or regionally extensive, with moderate filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)
- 3. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)

WB 49 -

Morphological diagnosis

- 1. Branchitis, moderate to severe, distal, focally extensive, hyperplastic, with numerous intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 2. Trematode, encysted intramuscular, skeletal (Phylum Platyhelminthes, Class Trematoda)

WB 50 -

Morphological diagnosis

- 1. Branchitis, moderate, distal, focally extensive, hyperplastic, with numerous intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 2. Branchitis, diffuse, with few lamellar protozoans

WB 51 -

Morphological diagnosis

- 1. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)
- 2. Branchitis, moderate, distal, focally extensive, hyperplastic
- 3. Branchitis, diffuse, with few lamellar protozoans

WB 52 -

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, focal to multifocal or regionally extensive, with numerous filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive) and Dermatitis, focal to multifocal or regionally extensive, with numerous filamental interepithelial bacterial microcolonies
- 3. Branchitis, moderate, distal, focally extensive, hyperplastic

WB 53 -

Morphological diagnosis

- 1. Cholangiohepatitis, focally extensive, with intraluminal cestodes and with hepatic atrophy (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, focal to multifocal or regionally extensive, with few numerous filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)
- 3. Branchitis, moderate to severe, distal, focally extensive, hyperplastic, with moderate intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)

WB 54 -

Intestine - One section of intestine is notably edematous.

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Mild multifocal epithelial hyperplasia and single section of a monogenetic trematode

WB 55 -

Skin - There is a focal lymphocytic dermatitis. No organisms are present.

Morphological diagnosis

- 1. Branchitis, focal to multifocal or regionally extensive, with few numerous filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)
- 2. Branchitis, moderate, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 3. Branchitis, diffuse, with numerous lamellar protozoans

WB 56 -

Morphological diagnosis

- 1. Cestodes, intraluminal, intestinal, numerous (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, moderate, distal, focally extensive, hyperplastic, with numerous intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)

WB 57 -

- 1. Cestodes, intraluminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, focal to multifocal or regionally extensive, with moderate numerous filamental interepithelial bacterial microcolonies (Rickettsia-like organism, presumptive)
- 3. Branchitis, multifocal with intralesional monogenetic trematode (not buried) (Phylum Platyhelminthes, Class Trematoda)
- 4. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)

5. Branchitis, diffuse, with numerous lamellar protozoans

WB 58 -

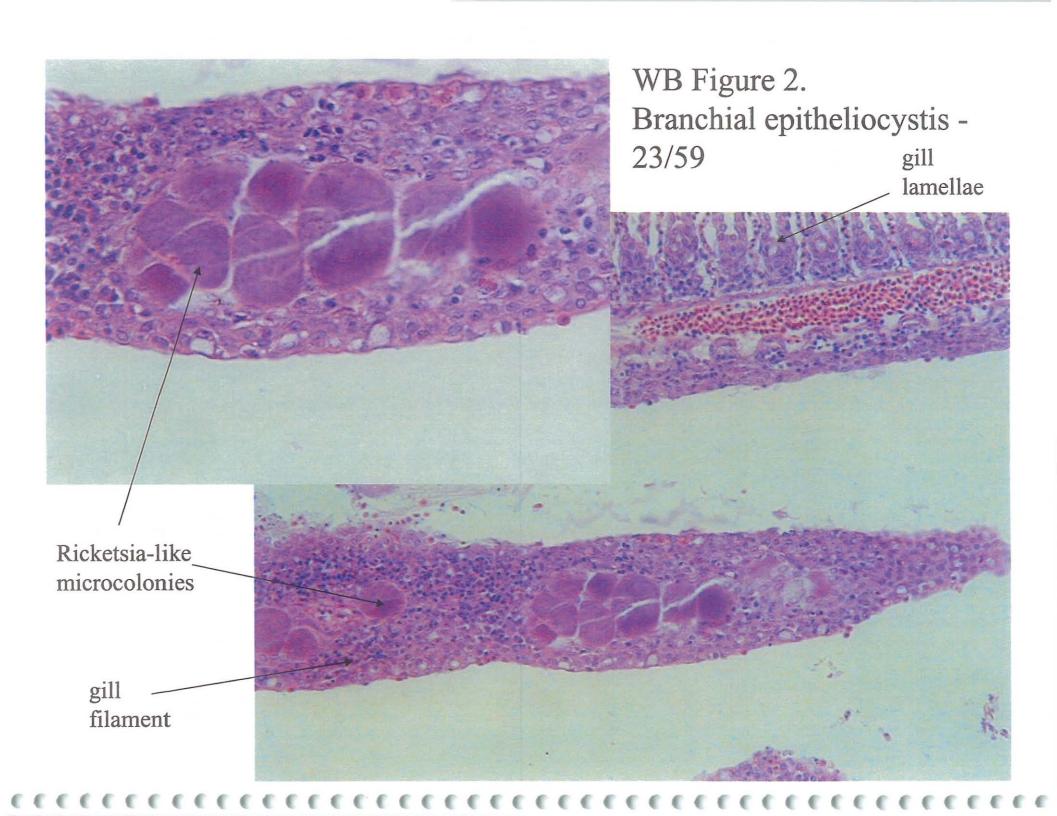
Morphological diagnosis

- 1. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 2. Branchitis, diffuse, with numerous lamellar protozoans

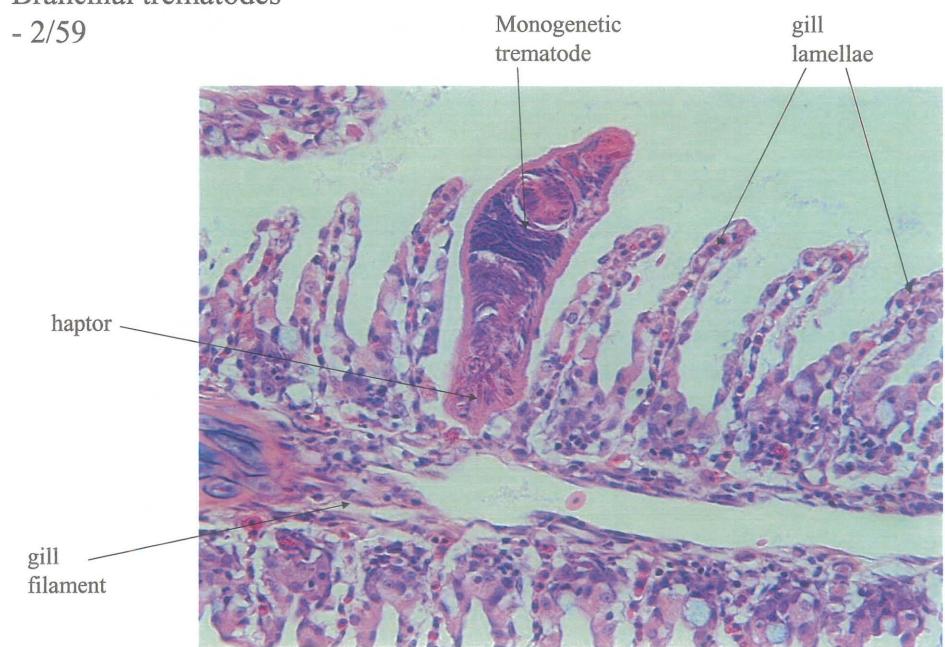
WB 59 -

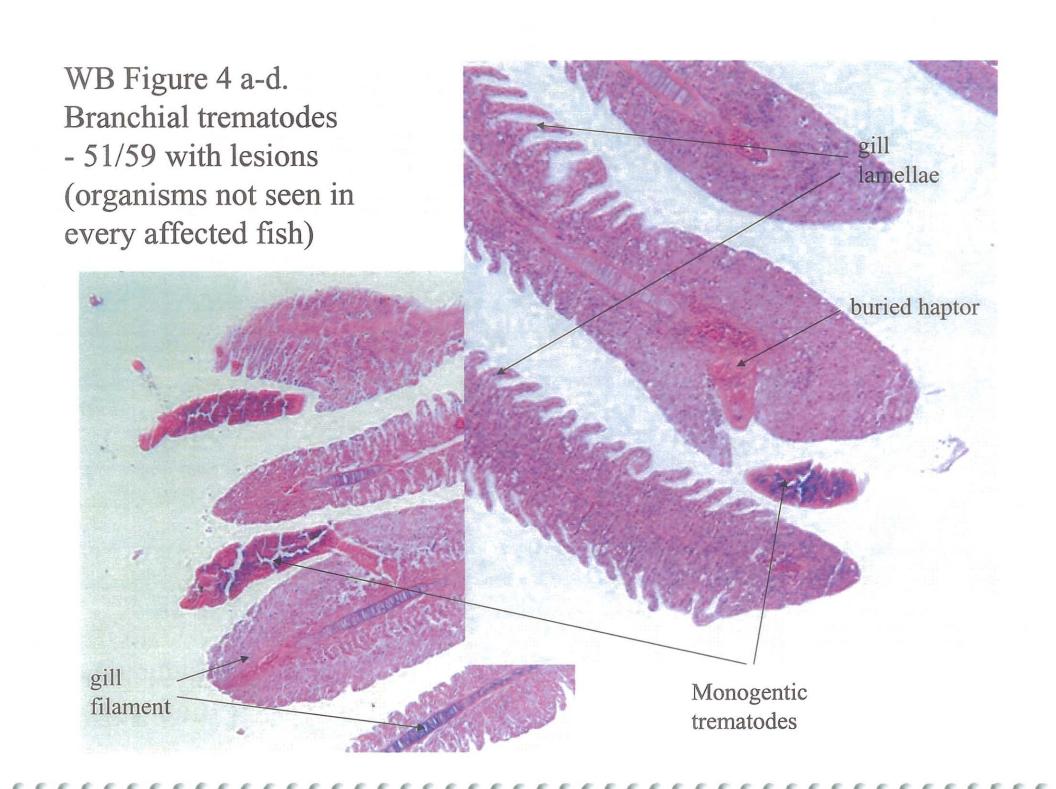
- 1. Branchitis, moderate, distal, focally extensive, hyperplastic, with numerous intralesional trematodes (Phylum Platyhelminthes, Class Trematoda)
- 2. Branchitis, diffuse, with few lamellar protozoans

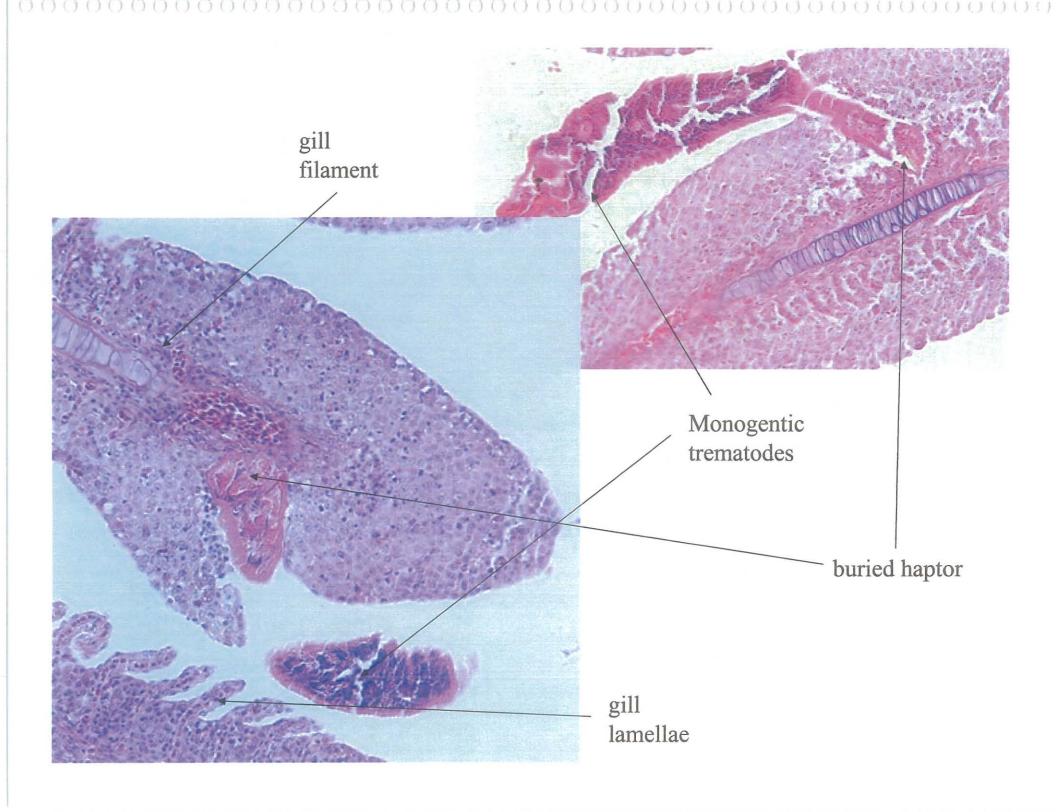
WB Figure 1 1a. Intestinal cestodes - 35/59 1b. Intrahepatic (biliary) - 2/59 focal epithelial intestine necrosis from scolex b. Cestodes liver dilated bile duct a.

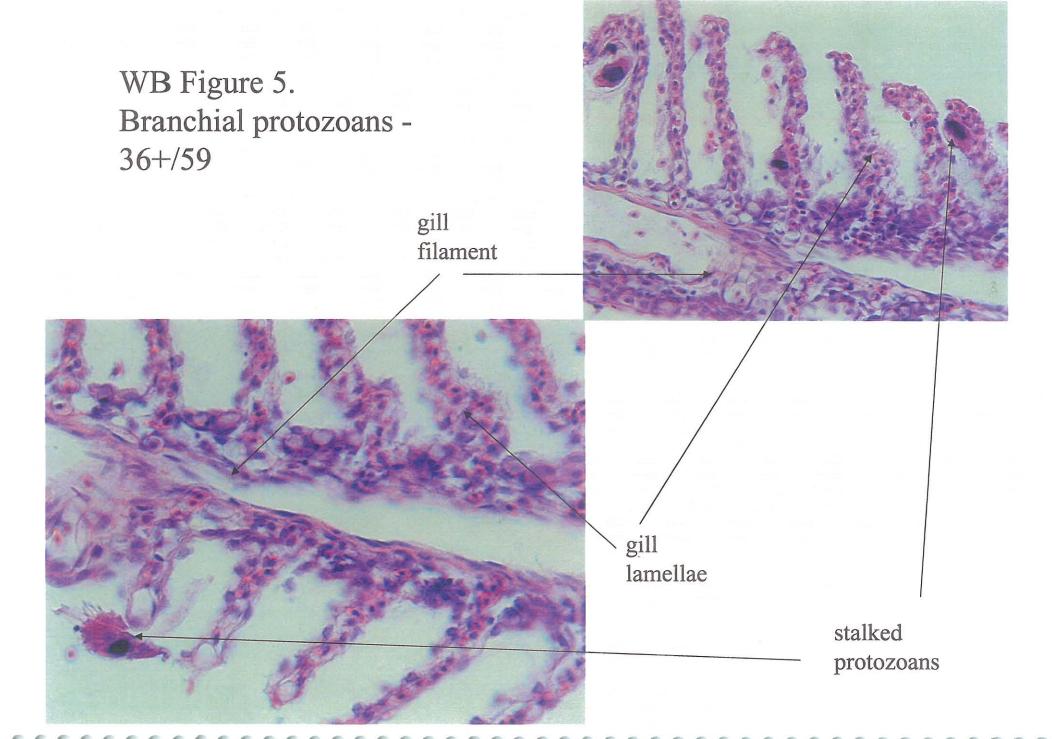


WB Figure 3. Branchial trematodes



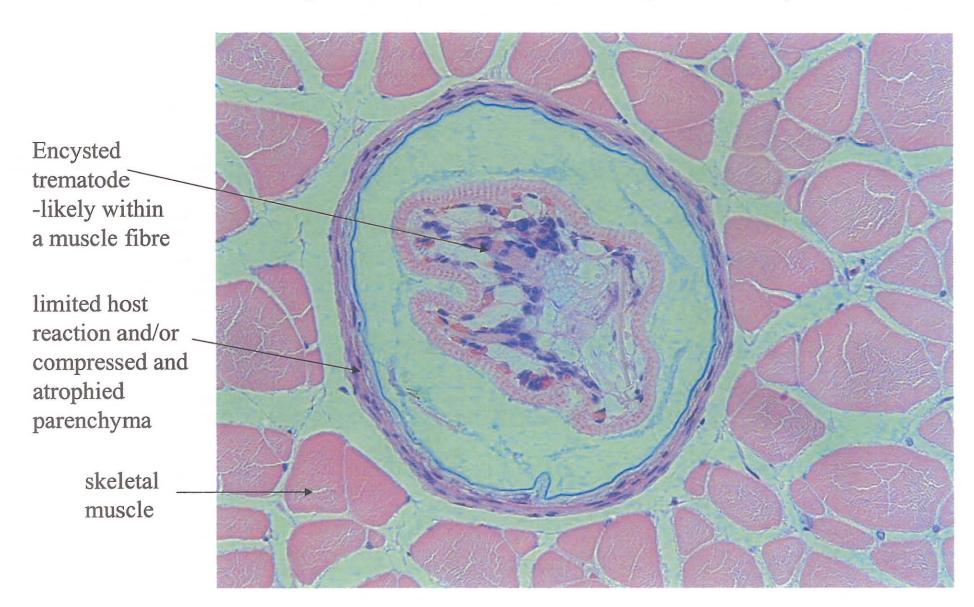


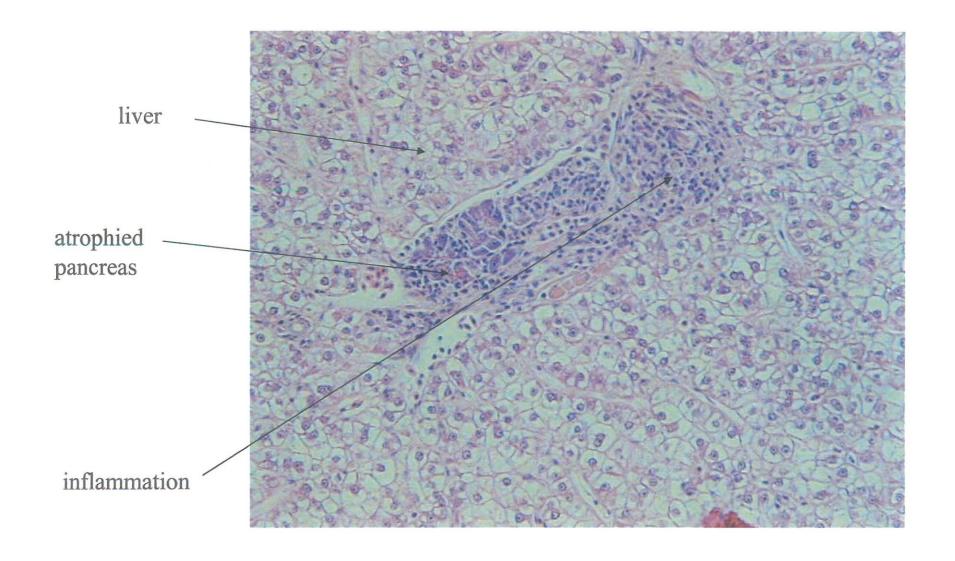




WB Figure 6. gill Branchial microsporean - 2/59 lamellae gill filament Microsporean cyst

WB Figure 7.
Intramuscular meta - 5/59
Granulomas/histocytic foci without contents (not shown) - 6/59





WB Figure 8.
Multifocal cholangiohepatitis - common (but not enumerated)

Channel cats (CC - Ictalurus punctatus)

Only seven fish of this species were caught. The sections consist of dissected tissues including skin, muscle, gill, heart, liver, spleen, pancreas, kidney, gastrointestinal tract (stomach and intestines), swim bladder, eye and ovary/testis.

The notable lesions and/or organisms are described in detail and pictures of these have been provided in two cases. Less notable alterations are occasionally included in the summary for lesions of each individual fish (see later).

The majority of the few fish examined have a diffuse branchitis of varying severity. Most types of leukocytes are present in all fish however the predominant cell type varies. The presence of a branchitis is not surprising given the range of organisms that are present on the gills of this species of fish.

Branchial trematodes - Branchitis, multifocal, mild to moderate with intra-lesional monogenetic trematodes Between lamellae of filaments there are occasionally focally prominent epithelial hyperplasia (with or without focal necrosis) that expands lamellae. Adjacent to affected lamellae there is often a metazoan organism that can occasionally be seen to possess scleritinized hooks and a loose reticular parenchyma and thin tegument (Phylum Platyhelminthes, Class Trematoda). ---- 5 of 7 affected.

CC Figure 1

Branchial myxosporeans - Branchitis, multifocal, with intra-lesional myxosporean cysts Within various sections of gill, there are few to several solitary myxosporean cysts between lamellae and surrounded by multifocal to regionally extensive epithelial hyperplasia admixed within increased numbers of leukocytes. The cysts, which range in size from ~50 to 400μm, contain both immature multinucleate sporoplasm, primarily located at the periphery of the cysts, and maturing spores and is surrounded by an ~5-10μm thick hyaline wall. The spores are fusiform in shape with a single polar capsule and are approximately 20μm in length. (Phylum Myxozoa, Class Myxosporea) ---- 3 of 7 affected.

CC Figure 2

Myo/epicarditis, multifocal, lymphocytic, mild. There are several to multiple foci of increased numbers of lymphocytes and fewer macrophages in either myocardial trabeculae or in the epicardium. In these areas there is also mild endocardial hypertrophy. No organisms are present. ---- 5 of 7 affected. (no photo).

Branchial protozoan – Branchitis, multifocal, mild with intra-lesional ciliated protozoan There are few to numerous ciliated protozoans that range in size from ~20-70µm that are located under the inter-lammelar epithelium. These have an eosinophilic granular cytoplasm and a basophilic lobed or perhaps horseshoe shaped nucleus. (Phylum Ciliophora, suspect *Ichthyopthirius* or similar) ---- 4 of 7 affected. (no photo)

CC 1-

- 1. Branchitis, multifocal, mild to moderate with a single monogenetic trematodes (Phylum Platyhelminthes, Class Trematoda)
- 2. Myocarditis, lymphocytic, multifocal, mild

CC 2-

Morphological diagnosis

- 1. Branchitis, multifocal, mild to moderate with few intra-lesional monogenetic trematodes (Phylum Platyhelminthes, Class Trematoda)
- 2. Myocarditis, lymphocytic, multifocal, mild

CC 3-

Gills – There are two presumptive crustaceans that have a chitinous exoskeleton and striated muscle and may have jointed appendages. These are closely apposed to the gills, which are physically distorted by their presence. Little damage or inflammation is apparent.

Morphological diagnosis

- 1. Branchitis, multifocal, mild to moderate with numerous intra-lesional monogenetic trematodes (Phylum Platyhelminthes, Class Trematoda)
- 2. Branchitis, multifocal, with several intra-lesional myxosporean cysts (Phylum Myxozoa, Class Myxosporea)
- 3. Myocarditis, lymphocytic, multifocal, mild
- 4. Branchitis, multifocal, mild with moderate intra-lesional ciliated protozoans (Phylum Ciliophora, suspect *Ichthyopthirius* or similar)
- 5. Branchial crustacea

CC 4-

Morphological diagnosis

- 1. Branchitis, multifocal, mild with intra-lesional ciliated protozoan (Phylum Ciliophora, suspect *Ichthyopthirius* or similar)
- 2. Epicarditis, lymphocytic, multifocal, mild

CC 5-

Morphological diagnosis

- 1. Branchitis, multifocal, with several intra-lesional myxosporean cysts (Phylum Myxozoa, Class Myxosporea)
- 2. Branchitis, multifocal, mild with moderate intra-lesional ciliated protozoans (Phylum Ciliophora, suspect *Ichthyopthirius* or similar)

CC 6-

Morphological diagnosis

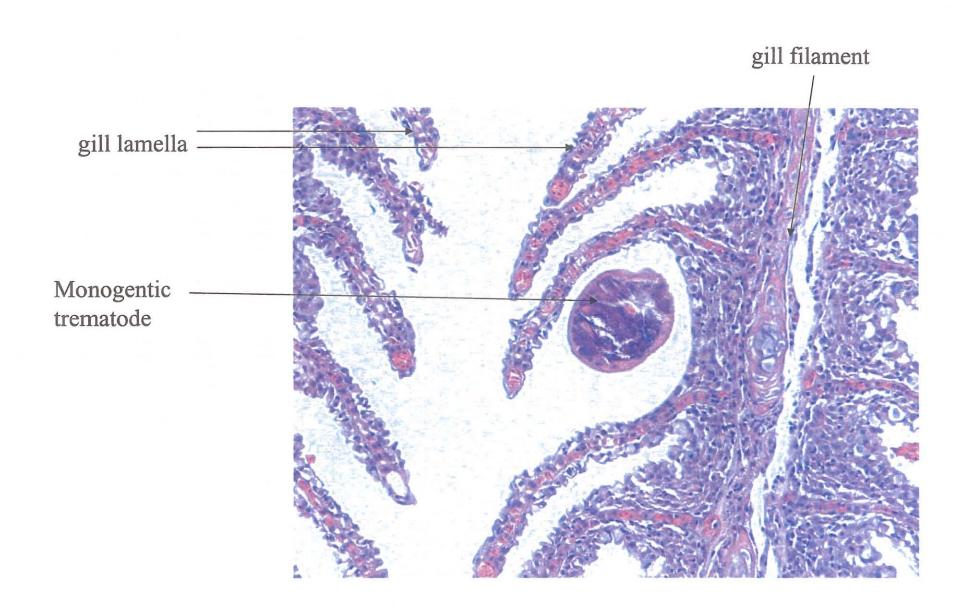
- 1. Branchitis, multifocal, mild to moderate with few intra-lesional monogenetic trematodes (Phylum Platyhelminthes, Class Trematoda)
- 2. Branchitis, multifocal, with a single intra-lesional myxosporean cysts (Phylum Myxozoa, Class Myxosporea)
- 3. Branchitis, multifocal, mild with few intra-lesional ciliated protozoans (Phylum Ciliophora, suspect *Ichthyopthirius* or similar)

CC 7-

Morphological diagnosis

1. Branchitis, multifocal, mild to moderate with a single intra-lesional monogenetic trematode (Phylum Platyhelminthes, Class Trematoda)

CC Figure 1. Branchial monogenetic trematodes - 5/7



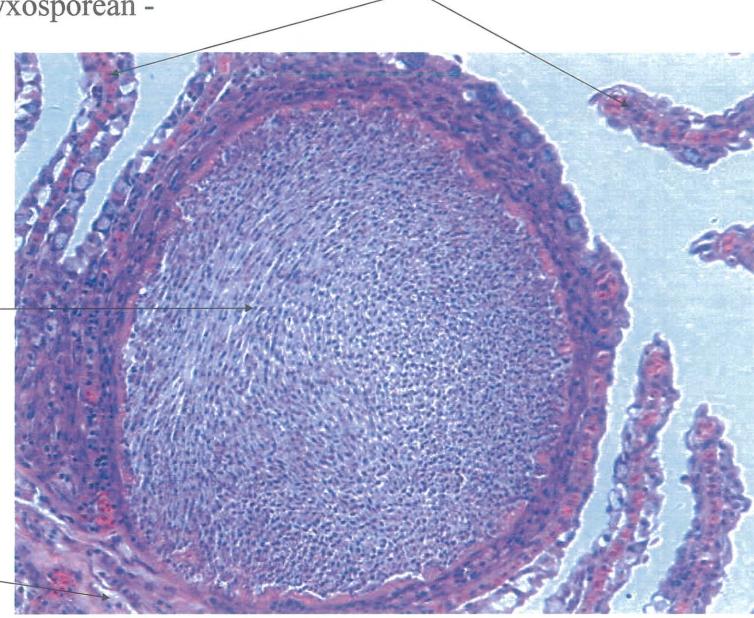
CC Figure 2.

Branchial myxosporean -

3/7

Microsporean_ cyst - filled with mature spores

gill filament



gill lamellae

Walleye (W - Sander vitreus)

Individual tissues were dissected in most cases. Tissues routinely examined included skeletal muscle, skin, spinal cord, brain kidney (caudal and less commonly cranial), liver, intestine, stomach, exocrine pancreas, heart, gills, swim bladder, and spleen. Spleen was the organ most often missing from the sections examined. Tissues less commonly or only occasionally in section included the endocrine pancreas, inter-renal and chromafin tissues and the corpuscles of Stannius.

A common lesion that was not evaluated in a systematic fashion was a mild (occasionally moderate) lymphocytic cholangiohepatitis and perihepatopancreatitis. Occasionally these foci are associated with mild atrophy and more uncommonly, necrosis of the hepatic or exocrine pancreatic parenchyma. No organisms were noted associated with this lesion. Similarly, numerous fish had a mild to moderate lymphocytic epicarditis that was often particularly cellular at the ventricular/bulbous notch. Many fish also had a mild to moderate and occasionally locally severe, lymphocytic (macrophages and plasma cells often common as well) submucosal gastritis. The spleen often contained excessive intracellular and extracellular hemosiderin (golden-brown pigment). The swim bladder occasionally contains few to several lymphoid foci.

The notable lesions and/or organisms are described in detail and pictures of these have been provided in most cases. Less notable alterations are occasionally included in the summary for lesions of each individual fish (see later).

The majority of the organisms are identified to the level of class with some exceptions, notably for the emerald shiner. Further work with available materials could narrow this further for the other species. Due to time constraints this work will not be included in the interim report for all species. For example, myxosporeans could be identified to genus in many cases by more detailed light microscopy including a broader range of special stains and electron microscopy of micro/myxosporeans would better identify these agents. Further sectioning of some of the metazoans would likely enable better anatomical detail and allow more precise classification. All measurements are approximations based on comparison with normal anatomic structures.

Intracardial trematode 'complex'

Intravascular metazoan 'eggs' – Gill, intravascular foreign body. Trematode egg, presumptive. There are few to numerous intravascular multicellular bodies that expand the lamellar pillar cell channel. The highly basophilic cells form a round ~7-10μm 'egg' packet that appears to be without a case. These same bodies are rarely (once S54) seen intimately associated with the multifocal myocardial lesions described below. ---- 27 of 60 affected

Multifocal necrotizing myocarditis – Myocarditis, multifocal, necrotizing.

There are multiple small hypercellular foci scattered in the myocardium of the atrium and ventricle. The bulbous is rarely affected. These have dense populations of small leukocytes (neutrophils presumably) and endocardial/myocardial cells that are undergoing degeneration and necrosis. Affected myocardial trabeculae are hypereosinophilic, often with loss of striations. In one fish, these are associated with multicelluar bodies similar to those described within intravascular channels in gill lamellae. There are no organisms associated with these lesions otherwise. ---- 39 of 60 affected

Proliferative endocarditis - Endocarditis, proliferative, focally extensive ---- 37 of 60 affected, with an intralesional trematode(s) ---- 9 of 60 affected.

There is a focally extensive hyperplastic plaque of hypertrophied endocardial cells subtended by fibrous hyperplasia. This is occasionally up to 100µm thick, is often thrown into papillary fronds and is typically found in the intraventricular lumen near the atrioventricular valves. The hyperplasia occasionally affects the

ventricular side of the valves and more uncommonly envelopes the valve and extends into the bulbous. There is cellular debris, fibrin and cellular thrombi, and rarely portions of a $\sim 100 \times 500 \mu m$ metazoan with a tegument and parenchymatous matrix but no calcareous corpuscles or intestine or cuticle (trematode, presumptive).

W Figures 1-4

Branchial protozoans - Branchitis, diffuse, with lamellar protozoans

There are single to very numerous (100's) of ~20x50um slightly dorsoventrally-flattened protozoans with a basophilic nucleus and thin ~2um multiple tentacles (these are not uniformly arranged and are opposite from the surface in contact with the epithelium) present on the lamellar epithelial surface. (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp (formerly *Trichophyra*).

Usually present in small to moderate numbers are similarly shaped and sized organism without stalks that appear to be holotrichous. Chilodonella or similar, presumptive (Phylum Ciliophora).

There are also small to very numerous $\sim\!25\mu m$ dorsoventrally flattened protozoans with a chitinous endoskeleton (denticular ring) and ring of cilia. *Trichodina* sp. (Phylum Ciliophora, Class Litostomatea, Family Trichodina)

Since these are common and numerous it was not practical to differentiate which fish had each of these protozoans. --- 36 of 56 affected

W Figure 5

Intestinal cestodes - Cestode, luminal intestinal

These are ~150-200µm wide and have a parenchymatous matrix and tegument, often thrown into distinct folds and occasionally have calcareous corpuscles (Phylum Platyhelminthes, Class Cestoidea). ---- 28 of 60 affected

W Figure 6a

Mural intestinal/gastric trematodiasis – Mural enteritis, multifocal, with intra-lesional trematodes. Single to multiple cross sections (200μm to 500μm) of a metazoan are encysted within various sections of the smooth muscle of the intestinal wall. The trematodes are characterized by having a thin eosinophilic tegument surrounding a parenchymatous matrix and the absence of calcareous corpuscles. In some sections, a digestive tract is present and in still others, reproductive organs are also present. The trematodes are surrounded by multiple layers of plump and attenuated macrophages (cyst wall), and variable, but often very large, numbers of more peripheral pigment-laden macrophages with fewer other leukocytes also present. (Phylum Platyhelminthes, Class Trematoda) ----- 3 of 60 affected (no photo)

W Figure 6b

Branchial crustacean - Branchitis, distal, focally extensive, hyperplastic, with (or without) intralesional crustaceans

Few to several filaments have regionally extensive epithelial hyperplasia that is particularly severe at the distal portion. There is a moderate infiltrate of morphologically mixed inflammatory cells surrounding a $\sim 400 \times 700 \mu m$ metazoan with a thin chitinous exoskeleton, jointed appendages with skeletal muscle and reproductive and digestive structures. The lesion is characteristic and was tallied even if the metazoan was not actually visible in the section (Phylum Arthropoda, Class Crustacea). ---- 6 of 60 affected

W Figure 7

Granulomatous enteritis - Granulomas, intestinal or gastric, lamina proprial to submucosal, focal to

There single to several, large up to ~1-2mm, masses composed of very large numbers of hypertrophied macrophages that surround eosinophilic amorphous content with necrotic cellular debris. Large of numbers of other leukocytes are present at the periphery of the lesion; which markedly expands the lamina propria/submucosa of the intestine, often resulting in effacement of the mucosa (artefactual in some cases), and protrusion into the lumen of the intestine. No organisms are typically present within these lesions however at least one has metazoan remnants present. The anatomic location and pattern of these granulomas are distinct from the mural trematodes (see below) suggesting that the lesions are caused by dissimilar organisms. ---- 3 of 60 affected

W Figure 8

Dermal sarcoma - Sarcoma, dermal, focal

The thinned epidermis is subtended by a markedly expanded dermis populated by cells that are spindle-shaped, are densely packaged in streams, have oval nuclei and eosinophilic cytoplasm with scant intercellular eosinophilic matrix. Mitotic figures are uncommon and areas of necrosis are present. One of these has strapshaped to bulbous cytoplasmic features resembling muscle cells. ---- 2 of 60 affected

W Figure 9

Lymphocystis – Cytomegaly, dermal fibroblasts with cytoplasmic iridoviral inclusions There are numerous markedly cytomegalic fibroblasts (~1-2mm in diameter) that expand the dermis and elevate the epidermis. The affected cells have a cytoplasm filled with basophilic granular material. There is a marked inflammatory response present between the virally-infected cells, (suggesting resolution). ---- 1 of 60 affected

W Figure 10

W1 -

Morphologic diagnosis

- 1. Gill, intravascular foreign body, few
- 2. Myocarditis, multifocal, necrotizing, mild
- 3. Endocarditis, proliferative, focally extensive, moderate with a single intralesional trematode
- 4. Branchitis, diffuse, with lamellar protozoans, numerous

Eye – There is a diffuse periscleral infiltration of lymphocytes. No organisms are present.

Morphologic diagnosis

1. Branchitis, diffuse, with lamellar protozoans, numerous.

W3-

- 1. Gill, intravascular foreign body, few
- 2. Branchitis, diffuse, with lamellar protozoans, few

- 3. Cestode, luminal intestinal, numerous (Phylum Platyhelminthes, Class Cestoidea)
- 4. Granulomas, intestinal, lamina proprial to submucosal, focal to multifocal
- 5. Mural enteritis, multifocal (Phylum Platyhelminthes, Class Trematoda)

W_{4} –

Morphologic diagnosis

- 1. Gill, intravascular foreign body, few
- 2. Myocarditis, multifocal, necrotizing, mild
- 3. Endocarditis, proliferative, focally extensive, moderate to severe with a single intralesional trematode
- 4. Branchitis, diffuse, with lamellar protozoans, few
- 5. Cestode, luminal intestinal, numerous (Phylum Platyhelminthes, Class Cestoidea)

W5 - NAF

$W_{6} -$

Morphologic diagnosis

1. Cestode, luminal intestinal, numerous (Phylum Platyhelminthes, Class Cestoidea)

W7-

Morphologic diagnosis

- 1. Gill, intravascular foreign body, few
- 2. Branchitis, diffuse, with lamellar protozoans, few

W8 -

Morphologic diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

W9-

Morphologic diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Branchitis, diffuse, with lamellar protozoans, moderate

W10 -

Morphologic diagnosis

- 1. Endocarditis, proliferative, focally extensive, mild
- 2. Branchitis, diffuse, with lamellar protozoans, few
- 3. Cestode, luminal intestinal, moderate (Phylum Platyhelminthes, Class Cestoidea)
- 4. Granulomas, gastric, lamina proprial to submucosal, focal to multifocal
- 5. Granulomas, hepatic, metazoan-type

W11 -

- 1. Gill, intravascular foreign body, few
- 2. Myocarditis, multifocal, necrotizing, mild
- 3. Endocarditis, proliferative, focally extensive

4. Branchitis, diffuse, with lamellar protozoans, few

W12 -

Morphologic diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Endocarditis, proliferative, focally extensive
- 3. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 4. Granulomas, gastric, lamina proprial to submucosal, focal to multifocal

W 13 -

Eye – There is a single lymphocytic focus in the choroidal rete.

Morphologic diagnosis

- 1. Gill, intravascular foreign body, few
- 2. Myocarditis, multifocal, necrotizing, mild
- 3. Endocarditis, proliferative, focally extensive, moderate to severe
- 4. Branchitis, diffuse, with lamellar protozoans, few
- 5. Granuloma, gastric, lamina proprial to submucosal, focal

W14 -

Morphologic diagnosis

- 1. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Granuloma, gastric, lamina proprial to submucosal, focal
- 3. Altered hepatocellular focus

W 15 -

Morphologic diagnosis

1. Gill, intravascular foreign body, few

W 16-

Morphologic diagnosis

- 1. Gill, intravascular foreign body, few
- 2. Myocarditis, multifocal, necrotizing, mild
- 3. Endocarditis, proliferative, focally extensive, extending into bulbous arteriosus, with a numerous intralesional trematodes
- 4. Cestode, luminal intestinal, moderate (Phylum Platyhelminthes, Class Cestoidea)

W17 -

Morphologic diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Endocarditis, proliferative, focally extensive, extending into bulbous arteriosus, with a single intralesional trematode
- 3. Branchitis, diffuse, with lamellar protozoans, few

W 18 -

Morphologic diagnosis

1. Myocarditis, multifocal, necrotizing, mild

- 2. Endocarditis, proliferative, focally extensive, moderate with a single intralesional trematode
- 3. Granuloma, gastric, submucosal, focal

W 19 -

Morphologic diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Endocarditis, proliferative, focally extensive, moderate.
- 3. Branchitis, diffuse, with lamellar protozoans, numerous
- 4. Branchitis, distal, focally extensive, hyperplastic, moderate
- 5. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

W20 -

Morphologic diagnosis

- 1. Myocarditis, multifocal, necrotizing, very mild
- 2. Branchitis, diffuse, with lamellar protozoans, numerous
- 3. Intestine, granuloma, focal, metazoan contents

W21 -

Morphologic diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Endocarditis, proliferative, focally extensive, moderate
- 3. Branchitis, diffuse, with lamellar protozoans, numerous
- 4. Cestode, luminal intestinal, numerous (Phylum Platyhelminthes, Class Cestoidea)

W 22 -

Morphologic diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Endocarditis, proliferative, focally extensive, mild
- 3. Branchitis, diffuse, with lamellar protozoans, few
- 4. Branchitis, distal, focally extensive, hyperplastic, moderate

<u>W 23</u> –

Morphologic diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Endocarditis, proliferative, focally extensive, severe

W 24 -

Morphologic diagnosis

- 1. Gill, intravascular foreign body, few
- 2. Myocarditis, multifocal, necrotizing, mild
- 3. Endocarditis, proliferative, focally extensive, severe with an intralesional trematode
- 4. Branchitis, diffuse, with lamellar protozoans, few
- 5. Cestode, luminal intestinal, numerous (Phylum Platyhelminthes, Class Cestoidea)

<u>W 25</u> -

- 1. Gill, intravascular foreign body, few
- 2. Endocarditis, proliferative, focally extensive, moderate with myocardial necrosis at the base of the AV valves
- 3. Branchitis, diffuse, with lamellar protozoans, few

W26 -

Morphologic diagnosis

- 1. Gill, intravascular foreign body, moderate
- 2. Endocarditis, proliferative, focally extensive, mild
- 3. Branchitis, distal, focally extensive, hyperplastic, moderate with intralesional crustaceans

W27 -

Morphologic diagnosis.

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Branchitis, diffuse, with lamellar protozoans, moderate
- 3. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

W28 -

There is a single focus of gliosis in the molecular layer of the optic lobe (W Figure 11).

Morphologic diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild to moderate
- 2. Endocarditis, proliferative, focally extensive, mild
- 3. Branchitis, diffuse, with lamellar protozoans, moderate
- 4. Branchitis, distal, focally extensive, hyperplastic, mild with intralesional crustaceans (Phylum Arthropoda, Class Crustacea)

W29 -

There are two wedge-shaped foci of necrosis in the compact myocardium and extending ~2mm into the spongy myocardium. Extensive hemorrhage is present. Resembles a recent penetrating wound.

Morphologic diagnosis

- 1. Myocarditis, multifocal, necrotizing, moderate
- 2. Endocarditis, proliferative, focally extensive, severe
- 3. Branchitis, diffuse, with lamellar protozoans, moderate

W30 -

Morphologic diagnosis

- 1. Myocarditis, multifocal, necrotizing, moderate
- 2. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

W31 -

Morphologic diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Endocarditis, proliferative, focally extensive, moderate
- 3. Cestode, luminal intestinal, numerous (Phylum Platyhelminthes, Class Cestoidea)

W32 -

- 1. Gill, intravascular foreign body, few
- 2. Myocarditis, multifocal, necrotizing, mild with a trematode in the ventricular lumen, but without proliferative endocarditis
- 3. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

W 33 -

Morphologic diagnosis

- 1. Gill, intravascular foreign body, few
- 2. Myocarditis, multifocal, necrotizing, mild
- 3. Endocarditis, proliferative, focally extensive, moderate to severe
- 4. Branchitis, diffuse, with lamellar protozoans, few
- 5. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

W 34-

Morphologic diagnosis

- 1. Gill, intravascular foreign body, few
- 2. Branchitis, distal, focally extensive, hyperplastic, moderate to severe with intralesional crustaceans (Phylum Arthropoda, Class Crustacea)
- 3. Cestode, luminal intestinal, numerous (Phylum Platyhelminthes, Class Cestoidea)

W35 -

Morphologic diagnosis

- 1. Gill, intravascular foreign body, numerous
- 2. Endocarditis, proliferative, focally extensive, moderate
- 3. Branchitis, diffuse, with lamellar protozoans, few

<u>W 36</u> –

Morphologic diagnosis

1. Branchitis, diffuse, with lamellar protozoans, numerous

W37 -

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Morphologic diagnosis

- 1. Endocarditis, proliferative, focally extensive, mild with portion of a metazoan
- 2. Cestode, luminal intestinal, numerous (Phylum Platyhelminthes, Class Cestoidea)
- 3. Dermis, fibrosarcoma

$W_{38} -$

Inter-renal tissue – There is diffuse vacuolation of the parenchyma.

Morphologic diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild to moderate
- 2. Endocarditis, proliferative, focally extensive, moderate to severe
- 3. Branchitis, diffuse, with lamellar protozoans, moderate
- 4. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

W39 -

Eye - There is sludging of leukocytes in the choroidal rete. There are also several 'intravascular bodies'

Morphologic diagnosis

- 1. Gill, intravascular foreign body, few
- 2. Endocarditis, proliferative, focally extensive, moderate to severe
- 3. Branchitis, diffuse, with lamellar protozoans, moderate

W40 -

Morphologic diagnosis

- 1. Gill, intravascular foreign body, numerous
- 2. Branchitis, diffuse, with lamellar protozoans, few
- 3. Cestodes, luminal, intestinal, numerous (Phylum Platyhelminthes, Class Cestoidea)

W41 -

Morphologic diagnosis

- 1. Gill, intravascular foreign body, few
- 2. Myocarditis, multifocal, necrotizing, moderate
- 3. Endocarditis, proliferative, focally extensive, moderate to severe
- 4. Branchitis, diffuse, with lamellar protozoans, very numerous
- 5. Dermal sarcomas, two (one has rhabdoid elements)

W42 -

Kidney – There is marked focally extensive proliferative arteritis (renal artery – similar in character to the proliferative endocarditis) with thrombosis and three intravascular trematodes

Morphologic diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Proliferative arteritis, renal artery with intravascular trematodes

W 43 -

Skeletal muscle – Intertwined between myofibres are several hypercellular foci of lymphocytes and macrophages. No organisms are present.

Morphologic diagnosis

1. Branchitis, diffuse, with lamellar protozoans, numerous

W 44 -

Stomach – There are two submucosal granulomas (metazoan-type) containing necrotic debris and one additional similar granuloma in the peripancreatic mesentery. No organisms were present.

Morphologic diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Endocarditis, proliferative, focally extensive, mild to moderate
- 3. Branchitis, diffuse, with lamellar protozoans, numerous

W45 -

- 1. Myocarditis, multifocal, necrotizing, moderate
- 2. Endocarditis, proliferative, focally extensive, moderate to severe
- 3. Branchitis, diffuse, with lamellar protozoans, few

W 46 -

Kidney – There are several multinucleate \sim 10µm organisms attached to the apical epithelium. No tissue reaction is present (prespore myxosporean or protozoan)

Morphologic diagnosis

- 1. Gill, intravascular foreign body, few
- 2. Myocarditis, multifocal, necrotizing, mild
- 3. Endocarditis, proliferative, focally extensive, moderate with intralesional trematode
- 4. Branchitis, diffuse, with lamellar protozoans, few
- 5. Branchitis, distal, focally extensive, hyperplastic, mild with intralesional crustaceans (Phylum Arthropoda, Class Crustacea)
- 6. Cestodes, luminal, intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

W47 -

Kidney – There are two foci, one that appears to be in the interstitium and the other that surrounds a glomerulus, that are \sim 60-80 μ m in diameter and are composed of structures similar to \sim 5-7 μ m multinucleate plasmodia arranged in rows. Presumptive myxosporean cyst containing unsporulated plasmodia. No spores and no inflammation is present.

Morphologic diagnosis

1. Cestode, luminal intestinal, numerous (Phylum Platyhelminthes, Class Cestoidea)

W 48 -

Morphologic diagnosis

- 1. Gill, intravascular foreign body, few
- 2. Myocarditis, multifocal, necrotizing, moderate
- 3. Branchitis, diffuse, with lamellar protozoans, few
- 4. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

W 49 -

Morphologic diagnosis

- 1. Gill, intravascular foreign body, few
- 2. Myocarditis, multifocal, necrotizing, mild
- 3. Endocarditis, proliferative, focally extensive
- 4. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

W50 -

000000000000000000000

Morphologic diagnosis

1. Branchitis, diffuse, with lamellar protozoans

W 51 -

Liver – There are several cavities that are rimmed by a thin layer of eosinophilic debris and/or edematous connective tissue (around pancreatic acini) infiltrated by moderate numbers of macrophages and other leukocytes. No other organisms are present.

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Endocarditis, proliferative, focally extensive
- 3. Branchitis, diffuse, with lamellar protozoans, few

W 52 -

Morphologic diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Branchitis, diffuse, with lamellar protozoans, numerous
- 3. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

W 53 -

Morphologic diagnosis

Endocarditis, proliferative, focally extensive

Branchitis, diffuse, with lamellar protozoans, numerous

W 54 -

Morphologic diagnosis

- 1. Gill, intravascular foreign body, moderate
- 2. Myocarditis, multifocal, necrotizing, mild
- 3. Endocarditis, proliferative, focally extensive, mild with an intralesional trematode
- 4. Branchitis, diffuse, with lamellar protozoans, numerous
- 5. Branchitis, distal, focally extensive, hyperplastic, mild with intralesional crustaceans (Phylum Arthropoda, Class Crustacea)

W55 -

Morphologic diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild to moderate
- 2. Endocarditis, proliferative, focally extensive, moderate to severe with several intralesional trematodes (several sections oriented to show clearly the flatworm shape and internal structures)
- 3. Branchitis, diffuse, with lamellar protozoans, numerous

W 56 -

Morphologic diagnosis

- 1. Gill, intravascular foreign body, few
- 2. Myocarditis, multifocal, necrotizing, mild
- 3. Endocarditis, proliferative, focally extensive, moderate
- 4. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

W57 -

Morphologic diagnosis

- 1. Gill, intravascular foreign body, moderate
- 2. Myocarditis, multifocal, necrotizing, mild
- 3. Endocarditis, proliferative, focally extensive, mild
- 4. Branchitis, diffuse, with lamellar protozoans, moderate
- 5. Mural enteritis, multifocal, with intra-lesional trematode (Phylum Platyhelminthes, Class Trematoda)
- 6. Cytomegaly, dermal fibroblasts with cytoplasmic iridoviral inclusions

W 58 -

- 1. Gill, intravascular foreign body, few
- 2. Myocarditis, multifocal, necrotizing, mild
- 3. Endocarditis, proliferative, focally extensive, moderate
- 4. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 5. Branchitis, distal, focally extensive, hyperplastic, moderate with intralesional crustaceans (Phylum Arthropoda, Class Crustacea)

W 59 -

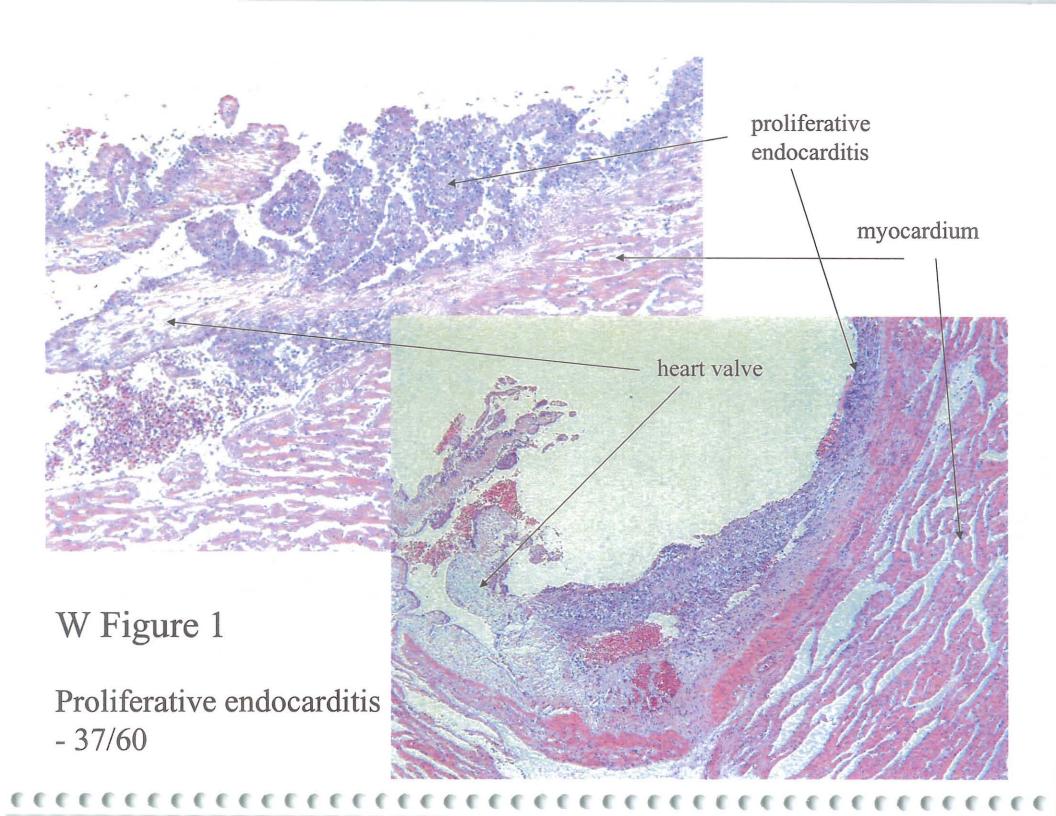
Intestine – There are two sections of a nematode, $\sim \! \! 150 \mu m$ in diameter with an eosinophilic cuticle and an intestine. No tissue reaction is visible.

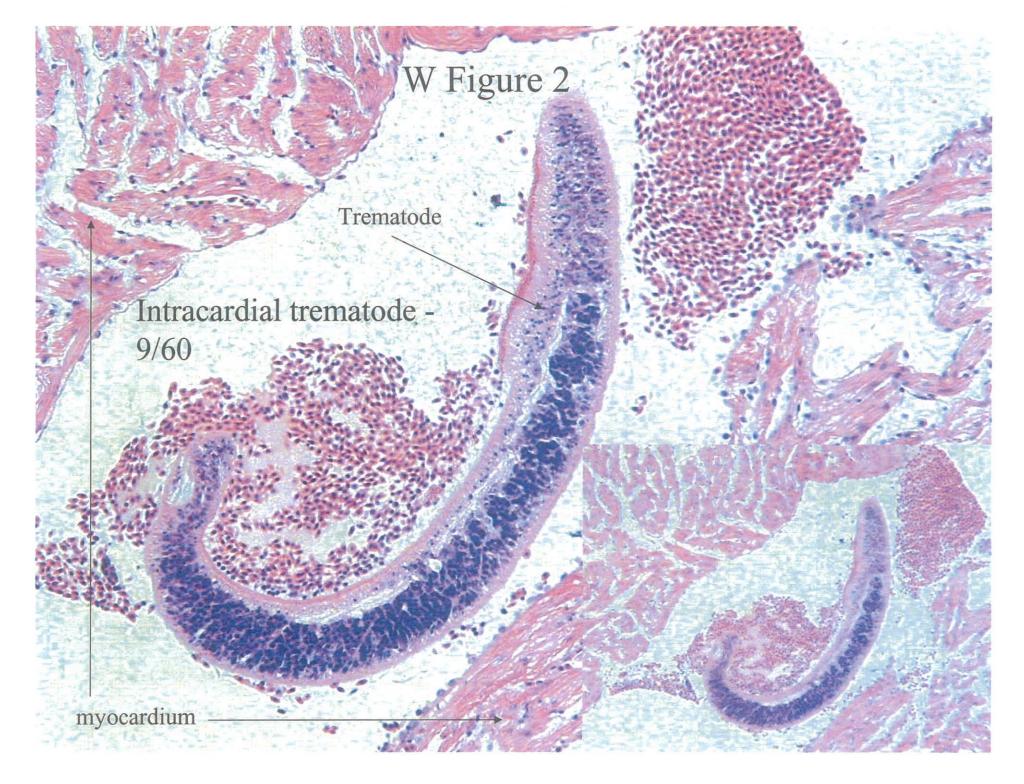
Morphologic diagnosis

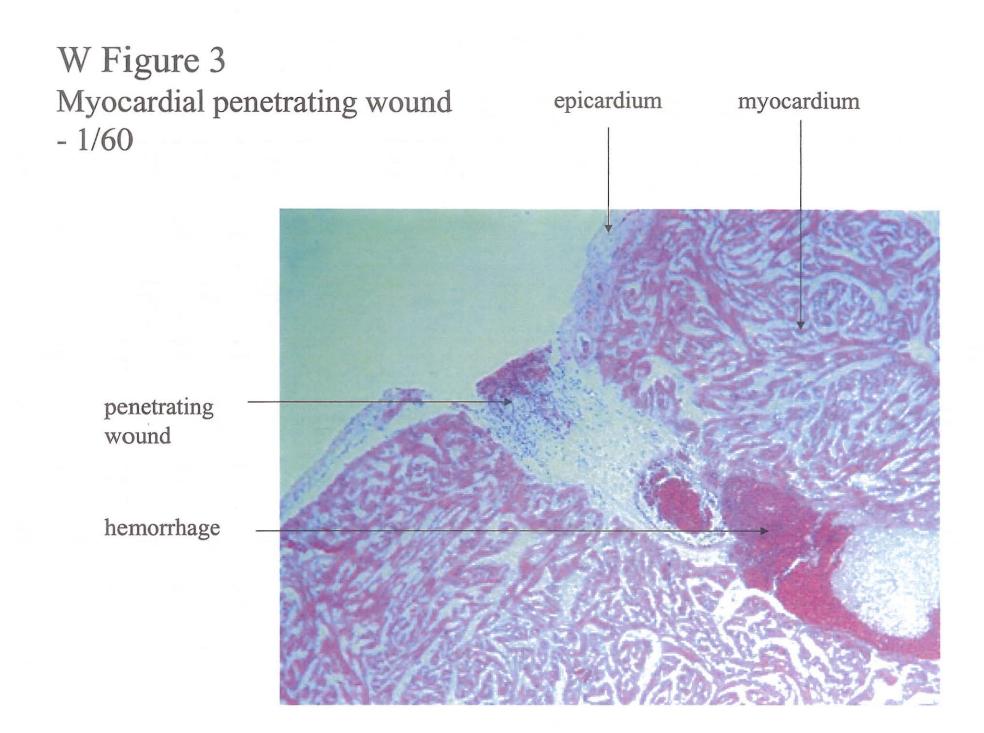
- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Endocarditis, proliferative, focally extensive, severe

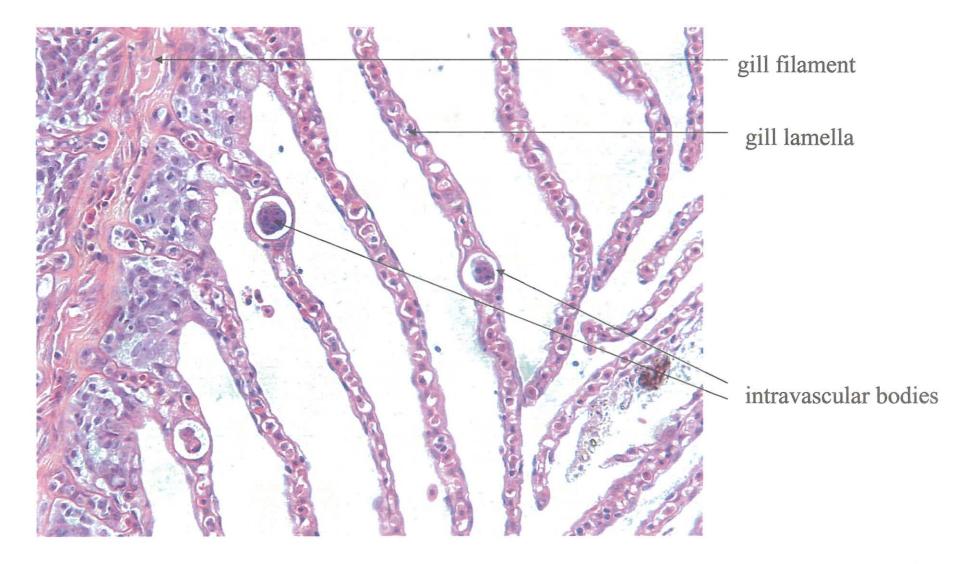
W 60 -

- 1. Gill, intravascular foreign body, few (one with a clear egg case)
- 2. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)





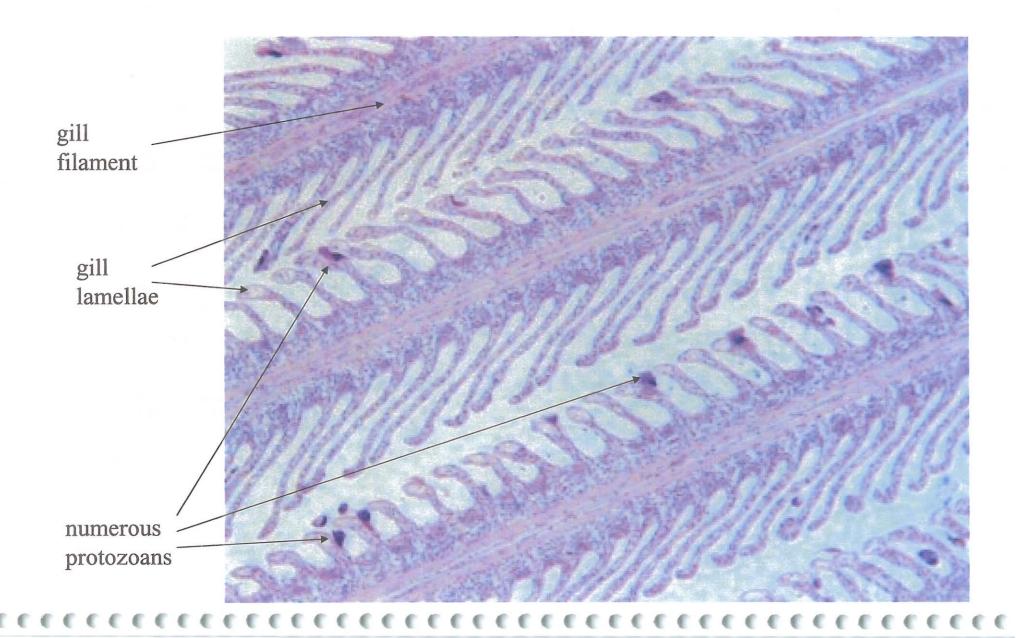




W Figure 4

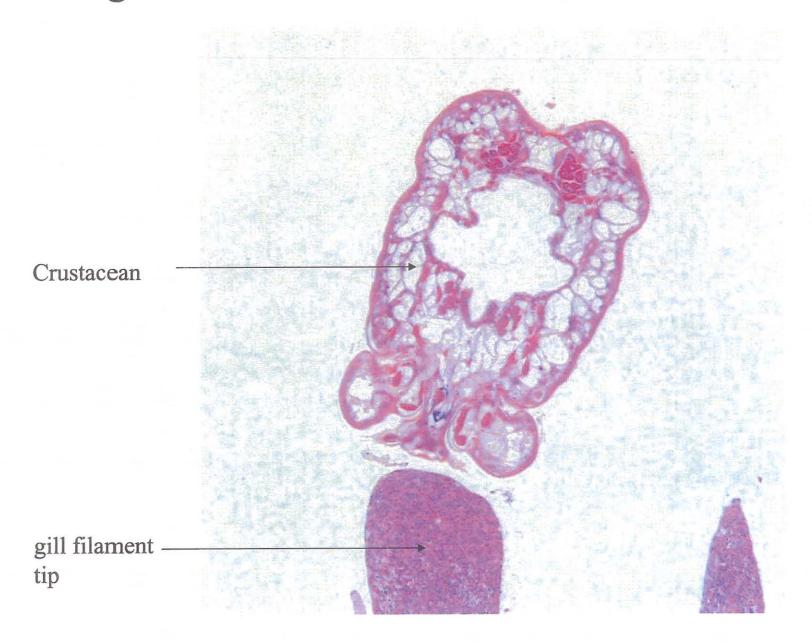
Branchial intravascular egg packets - 27/60

W Figure 5 Branchial protozoans - 36/60



6a. Luminal intestinal cestodes - 28/60 intestinal 6b. Gastric muscular lumen trematode - 3/60 histiocytic inflammation Cestode stomach wall Trematode W Figure 6

W Figure 7 Branchial crustaceans - 6/60

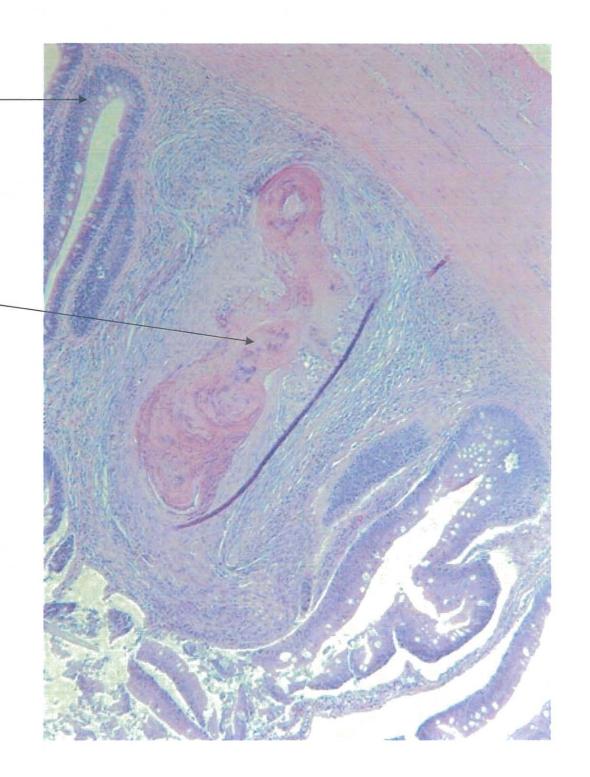


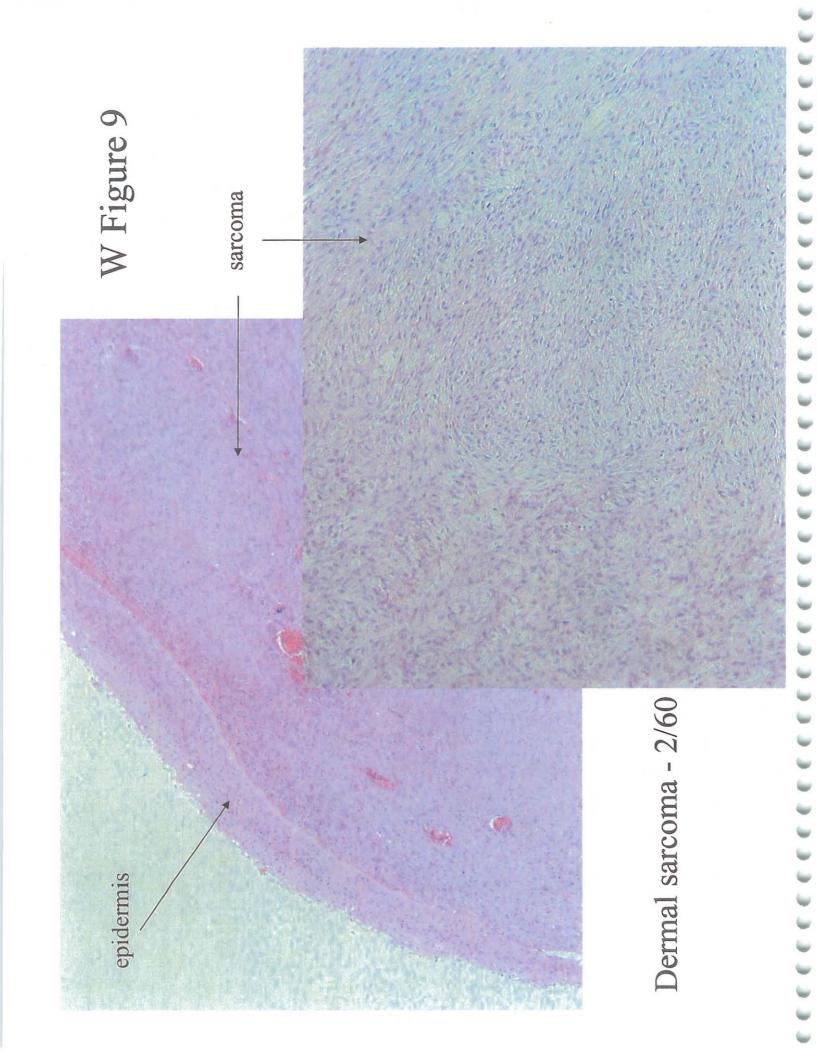
intestine

granuloma
with amorphous
eosinophilic
content (typical
of metazoan-type
granulomas)

W Figure 8

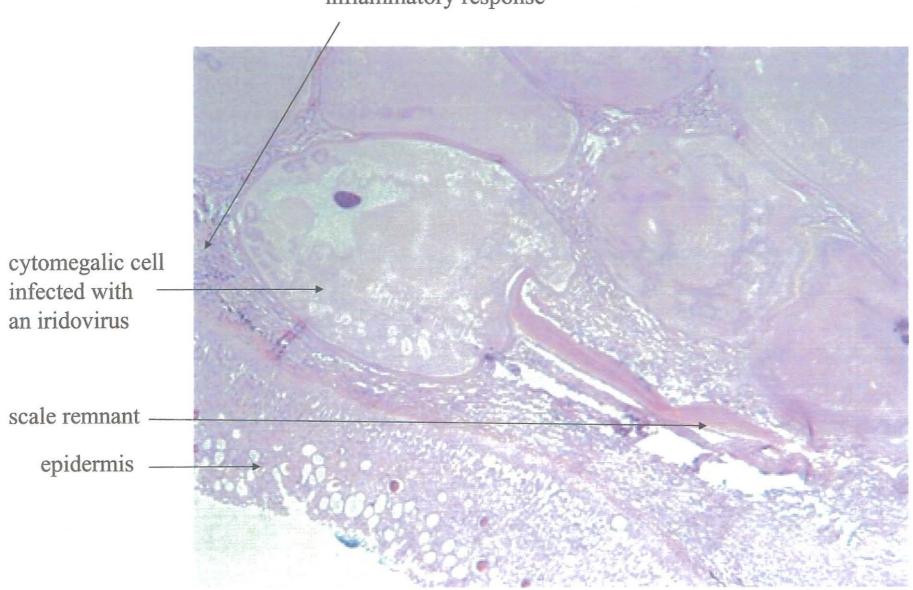
Granulomatous enteritis - 3/60





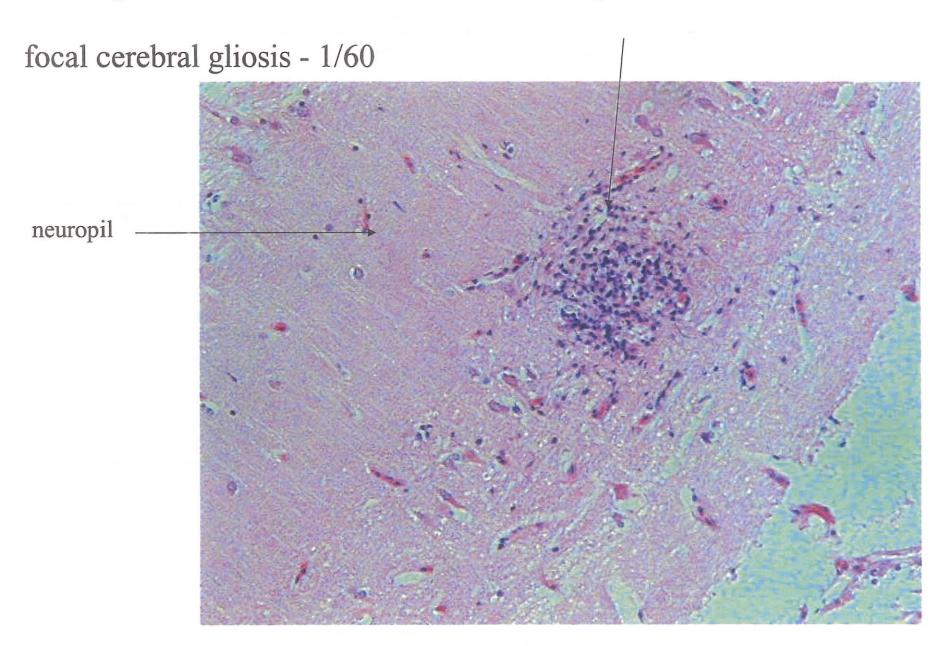
W Figure 10 Lymphocystis - 1/60

inflammatory response



W Figure 11

gliotic focus



Sauger (S - Sander canadensis)

Individual tissues were dissected in most cases. Tissues routinely examined included skeletal muscle, skin, spinal cord, brain kidney (caudal and less commonly cranial), liver, intestine, stomach, exocrine pancreas, heart, gills, swim bladder, and spleen. Spleen was the organ most often missing from the sections examined. Tissues less commonly or only occasionally in section included the endocrine pancreas, inter-renal and chromafin tissues and the corpuscles of Stannius.

The notable lesions and/or organisms are described in detail and pictures of these have been provided in most cases. Less notable alterations are occasionally included in the summary for lesions of each individual fish (see later).

The majority of the organisms are identified to the level of class with some exceptions, notably for the emerald shiner. Further work with available materials could narrow this further for the other species. Due to time constraints this work will not be included in the interim report for all species. For example, myxosporeans could be identified to genus in many cases by more detailed light microscopy including a broader range of special stains and electron microscopy of micro/myxosporeans could speciate these agents. Further sectioning of some of the metazoans would likely enable better anatomical detail and allow more precise classification. All measurements are approximations based on comparison with normal anatomic structures.

Intracardial trematode 'complex'

Intravascular metazoan 'eggs' – Gill, intravascular foreign body. Trematode egg, presumptive. There are few to numerous intravascular multicellular bodies that expand the lamellar pillar cell channel. The highly basophilic cells form a round ~7-10um 'egg' packet that appears to be without a case. These same bodies are rarely (once S54) seen intimately associated with the multifocal myocardial lesions described below. ----11 of 60 affected

Multifocal necrotizing myocarditis - Myocarditis, multifocal, necrotizing.

There are multiple small hypercellular foci scattered in the myocardium of the atrium and ventricle. The bulbous is rarely affected. These have dense populations of small leukocytes (neutrophils presumably) and endocardial/myocardial cells that are undergoing degeneration and necrosis. Affected myocardial trabeculae are hypereosinophilic, often with loss of striations. In one fish, these are associated with multicelluar bodies similar to those described within intravascular channels in gill lamellae. There are no organisms associated with these lesions otherwise. ---- 25 of 60 affected

Proliferative endocarditis - Endocarditis, proliferative, focally extensive ---- 6 of 60 affected, with a single intralesional trematode ---- 2 of 60 affected.

There is a focally extensive hyperplastic plaque of hypertrophied endocardial cells subtended by fibrous hyperplasia. This is occasionally up to 30-40 μ m thick and is typically found in the intraventricular lumen near the atrioventricular valves. The hyperplasia occasionally affects the ventricular side of the valves. There is cellular debris, fibrin and cellular thrombi, and rarely portions of a ~100x500 μ m metazoan with a tegument and parenchymatous matrix but no calcareous corpuscles or intestine (trematode, presumptive).

S Figure 1, 2 and 3

Intestinal cestodes - Cestode, luminal intestinal.

These are \sim 150-200 μ m wide and have a parenchymatous matrix and tegument and occasionally have calcareous corpuscles (Phylum Platyhelminthes, Class Cestoidea). ---- 25 of 60 affected

S Figure 4

Mural intestinal trematodiasis – Mural enteritis, multifocal, with intra-lesional trematodes.

Single to multiple cross sections (200µm to 500µm) of a metazoan are encysted within various sections of the smooth muscle of the intestinal wall. The trematodes are characterized by a thin eosinophilic tegument surrounding a parenchymatous matrix with the absence of calcareous corpuscles. In some sections, a digestive tract is present and in still others, reproductive organs are also present. The trematodes are surrounded by multiple layers of plump and attenuated macrophages (cyst wall), and variable number of more peripheral pigment-laden macrophages with fewer other leukocytes also present. Occasionally these encysted metazoans are also present within ceolomic connective tissue. (Phylum Platyhelminthes, Class Trematoda) ----- 11 of 60 affected

S Figure 5

Endomeningial trematodiasis - Trematode, endomeningial, encysted, focal

A single cross sections of a metazoan that is $\sim 100 \mu m$ in diameter is present within the ventricle of the brain. The trematode is encysted by single to few layers of flattened macrophages and characterized by a thin eosinophilic tegument and a parenchymatous matrix with no calcareous corpuscles (Phylum Platyhelmintheses, Class Trematoda). ----- 1 of 60 affected

S Figure 6

Intestinal nematodes – Nematodes, few, intestinal, luminal.

There were few to moderate numbers of thin $\sim 100 \mu m$ in diameter nematodes with a thin chitinous exoskeleton, and a thick muscular esophagus. An intestine was also occasionally visible. No host reaction was associated with the presence of these nematodes. (Phylum Nemathelminthes, Class Nematoda). ---- 1 of 60 affected

S Figure 7

Branchial protozoan - Trichodina sp., few, lamellar.

There were very small numbers of $\sim 20 \mu m$ protozoans that were dorsoventrally flattened, had cilia and a denticular ring. No tissue reaction was associated with the presence of these organisms (Phylum Ciliophora, Class Litostomatea, Family Trichodina). ---- 1 of 60 affected (no photo)

Branchial crustacean - Branchitis, distal, focally extensive, hyperplastic, with (or without) intralesional crustaceans

Few to several filaments have regionally extensive epithelial hyperplasia that is particularly severe at the distal portion. There is a moderate infiltrate of morphologically mixed inflammatory cells and mild necrosis surrounding a $\sim 400 \times 700 \mu m$ metazoan with a thin chitinous exoskeleton, jointed appendages with skeletal muscle and reproductive and digestive structures. The lesion is characteristic and was tallied even if the metazoan was not actually visible in the section (Phylum Arthropoda, Class Crustacea). ---- 25 of 60 affected

S Figure 8

S 1-

Morphological diagnosis

1. Branchitis, distal, focally extensive, hyperplastic, moderate with intralesional crustaceans (Phylum Arthropoda, Class Crustacea)

S 2-

Morphological diagnosis

- 1. Branchitis, distal, focally extensive, hyperplastic, moderate
- 2. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

S 3-

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Morphological diagnosis

1. Branchitis, distal, focally extensive, hyperplastic, moderate with intralesional crustaceans (Phylum Arthropoda, Class Crustacea)

S 4-

Morphological diagnosis

- 1. Gill, intravascular foreign body, few
- 2. Myocarditis, multifocal, necrotizing, mild

S 5-

Morphological diagnosis

1. Mural enteritis, multifocal, with intralesional trematode, few (Phylum Platyhelmintheses, Class Trematoda)

S 6-

Morphological diagnosis

1. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

S 7-

Morphological diagnosis

- 1. Branchitis, distal, focally extensive, hyperplastic, moderate with intralesional crustaceans (Phylum Arthropoda, Class Crustacea)
- 2. Cestode, luminal intestinal, moderate (Phylum Platyhelminthes, Class Cestoidea)

S 8-

Morphological diagnosis

- 1. Endocarditis, proliferative, focally extensive, moderate with a single intralesional trematode (Phylum Platyhelmintheses, Class Trematoda)
- 2. Myocarditis, multifocal, necrotizing, mild

S 9- NAF

S 10-

- 1. Mural enteritis, multifocal, with intralesional trematodes, moderate (Phylum Platyhelmintheses, Class Trematoda)
- 2. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

S 11-

Morphological diagnosis

1. Myocarditis, multifocal, necrotizing, mild

S 12-

Morphological diagnosis

- 1. Cestode, luminal intestinal, moderate (Phylum Platyhelminthes, Class Cestoidea)
- 2. Myocarditis, multifocal, necrotizing, mild
- 3. Branchitis, distal, focally extensive, hyperplastic, mild

S 13-

Morphological diagnosis

- 1. Endocarditis, proliferative, focally extensive, mild
- 2. Myocarditis, multifocal, necrotizing, mild
- 3. Cestode, luminal intestinal, moderate (Phylum Platyhelminthes, Class Cestoidea)

S 14-

Morphological diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Cestode, luminal intestinal, moderate (Phylum Platyhelminthes, Class Cestoidea)
- 3. Branchitis, distal, focally extensive, hyperplastic, mild

S 15-

Morphological diagnosis

- 1. Myocarditis, multifocal, necrotizing, moderate to severe
- 2. Gill, intravascular foreign body, few
- 3. Branchitis, distal, focally extensive, hyperplastic, mild with intralesional crustaceans (Phylum Arthropoda, Class Crustacea)

S 16-

Morphological diagnosis

1. Branchitis, distal, focally extensive, hyperplastic, mild with intralesional crustaceans (Phylum Arthropoda, Class Crustacea)

w

2. Mural enteritis, multifocal, with intralesional trematodes, few (Phylum Platyhelmintheses, Class Trematoda)

S 17-

Morphological diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 3. Branchitis, distal, focally extensive, hyperplastic, moderate

S 18-

Morphological diagnosis

1. Gill, intravascular foreign body, few

S 19-

Morphological diagnosis

- 1. Endocarditis, proliferative, focally extensive with a single intralesional trematode (Phylum Platyhelmintheses, Class Trematoda)
- 2. Myocarditis, multifocal, necrotizing, mild
- 3. Mural enteritis, multifocal, with intralesional trematodes, moderate (Phylum Platyhelmintheses, Class Trematoda)
- 4. Branchitis, distal, focally extensive, hyperplastic, moderate with intralesional crustaceans (Phylum Arthropoda, Class Crustacea)

S 20-

Morphological diagnosis

- 1. Gill, intravascular foreign body
- 2. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

S 21-

Stomach – There is serosal mesothelial proliferation and focal submucosal histiocytic inflammation. No organisms are present.

Morphological diagnosis

- 1. Myocarditis, multifocal, necrotizing, moderate
- 2. Branchitis, distal, focally extensive, hyperplastic, moderate with intralesional crustaceans (Phylum Arthropoda, Class Crustacea)
- 3. Gastritis, histiocytic, focal

S 22-

Morphological diagnosis

- 1. Gill, intravascular foreign body, few
- 2. Branchitis, distal, focally extensive, hyperplastic, mild

S 23- NAF

S 24-

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Morphological diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Branchitis, distal, focally extensive, hyperplastic, mild

S 25-

There is a moderate infiltrate of lymphocytes and macrophages in the epicardium. No organisms are present. There is a mild submucosal, macrophage-rich gastritis. No organisms are present.

- 1. Nematodes, few, intestinal, luminal (Phylum Nemathelminthes, Class Nematoda)
- 2. Epicarditis, lymphohistiocytic, moderate
- 3. Gastritis, histocytic

S 26-

Intestine - There is a single metazoan-type granuloma at the base of the mucosa. No organisms are present. Morphological diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 3. Granulomas, intestinal, focal

S 27- NAF

S 28-

Connective tissue – There is a large $\sim 500 \mu m$ thin-walled granuloma with amorphous basophilic contents. No organisms are present.

Skeletal muscle – There is a focally extensive histocytic myositis that encompasses several small discrete granulomas. These resemble metazoan-type granulomas.

Morphological diagnosis

- 1. Myocarditis, multifocal, necrotizing, moderate
- 2. Cestode, luminal intestinal, moderate (Phylum Platyhelminthes, Class Cestoidea)
- 3. Granulomas, connective tissue, multifocal
- 4. Myositis, focal histiocytic with multifocal granulomas

S 29-

Intestine - There are several metazoan-type granulomas present at the base of lamina propria.

Morphological diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Mural enteritis, multifocal, with intralesional trematodes, numerous (Phylum Platyhelmintheses, Class Trematoda)
- 3. Granulomas, intestinal, multifocal

S 30-

Morphological diagnosis

- 1. Cestode, luminal intestinal, numerous (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, distal, focally extensive, hyperplastic, mild

S 31-

Morphological diagnosis

- 1. Endocarditis, proliferative, focally extensive, mild
- 2. Myocarditis, multifocal, necrotizing, moderate to severe
- 3. Gill, intravascular foreign body, few
- 4. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 5. Trichodina sp., few, lamellar (Phylum Ciliophora, Class Litostomatea, Family Trichodina)

S 32-

Morphological diagnosis

1. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

S 33 – NAF

S 34-

Kidney - There are numerous metazoan-type granulomas. No organisms are present.

Morphological diagnosis

- 1. Myocarditis, multifocal, necrotizing, moderate
- 2. Branchitis, distal, focally extensive, hyperplastic, mild
- 3. Mural enteritis, multifocal, with intralesional trematodes, few (Phylum Platyhelmintheses, Class Trematoda)
- 4. Granulomas, renal, interstitial, multifocal

S 35-

Morphological diagnosis

- 1. Gill, intravascular foreign body, few
- 2. Branchitis, distal, focally extensive, hyperplastic, moderate with intralesional crustaceans (Phylum Arthropoda, Class Crustacea)

S 36-

Morphological diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Mural enteritis, multifocal, with intralesional trematodes, moderate and Ceolomitis, multifocal, granulomatous, with intralesional trematodes, moderate (Phylum Platyhelmintheses, Class Trematoda)

S 37- NAF

S 38-

Ceolomic fat – There is a single histiocyte-rich focus of inflammation. No organisms are present.

Morphological diagnosis

- 1. Mural enteritis, multifocal, with intralesional trematodes, numerous (Phylum Platyhelmintheses, Class Trematoda)
- 2. Steatitis, focal, histiocytic

S 39-

Morphological diagnosis

1. Cestode, luminal intestinal, moderate (Phylum Platyhelminthes, Class Cestoidea)

S 40-

Morphological diagnosis

1. Myocarditis, multifocal, necrotizing, mild

S 41-

Morphological diagnosis

- 1. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, distal, focally extensive, hyperplastic, moderate with intralesional crustaceans (Phylum Arthropoda, Class Crustacea)

S 42-

Ceolomic fat – There is a single histiocyte-rich focus of inflammation. No organisms are present.

Morphological diagnosis

1. Steatitis, focal, histiocytic

S 43-

Morphological diagnosis

1. Gill, intravascular foreign body, few

S 44-

Morphological diagnosis

- 1. Endocarditis, proliferative, focally extensive, mild
- 2. Myocarditis, multifocal, necrotizing, mild to moderate
- 3. Branchitis, distal, focally extensive, hyperplastic, moderate with intralesional crustaceans (Phylum Arthropoda, Class Crustacea)
- 4. Mural enteritis, multifocal, with intralesional trematodes, few (Phylum Platyhelmintheses, Class Trematoda)
- 5. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

S 45-

Morphological diagnosis

- 1. Branchitis, distal, focally extensive, hyperplastic, mild
- 2. Myocarditis, multifocal, necrotizing, mild

S 46-

Liver – There are several metazoan-type granulomas in the hepatic parenchyma that marginally compress the surrounding tissue. No organisms are present.

Morphological diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Granulomas, hepatic, multifocal

S 47- NAF

S 48-

Morphological diagnosis

- 1. Branchitis, distal, focally extensive, hyperplastic, mild with intralesional crustaceans (Phylum Arthropoda, Class Crustacea)
- 2. Cestode, luminal intestinal, moderate (Phylum Platyhelminthes, Class Cestoidea)

S 49-

Morphological diagnosis

- 1. Extensive proliferative endocarditis (and bulbitis)
- 2. Myocarditis, multifocal, necrotizing, moderate
- 3. Gill, intravascular foreign body, few
- 4. Branchitis, distal, focally extensive, hyperplastic, mild
- 5. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

S 50-

Morphological diagnosis

1. Branchitis, distal, focally extensive, hyperplastic, mild

S 51-

Morphological diagnosis

1. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

S 52-

Morphological diagnosis

1. Branchitis, distal, focally extensive, hyperplastic, mild with intralesional crustaceans (Phylum Arthropoda, Class Crustacea)

S 53-

Morphological diagnosis

- 1. Gill, intravascular foreign body, few
- 2. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)
- 3. Branchitis, distal, focally extensive, hyperplastic, mild

S 54-

Morphological diagnosis

- 1. Gill, intravascular foreign body, moderate
- 2. Myocarditis, multifocal, necrotizing, moderate with similar structures to those found in the gill 'intravascular bodies', suspect trematode egg packets
- 3. Mural enteritis, multifocal, with intralesional trematodes, few (Phylum Platyhelmintheses, Class Trematoda)
- 4. Trematode, likely endomeningial, encysted, focal (Phylum Platyhelmintheses, Class Trematoda)

S 55-

Morphological diagnosis

- 1. Cestode, luminal intestinal, moderate (Phylum Platyhelminthes, Class Cestoidea)
- 2. Branchitis, distal, focally extensive, hyperplastic, mild
- 3. Gill, intravascular foreign body, few

S 56-

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Heart - There is a single epicardial, metazoan-type granuloma. No organisms are present.

Heart - There is an influx of lymphocytes and fewer macrophages in the epicardium.

Morphological diagnosis

- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Epicarditis, lymphohistiocytic, moderate
- 3. Granuloma, epicardial, focal

S 57-

- 1. Branchitis, distal, focally extensive, hyperplastic, mild with intralesional crustaceans (Phylum Arthropoda, Class Crustacea)
- 2. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

S 58-

Kidney - There is a single focus of tightly-packed tubules that have a moderately expanded epithelium. Each hypertrophied epithelial cell contains multiple, often multinucleate, $\sim \! \! 10 \mu m$ in diameter protozoans. Morphological diagnosis

- 1. Branchitis, distal, focally extensive, hyperplastic, moderate with intralesional crustaceans (Phylum Arthropoda, Class Crustacea)
- 2. Mural enteritis, multifocal, with intralesional trematodes, moderate (Phylum Platyhelmintheses, Class Trematoda)
- 3. Cestode, luminal intestinal, moderate (Phylum Platyhelminthes, Class Cestoidea)
- 4. Nephritis, intraepithelial, tubular with intracellular protzoans

S 59-

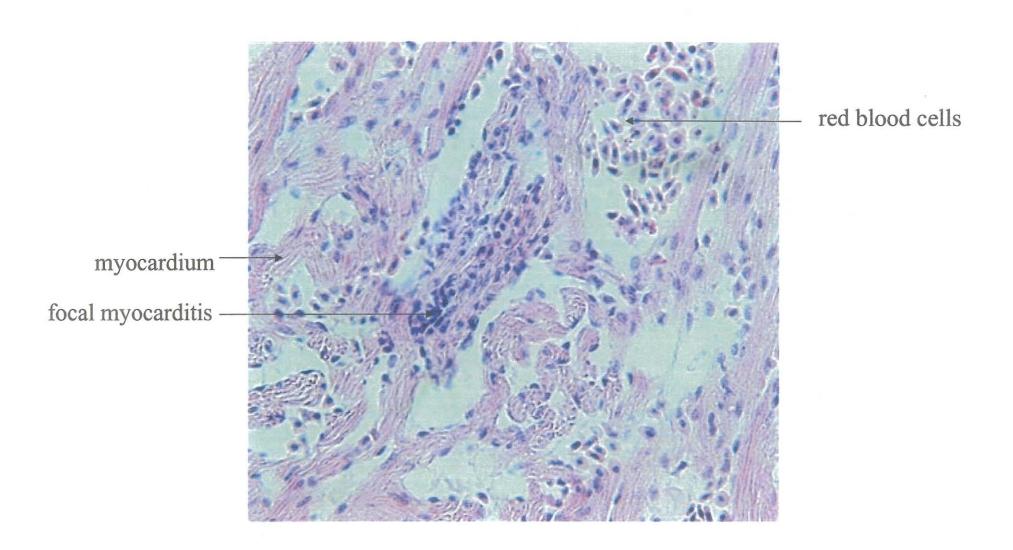
Morphological diagnosis

- 1. Endocarditis, proliferative, focally extensive, mild
- 2. Myocarditis, multifocal, necrotizing, mild
- 3. Branchitis, distal, focally extensive, hyperplastic, mild with intralesional crustaceans (Phylum Arthropoda, Class Crustacea)

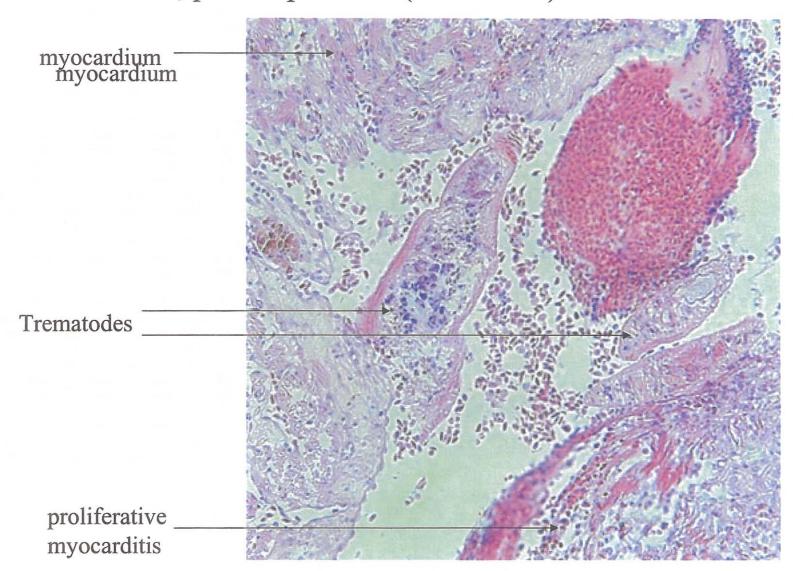
S 60-

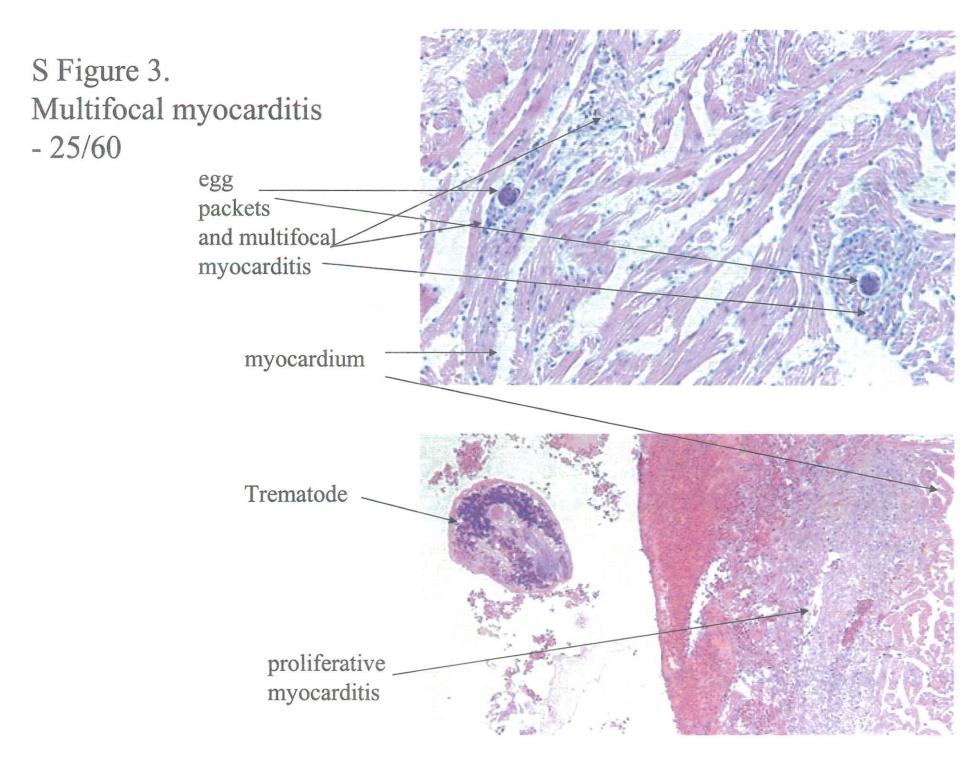
- 1. Myocarditis, multifocal, necrotizing, mild
- 2. Cestode, luminal intestinal, few (Phylum Platyhelminthes, Class Cestoidea)

S Figure 1. Multifocal myocarditis

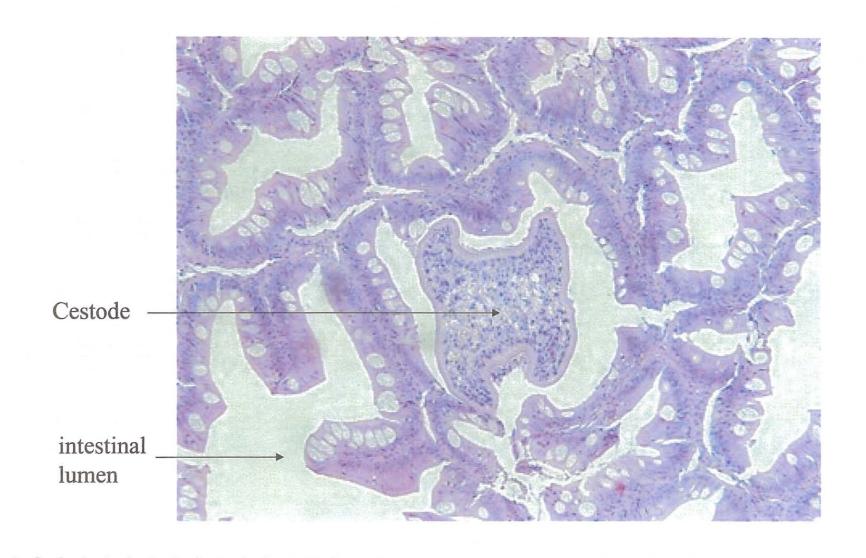


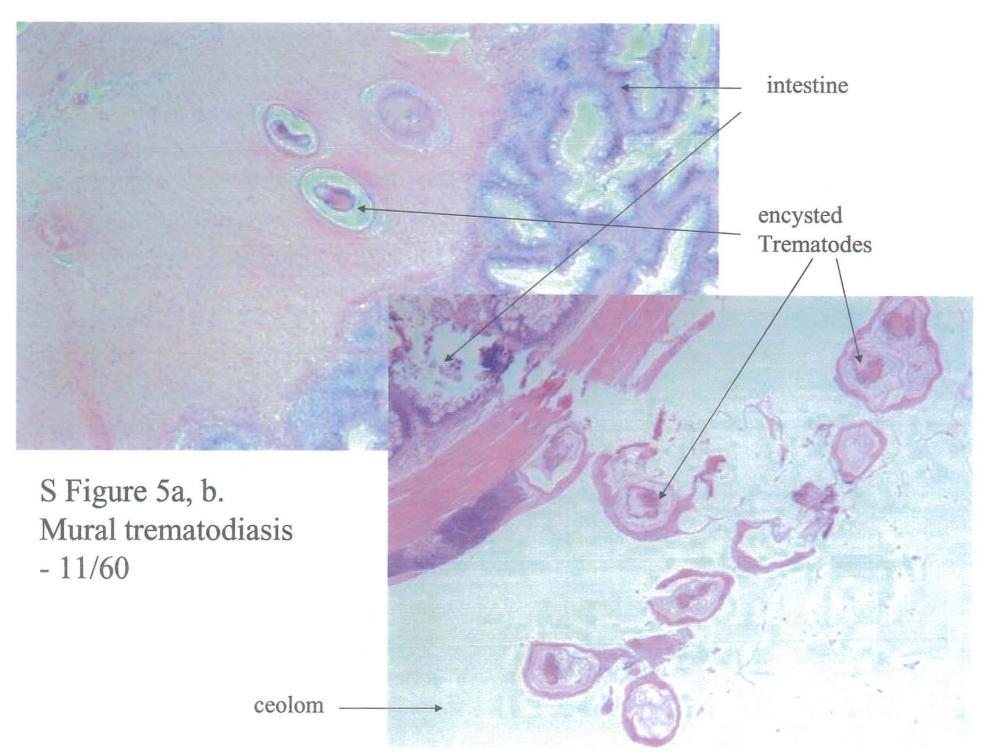
S Figure 2.
Proliferative endocarditis - 6/60
Intracardial trematode, presumptive - 2 (of 6 above)/60

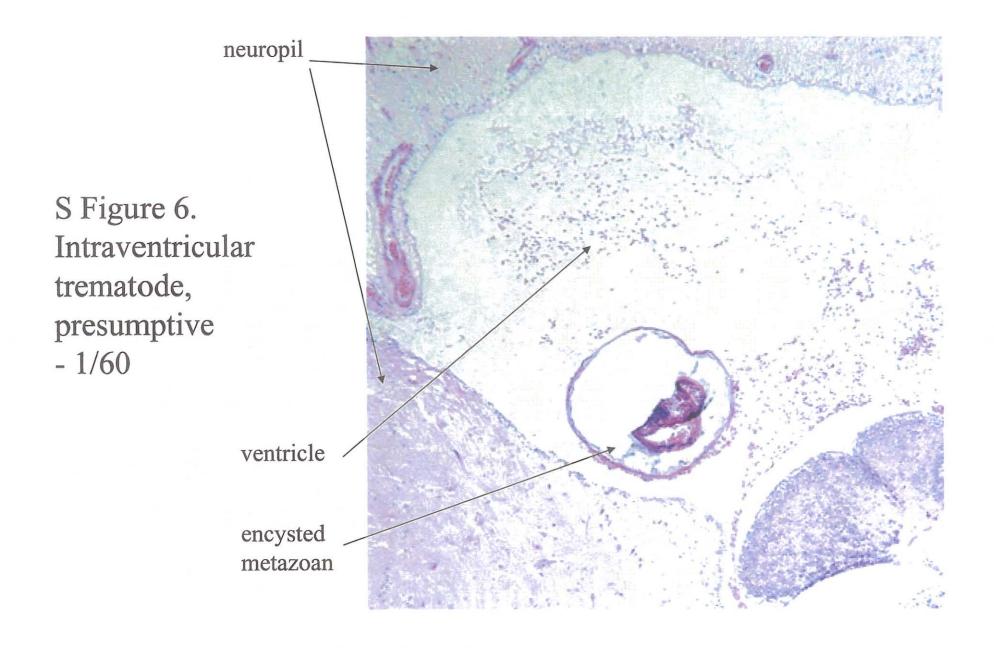




S Figure 4. Intestinal cestodiasis - 25/60







S Figure 7 - Intestinal nematodiasis - 1/60

