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

FINAL REPORT FOR LAKE WINNIPEG FISH HEALTH SURVEY – 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;
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 was to perform a fish health survey of 10 species of fish from Lake Winnipeg using light microscopy. Sixty fish from each species were examined in summer of 2006 and an additional 30 fish of each species from the spring of 2007.

Summaries for each species, including a table of lesions and/or organisms noted, are found 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. Organisms or lesions that affected fish at both time points examined are listed first (see methods); followed by those that were noted only in summer and finally those noted only from fish examined during the spring. For each of these a morphologic diagnosis (summary phrase) is also included. The majority of these lesions/organisms are illustrated with a photograph. The remainder of the document is a list of the morphological diagnoses that were noted for each individual fish. Fish examined during the summer (2006) are listed first and those examined during the spring (2007) are listed last. For easier orientation when scrolling through the document, fish examined during the summer are underlined and those examined during the spring are not. Occasionally, a short description is given for an additional lesion affecting a fish that wasn't common or more noteworthy.

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 by Dr. Terry Dick, University of Manitoba. All of the types of fish examined by light microscopy here had intestinal cestodes likely representing numerous different metazoan species. 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 possible 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, protozoans, etc.

Lesions associated with bacterial and viral agents were rare. Evidence for bacterial infections were limited to epitheliocystis (chlamydia-like intracellular bacteria) in white bass, yellow perch, fathead minnow and channel catfish. These organisms produce microcolonies, typically in the gill epithelium, that are visible by light microscopic sections. Evidence for virus was limited to the detection of lymphocystis in walleye, caused by a piscine iridovirus that produces characteristic histological lesions and dermal sarcomas in two walleye. Similar lesions are caused by the walleye dermal sarcoma virus; an oncogenic retrovirus that has a wide distribution in North America. The lymphocyte-intense lesion noted in the dermis of one Northern pike may be an early lymphosarcoma, which in this species can be caused by a separate oncogenic retrovirus.

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

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 of those found that would likely have the greatest impact on fish health (but this could still be relatively minimal) is listed for each species below.

Those agents judged to have the most significant impact on fish health for each species are;
Brook stickleback – intralenticular *Diplostomulum* sp., although very few fish were affected.
Channel catfish – no clear agent.
Emerald Shiner – branchial myxosporeans or intestinal cestodes.
Fathead minnow – unclear or possibly hepatic nematodes.
Goldeye – intestinal cestodes.
Northern pike – unclear.
Sauger – intracardial *Sanguinicola* sp.,
Walleye – intracardial *Sanguinicola* sp.
White bass – branchial monogenetic trematodes and/or epitheliocystis and/or intestinal cestodes.
Yellow perch – intestinal cestodes.

Finally, even with the limited utility of light microscopy for exact classification of parasites, there appears to be numerous organisms identified in this study that are either not previously recorded for a species of fish or not recorded from a particular species within the Hudson Bay watershed that includes Lake Winnipeg. An exhaustive literature review and in many cases coupled with additional efforts, such as electron microscopy, would be required to clarify the exact status of the organisms in question.

Methods

Ten species of fish: brook stickleback (*Culea inconstans* – **BS**), emerald shiner (*Notropis atherinoides* – **ES**), fathead minnow (*Pimephales promelas* – **FM**), yellow perch (*Perca flavescens* – **YP**), goldeye (*Hiodon allosoides* – **G**), Northern pike (*Esox lucius* – **NP**), white bass (*Morone chyrsops* – **WB**), channel catfish (*Ictalurus punctatus*- **CC**), walleye (*Sander vitreus* – **W**), and sauger (*Sander canadensis* – **S**) were examined by light microscopy. Sixty fish were examined during the summer of 2006 and thirty additional fish of each species were examined during the spring of 2007. The exceptions to these numbers were that only seven channel catfish were caught during the summer of 2006 and no brook stickleback were caught during the spring of 2007.

Protocols for dissection, processing and trimming of tissues are outlined in Appendix 1. Specific comments on the tissues, orientation and trimming techniques used for each fish species are summarized at the start of each section. Formalin-fixed tissues were embedded in paraffin and processed by routine methods, sectioned at 5 mm and stained with hematoxylin and eosin (H&E) unless indicated otherwise. Giemsa stains were also performed on selected tissues.

The majority of the organisms are identified to the level of class with some exceptions, where further specificity is possible. Giemsa staining allowed better definition of the morphology of some of the myxosporeans. Electron microscopy of micro/myxosporeans would be required to completely classify these agents. This is painstaking and time consuming work since many of the spores/cysts/lesions are easily lost with additional processing. 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. Measurements taken from fixed tissues are generally smaller than expected due to tissue shrinkage during fixation and processing.

Apart from the classical techniques (bacteriology, virology, parasitology) used as part of the larger study, the most precise way to determine identity, and to be absolutely certain that any organisms identified in the United States and Canada are indeed 'identical' would be to obtain sequence of 16S ribosomal DNA from organisms either cultured (bacteria), isolated (parasites), or observed (light microscopy). Laser microdissection of organisms observed in section is becoming a more widely used technique. We intend to use laser microdissection to attempt to further identify some of the organisms that have been found using light microscopy. This effort is well beyond the time and funding available for the present contract and the required sequences are still being generated for many groups of organisms.

For the purposes of this report, organisms are treated as 'similar' based on classification as achieved using light microscopy here and tissue location. For example, cestodes and trematodes, with very few exceptions, cannot be classified further in 5 mm sections. If cestodes found in the intestine of the yellow perch were present during both the summer and spring collections they were classified as 'similar' organisms here to allow some degree of comparison, however superficial.

The literature sources used were primarily: 1. Hoffman GL. Parasites of North American Freshwater Fishes, 2nd edition, 1999; 2. Margolis L. and Arthur JR. Synopsis of the Parasites of Fishes of Canada. 1979; 3. McDonald TE and Margolis L. Synopsis of the Parasites of Fishes of Canada. Supplement (1978 - 1993) and 4. Dick TA, Choudhury A, Souter B. Parasites and pathogens of fishes in the Hudson Bay drainage. 2001. In addition, Google and two abstract search engines, CAB abstracts and Biological abstracts, were used. In certain cases, e.g.

microsporeans, myxosporeans, epitheliocystis, *Sanguinicola* sp., *Diplostomum* sp., etc. a more extensive literature search was performed. There may be errors or omissions since the search was not exhaustive. A comprehensive literature review was beyond the scope of the contract and only a limited number of organisms and/or lesions will be discussed further. If an organism was identified or highly likely identified to the genus level or was notable for another reason, a brief review and discussion is included below. Otherwise select comparisons for a class of organism (e.g. summer vs. spring) are included.

Four species of fish surveyed were noted to have epitheliocystis. This included the channel catfish, fathead minnow, white bass and the yellow perch. A single channel catfish and fathead minnow was found to be infected and small numbers of yellow perch (4/60; 3/30), however infection was common in the white bass (23/59; 2/30). The channel catfish has been previously documented to have epitheliocystis (Zimmer et al. 1984). To my knowledge the other three species of fish have not previously been noted to have epitheliocystis. Epitheliocystis is a term used for an infection with a gram-negative, facultative intracellular chlamydia-like bacteria that is typically restricted to the epithelium of the gills and skin (for a review see Nowak and LaPatra, 2006). The morphology of the microcolonies are fairly characteristic, however it is not known if more than one bacterial species can infect the same species of fish. Over 50 species of freshwater and marine fish have been noted to be affected by epitheliocystis (Nowak and LaPatra, 2006; Paperna and Sabnai, 1980; Fryer and Lannan, 1994), however this number would increase markedly if further effort was made to perform light microscopy more routinely. Since the organisms have yet to be cultured, it is difficult to fulfill Koch's postulates or perform research to answer many of the unknowns associated with these organisms. Recent molecular research has been revealing.

There have been surveys performed consisting of moderate to large numbers of fish of a given species where epitheliocystis has not been detected (Nowak and LaPatra, 2006). It is likely that most species of fish can be infected by a chlamydia-like bacterium. Infections are highly species specific and generalizations regarding pathogenesis, epidemiology, etc. should be dealt with cautiously (Nowak and LaPatra, 2006).

Surveys to examine the seasonal prevalence of epitheliocystis have been performed, mostly in salmonids (Frances, Nowak, Allan 2000). Atlantic salmon were more heavily infected during the summer (at higher water temperatures) while the opposite has also been found for cultured amberjack where infection was highest at low water temperatures (Crespo et al. 1990). The only species in the present survey that had a sufficient rate of infection was the white bass in which fish examined from the summer sampling were commonly affected. As well, mixed gill infections (infection with multiple different organisms) were the rule in white bass, and epitheliocystis mixed with other branchial infections has also been frequently recorded (Nowak and LaPatra, 2006), although it should be noted that mixed branchial infections are commonly noted without including epitheliocystis. Three common organisms were noted in the white bass during the summer; protozoans, epitheliocystis and a monogenetic trematode.

The significance of infection with epitheliocystis is often difficult to determine and there are conflicting claims to their pathogenicity (reviewed by Nowak and LaPatra, 2006). The majority of infections noted are limited to small numbers of organisms with mild lesions. These cases are likely of minimal clinical significance; similar to the yellow perch and fathead minnow in the present survey. Very large numbers can produce proliferative lesions, which can even be seen grossly, with a severe influx of inflammatory cells and variable necrosis and mortality. The white

bass in the present study had numerous agents detected in the gills, however the anatomic placement and pattern of tissue damage suggest that the epitheliocystis was affecting gill function (clinical significance is uncertain). The lesions present suggest explosive 'release' of microcolonies as a mechanism of spread to the environment.

Of those agents specifically noted for each fish species above, 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 substantial splenic hemosiderin accumulation, and would be less fit. A morphologically similar trematode and lesion was also seen in the sauger, however the number of fish affected and the severity of the lesions were less.
The adult trematode found in the heart and blood vessels of the walleye and sauger are likely a *Sanguinicola* sp. based on the intravascular location, the small size of the worm (approx. 1-1.3 mm), the lack of a sucker, the eggs containing miricidia in the gills of infected fish and with the eggs having very thin walls. The preservation of the worms in the sauger was relatively poor and there were fewer to examine in section so the majority of these characteristics are best visible in the specimens in the walleye. *Sanguinicola occidentalis* has been described in the heart of walleye several times including in the states of Wisconsin (Fischthal 1952; in Hoffman, 1999) and New York (Van Cleave and Mueller, 1932; in Hoffman, 1999) but more recently from Lake Erie where 5 of 27 walleye examined were infected (Dechtiar 1972) and from the Lakes of Ontario, Superior and Huron (Dechtiar and Christie, 1988; Dechtiar and Lawrie, 1988; Dechtiar et al. 1988, respectively). There is no record of any *Sanguinicola* detected from sauger to my knowledge. *S. occidentalis*, which is also found in the heart, gills and the body cavity of the yellow perch (Muzzall, 2000), has been described to be a pathogen however this is largely based on its location rather than evidence. Up to 50% of yellow perch were determined to be infected over multiple survey years and the intensity of infection increased from July to September (Muzzall, 2000). The intermediate host of *S. occidentalis* found in yellow perch is the snail *Campeloma decisum* (Muzzall, 2000). Walleye were more commonly affected in the summer (proliferative endocarditis 37/60; 6/30 with 9/60 and 1/30 fish having intracardial or intravascular trematodes, respectively) however the size variation in these species between the summer and spring surveys was marked. The majority of fish collected in the spring were young of the previous year while the fish collected in the summer were mature. No small walleye or sauger was noted that were infected with the trematode.

A *Diplostomulum* sp. (adult: *Diplostomum*) was identified in brook stickleback. Only two fish were seen to have these intralenticular trematodes present. Both eyes affected had significant lesions (cataracts) and would likely have had either reduced vision or been blind in one eye. The brook stickleback is known to be a host for at least three different *Diplostomulum* sp. and the presence of the trematode in the lens suggests that it may be *D. spathaceum* although other means would be required to confirm this. One emerald shiner lens contained a single morphologically-similar trematode. A *Diplostomum* sp. has been noted in emerald shiner from Manitoba. There were no lesions visible in the single lens examined. While the eyes of the fish in

the present study were targeted for examination, typically only one was trimmed and it is very possible that trematodes of the same genus were not seen in other species for this reason. In addition, if there are low numbers of worms present and limited lesions generated, a trematode (and lesion) may be present in the eye under examination, but out of the plane of section.

Four species of fish were found that had endomeningeal trematodes present. These generated little to no tissue reaction and typically cause no clinical signs, even when large numbers are present. The brook stickleback, fathead minnow, sauger and Northern pike had endomeningeal larval trematodes present. The brook stickleback is known to have *Diplostomulum baeri eucaliae* present in the brain. The other three species are also recorded to have *Diplostomulum* sp. in North America but only the Northern pike in the Hudson Bay drainage is recorded as a host. There are however other trematode larvae that encyst in the brain, such as *Ornithodiplostomum ptychocheilus*, which has been demonstrated to cause expansion of the cranium of fathead minnows (Sandland and Goater, 2001). Only one fish each of the sauger and Northern pike from the present study had the trematode present, while there were common in the fathead minnow during summer (31/60) and less so in spring (6/30).

Several protozoans, including those that morphologically resembled *Trichodina* sp. (numerous species of fish affected and at least three morphologically distinct *Trichodina* sp. were seen and were also noted on the skin of yellow perch), *Caprinia* (or *Capriniana*) sp. (on numerous fish species), and two ciliates, Chilodonella-like (walleye) and Ichthyopthiriasis-like (channel catfish) were found on the gills of the nine of the ten species of fish included in the study. Only the emerald shiner lacked visible protozoans. Further sectioning of gill tissue would, without doubt, allow this species to be included as having branchial protozoans as well. The first two agents, *Trichodina* sp. and *Caprinia* sp., are essentially commensals and are of no or extremely limited pathogenic potential even when very large numbers of organisms are present. Both *Chilodonella* and *Ichthyopthiriasis* have greater potential for tissue damage and adverse health effects, particularly when present in moderate to large numbers. They were seen in only small numbers on the fish examined here. Speciation of these organisms should be performed using gill biopsies and skin scrapes from freshly caught fish, if there is further need. If a fish species included this study has not previously been identified to have one of these organisms it is due to a lack of examination of suitable tissue preparations as these are ubiquitous organisms. Certain species of protozoan, e.g. *Trichodina*, will more likely found on some species of fish, especially those fish that prefer shallower and more productive zones of water.

Branchial monogenetic trematodes were also commonly noted in several species of fish. The term monogenes will be used to refer to these organisms here. Monogenes were found in channel catfish, emerald shiner, fathead minnow, Northern pike, sauger and white bass. The last species had two morphologically distinct monogenes present and one of these was present in 51 of 59 fish examined during summer. None were positively confirmed on this species collected during the spring sample period. These monogenes were large, present in substantial numbers on each fish and had a haptor that was deeply buried in the branchial filament with a moderate, but localized, inflammatory reaction. Monogenetic trematodes are very common and one is the only parasite to be listed by the OIE as a reportable agent. As with many micro-organisms, including parasites, it is often difficult to assess the degree of clinical or sub-clinical impact, particularly in an organ like the gill in which there is substantial reserve function. Multifocal lesions are less

likely to be clinically significant unless the lesions, and the causal agent, are present in very large numbers. The large monogenes noted on white bass during summer were common enough to have some effect. These may be the same as that identified by the parasitology survey (*Onchocleidus chrysops*), which have been previously recorded on white bass from the Hudson Bay drainage. At least two genera have been noted on white bass in North America, including *Onchocleidus* and *Urocleidus*.

Every fish species examined had intestinal cestodes identified. It is important to note that the real proportions of fish that have cestode burdens will be underestimated by microscopy. In addition, while the genus and species cannot be determined using light microscopy, these will have been identified by the concurrent parasite survey. Some general comments are likely appropriate. The brook stickleback, channel catfish and fathead minnow had few intestinal cestodes identified. In the groups of emerald shiner, goldeye, northern pike, sauger, walleye and white bass examined during the summer, at least 30% of the population had a cestode burden. The proportion of yellow perch with intestinal cestodes at this time was slightly less (17/60). The proportion of fish noted to have intestinal cestodes during the spring sample dropped substantially for all of these species, except the Northern pike and goldeye. The species in which individual fish had particularly heavy burdens were the yellow perch, goldeye and emerald shiner; in which intestinal dilation and thinning were occasionally noted due to the large numbers of cestodes present. There were few other lesions noted that were associated with the presence of cestodes or likely to be associated with cestodes. There were rarely fortuitous sections seen with a scolex clamping intestinal mucosa. Occasionally, cestodes were found in other locations such as the pancreas, pancreatic ducts or in the liver within bile ducts in small numbers. Presumably these have migrated from the intestine and were found to cause local expansion of ducts and limited inflammation.

Branchial crustaceans were found on the sauger in the gills and were common in the summer sample and not noted during the spring. This may be an *Ergasalis* sp. and several species of this crustacean have been identified on sauger previously including *Egasalis luciopercarum* from Manitoba. The other crustacean identified was found on the gills of walleye and morphologically resembled an *Argulus* sp. however few good sections were available. These crustaceans can be a problem, particularly on captive fish populations, and treatment of similar crustaceans is a substantial issue in aquaculture. The sauger had moderate numbers present while the walleye had few. In the numbers found, they are likely of minimal significance.

Only three species of fish were noted to be infected with microsporeans, however the likelihood of finding small widely-spread cysts (or parasitophorous vacuoles/xenomas) in a single 5 μm section for light microscopy is relatively unlikely, particularly without lesions of sufficient size to direct trimming. While it is very likely that some were missed, four separate instances of likely microsporean infection were found. One of these was infecting a neuron in the spinal column of the brook stickleback. These were arranged in an interesting pattern around the periphery of the expanded neuronal cell body. Further characterization, let alone speciation, is not possible without transmission electron microscopy. In addition, the small size of the lesion precluded extensive trimming for additional examination using alternate stains. This fish species has been noted previously to be infected with the microsporean *Glugea anomala* (McDonald and Margolis, 1995; Lom and Dykova, 2005) and infection is common and widespread although

localization in the spinal cord doesn't seem to be. Branchial microsporeans (or suspect microsporeans) were seen in the white bass and channel catfish, with the latter species having two morphologically distinct types. Since the organisms seen in the white bass weren't commonly sporulated they should be characterized as suspect or at best presumptive. A *Pleistophora* sp. is listed as infecting the channel catfish and the tissue affected was the liver (Herman and Putz, 1970; in Hoffman 1999). Neither of the microsporeans were found in this location however more importantly at least one of the organisms found is unlikely a *Pleistophora* sp. as a distinct cyst was formed. No references for a microsporean of white bass were found in the reference texts used or in the literature using two common search engines. Electron microscopy is needed to characterize the spore morphology and intracellular arrangement to allow a complete description of these organisms.

Myxosporeans were noted very often and only the goldeye and white bass did not have pre-spore or sporulated examples of myxosporeans present in tissues. The emerald shiner and yellow perch had three, or four depending interpretation, myxosporeans found at separate anatomical sites. In the fathead minnow they were noted at six separate anatomical sites. Prespore forms of myxosporeans are very difficult to identify, in fact they could also be apicomplexans or other protozoans. For the purposes of this report they are all grouped as prespore myxosporeans for convenience. For example, the renal intratubular plasmodia or 'protozoan'-like organisms found in the Northern pike are likely pre-spore myxosporeans and this is a relatively common location and appearance for same. Many of the cysts were relatively small and were trimmed through and unavailable for staining by Giemsa to aid in further classification. For example, the spinal cord cyst of yellow perch was lost on re-trimming and this was true for most of the microsporean or suspected microsporean lesions as well. Where cysts or free spores were available for staining with Giemsa, in most cases they were of a similar morphology. The fathead minnow intramuscular, thymic and pericardial myxosporeans were all similar to a *Myxobolus* in appearance. While more than one *Myxobolus* sp. can infect a species of fish at the same time the techniques used here are insufficient to differentiate between *Myxobolus* species. In fact, with three possible exceptions, including the branchial myxosporean of the catfish and the renal and perineural myxosporeans of the emerald shiner, all of the sporulated myxosporeans available for staining with Giemsa in the present study were morphologically similar to a *Myxobolus* sp. (emerald shiner - branchial and pericardial; yellow perch – hepatic). The branchial myxosporean of the channel catfish most closely resembled a *Henneguya* sp. although the Giemsa staining was very poor. This was also true for the renal myxosporean of the emerald shiner, which more closely resembled a *Myxidium* sp. although few spores were present and were poorly stained. While not stained by Giemsa the morphology of the spinal myxosporean of the yellow perch suggested that they were less likely to be a *Myxobolus* sp. Channel catfish are host to several species of *Henneguya* sp. and the gills are a location for more than one species. The emerald shiner is known to be infected by both *Myxidium* and *Myxobolus* sp., some of which are only classified to genus level. Numerous *Myxobolus* sp. are known to infect the yellow perch. Further classification of the myxosporeans found here would allow the establishment of new parasite records for the Hudson Bay drainage.

Dermal sarcoma of walleye was found in two fish collected during the summer. The fish collected during this sampling were larger and expression of visible neoplasia in larger fish is much more likely. This neoplasm is caused by walleye dermal sarcoma virus (Bowser *et al.*

2002), which is a well-studied virus that is common in North America. Typically it causes expansive non-invasive neoplasms however rare invasive examples have been noted (Bowser *et al.* 2002). The virus can be transmitted horizontally in the water (Bowser *et al.* 1999) and may also affect yellow perch (Bowser *et al.* 2005). The lesions are known to be present in walleye in Lake Winnipeg (B. Souter and D. Donald, personal communication) although I was unable to find ready documentation of this fact, which is likely a reflection of the depth of review undertaken. My laboratory has previously been sent walleye with similar lesions from Lake Winnipeg and surrounding lakes.

Characteristic lesions associated with lymphocystis were found in one walleye sampled during the summer. Lymphocystis is caused by a fish iridovirus that likely has a world-wide distribution and affects fish in salt and freshwater. This iridovirus is also known to be present in Lake Winnipeg (B. Souter) and my laboratory has also examined cases submitted from there. It is known to occur in nearby Lake Dauphin (Szalai and Dick, 1991) and affected walleye have been documented from Western Canada (Yamamoto *et al.* 1985). It is typically a self-limiting infection in most fish however increased incidence (outbreaks) of lymphocystis has been documented occasionally in wild fish (Mellergaard and Nielsen, 1997).

Three examples of apicomplexans were noted, one each in the yellow perch, sauger and emerald shiner. In the latter species, approximately half of the fish examined during the summer had few scattered oocysts present in the intestinal epithelium (none were present in the spring sample), allowing a tentative identification of a member of the Calyptosporidae or Emeriidae. Isolation of the sporulated oocysts would be required for more precise identification. Minimal tissue reaction was present. *Eimeria* sp. and *Goussia* sp., for example, are commonly recognized in other *Notropis* species (shiners) but a record of an apicomplexan in one of the four reference sources listed above for the emerald shiner wasn't found. Developmental stages, but no sporulated oocysts were noted in the yellow perch, precluding further identification. Numerous apicomplexans from several different genera, have been found in the yellow perch. Large structures resembling shizonts packed with merozoites (or other zoite type) were found in the submucosa of the intestine of two sauger. Further identification wasn't possible using light microscopy alone. Reference to apicomplexans in sauger weren't found in the major reference texts used.

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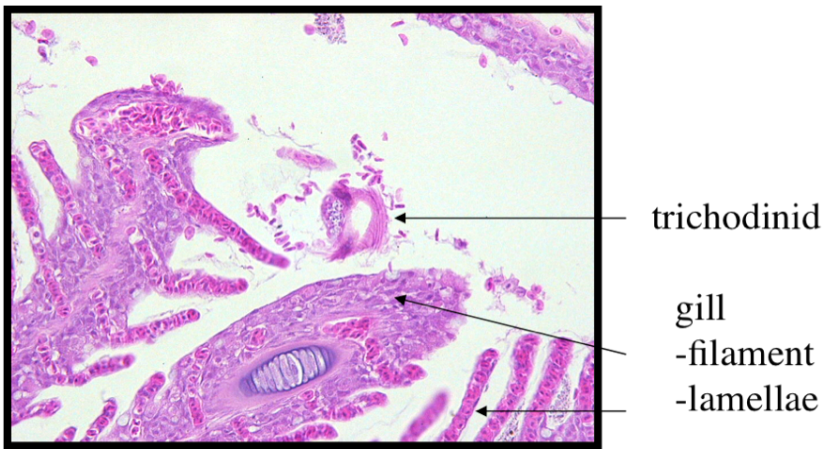
Thanks to Environment Canada for persevering with funding.

Table 1. Summary of notable lesions and organisms in the brook stickleback (*Culea inconstans* – BS) collected during the summer of 2006 (n=60) from Lake Winnipeg and surrounding waters.

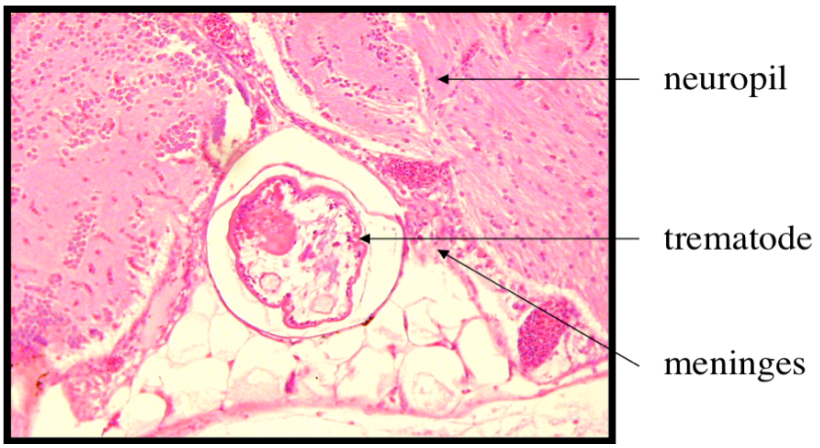
Brook Stickleback Tissue/agent	Summer 2006 – Sixty fish	Spring 2007 – None caught
Gills	1.*Trichodina* sp. (6/60)	
Stomach/ Intestine/ peritoneum	1. Granuloma, nematode (6/60) 2. Intestinal mural nematodes (1/60) 3. Intestinal cestode (2/60)	
Skeletal muscle and skin	1. Muscular nematode (1/60) 2. Intradermal nematode (1/60)	
Brain and meninges	1. Meningeal trematode (2/60) 2. Intra-neuronal protozoan (suspect microsporean) (1/60)	
Liver and bile ducts	1. Nematode (6/60)	
Other	1. Lens *Diplostomulum* sp. (2/60) 2. Splenic nematode (1/60) 3. Pancreatic duct myxosporean (1/60) 4. Granulomas, numerous tissues, metazoan 5. Trematode, oral cavity connective tissue (1/60) 6. Peritoneal nematode (1/60)	

The histological sections of this species 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. Not all of the tissues listed above were present on each slide examined.

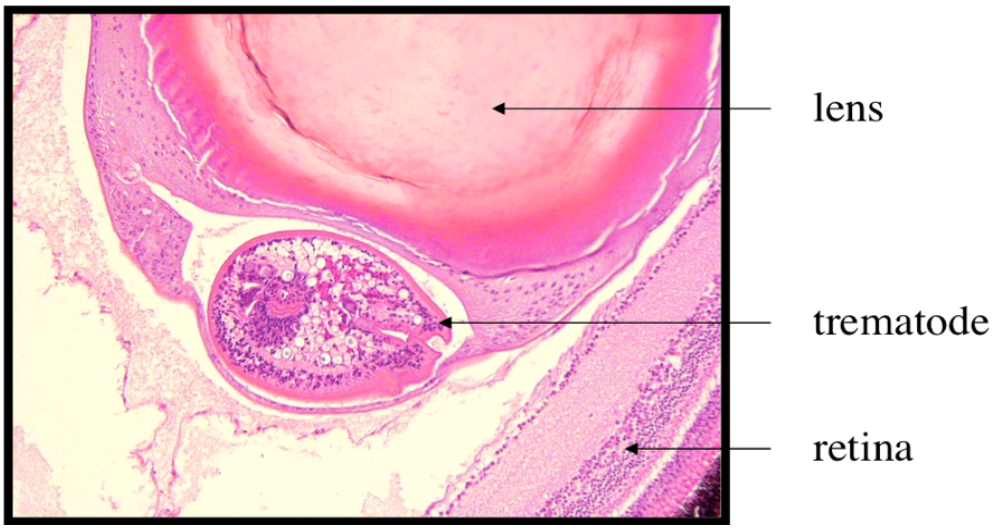
Branchial Trichodina - Trichodinosis, branchial cavity.
Within the branchial chamber there are two sections of round, approximately 45µm in diameter ciliated protozoan possessing a circular denticular ring – *Trichodina* sp. (Phylum Ciliophora, Class Litostomatea, Family Trichodina). ---- 6 of 60 affected.



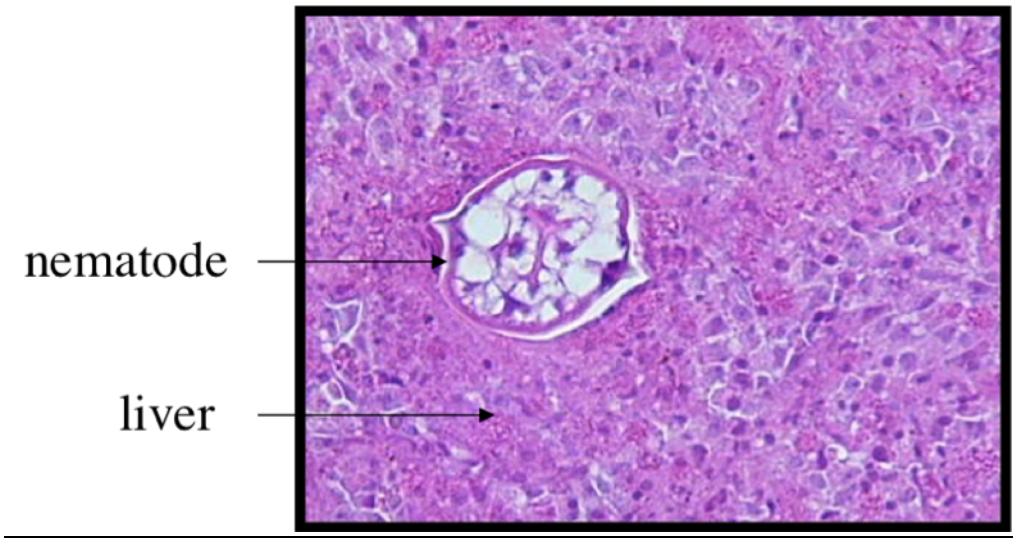
Endomeningial trematode - Trematodiasis, endomeningial, encysted
Within endomeningial connective tissue and adipose tissue of the cranium, sections of a trematode organism are surrounded by one to two layers of flattened macrophages/fibrocytes. The encysted trematode is approximately 80 to 100 µm in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



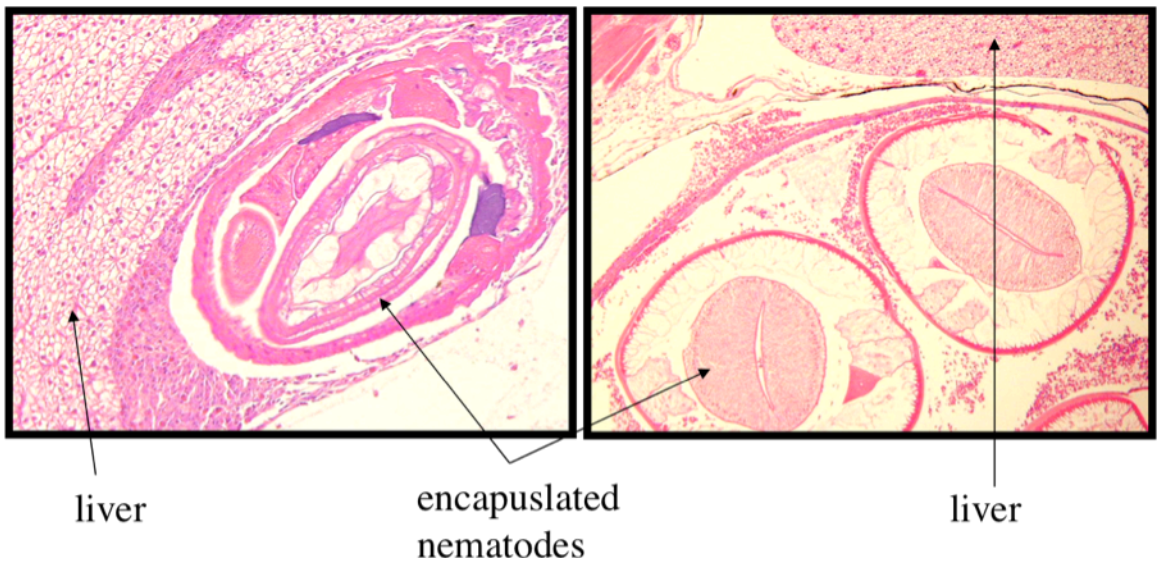
Eye fluke – Trematodiasis, intralenticular. Likely *Diplostomulum* sp. possibly *D. spathaceum*. Since a key for *Diplostomulum* indicates that a larval trematode in the eye and more specifically in the lens is likely this species.
Embedded under the cuboidal epithelium of the lens, there are sections of a trematode that is approximately 70 by 100 µm characterized by thin eosinophilic tegument and that lacks a body cavity and calcareous corpuscles. There appears to be a pseudosucker present. (Phylum Platyhelmintheses, Class Trematoda, Order Strigeidida, likely Genus *Diplostomulum*). There is a substantial accumulation of bladder cells surrounding the trematode (cataract) and the lens is distorted. ---- 2 of 60 affected.



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 50 µm in diameter are characterized by a bright eosinophilic cuticle, hypodermis, digestive tract and lateral alae (Phylum Nemathelminthes, Class Nematoda). There is mild to moderate hepatic necrosis where the nematodes have migrated and mild compression of unaffected hepatocytes surrounding the granulomas. ---- 6 of 60 affected.



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.



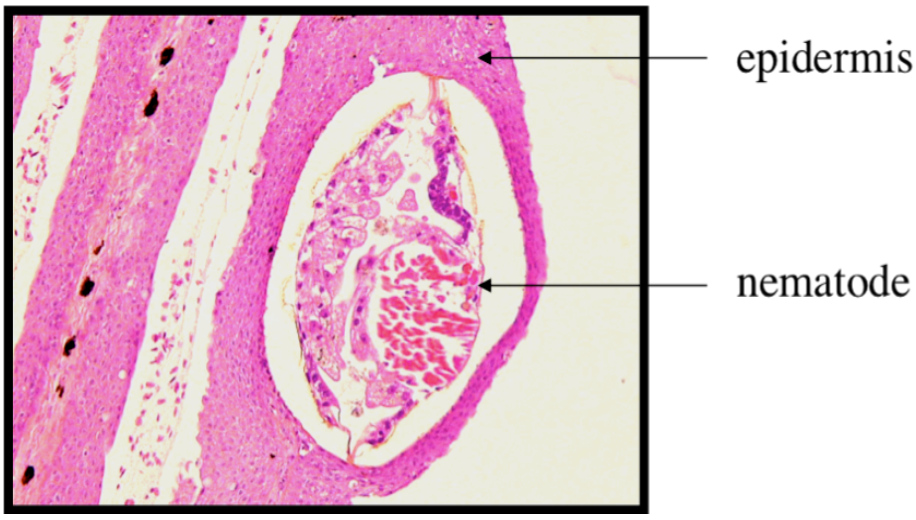
Intramuscular nematodes –Myositis, focal to multifocal, mild to moderate with intra-lesional nematodes.
Sections of a nematode organism morphologically similar to those found within the coelom (above) are encysted in the skeletal musculature of the body wall. The nematodes possess

broadly similar characteristics but lateral alae are also visible. (Phylum Nemathelminthes, Class Nematoda) ---- 1 of 60 affected. (no photo)

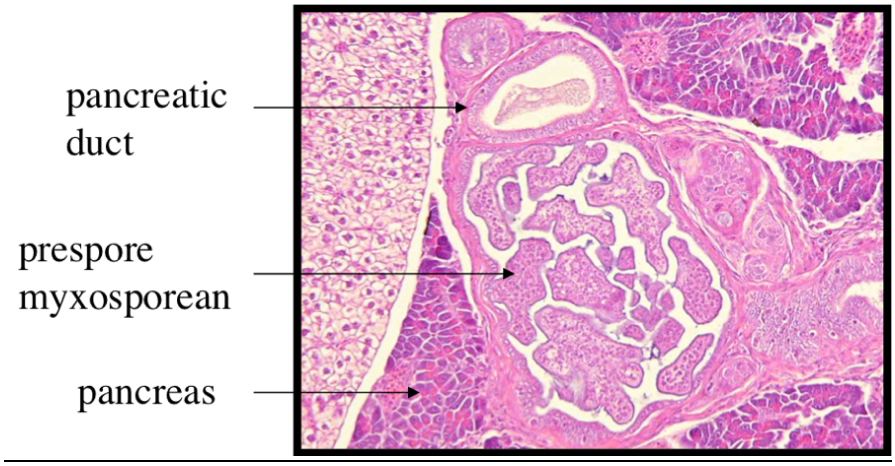
Intrasplenic nematodes – Splenitis, multifocal, mild to moderate with intra-lesional nematodes. There are multiple cross sections of a nematode parasite surrounded by small to moderate numbers of macrophages and lesser numbers of lymphocytes. The nematodes are similar morphologically and in size to those 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)

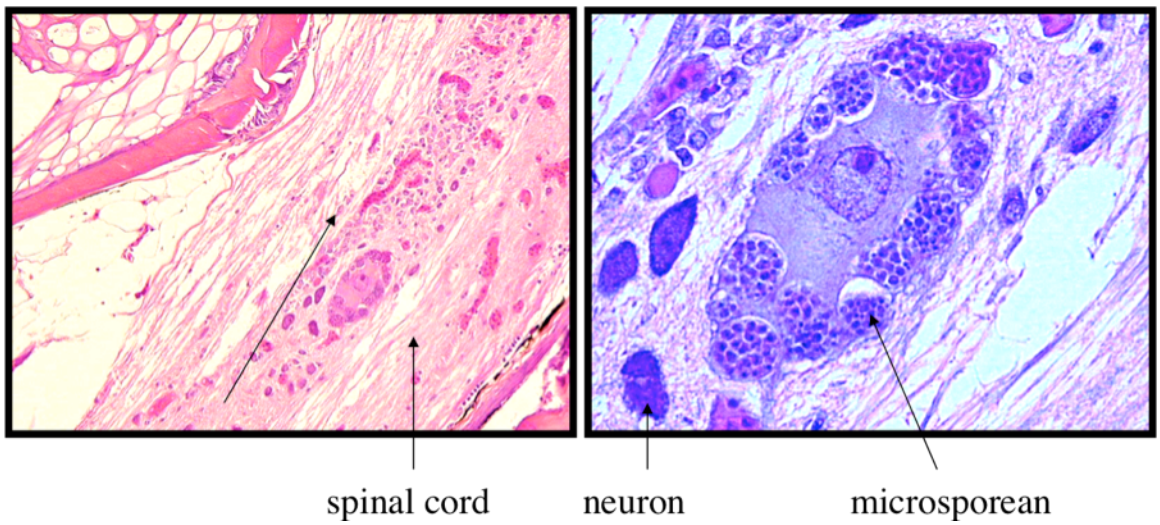
Intradermal nematodes – Nematodiasis, dermal, focal
Embedded within the dermis of a caudal spine, there is a cross section of a solitary nematode organism characterized by lateral alae, a thin eosinophilic cuticle and a psuedocoelom (Phylum Nemathelminthes, Class Nematoda). Little to no host inflammatory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



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, 5-7 µm 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.



Intraneuronal protozoan – Protozoan, intraneuronal, 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 µm in diameter. A single dark basophilic nucleus like organ (~1µm in diameter) is present within variable numbers of these organisms. ---- 1 of 60 affected.



Intestinal cestodes – Cestodes, intraluminal, 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)

Summaries of lesions or organisms noted in individual fish.

<u>BS 1</u> –
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. Coelomitis, mild to moderate with intralesional remnants of a metazoan organism (presumptive nematode)

BS 2 – No abnormal findings (NAF)

BS 3 – NAF

BS 4 - NAF

BS 5 – NAF

BS 6 –
Morphological diagnosis

1. Coelomitis, granulomatous, multifocal, moderate with intralesional nematodes
2. Myositis, focal, mild with intralesional nematode

BS 7 –
Morphological diagnosis

1. Coelomitis, granulomatous, focal, mild with intralesional nematode
2. Trematodiasis, intraocular, focal

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

BS 9
Morphological diagnosis

1. Nematodiasis, dermal, focal

BS10
Morphological diagnosis

1. Cestodes, intraluminal, intestinal

BS 11 – NAF

BS 12 -
Morphological diagnosis

1. Hepatitis, multifocal, mild to moderate with intralesional nematodes
2. Coelomitis, granulomatous, multifocal, mild with intralesional nematodes

BS 13 – NAF

BS 14 –
Morphological diagnosis
1. Trichodinosis, branchial cavity, small numbers

BS 15 –
Morphological diagnosis
1. Trematodiasis, endomeningial, encysted, solitary
2. Trichodinosis, branchial cavity, small numbers

BS 16 -
Morphological diagnosis
1. Coelomitis, granulomatous, focal, mild with intralesional nematodes

BS 17 –
Coelom – 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
2. Coelomitis, histiocytic, multifocal, mild

BS 18 - NAF

BS 19 -
Morphological diagnosis
1. Hepatitis, multifocal to regionally extensive, mild to moderate with intralesional nematodes

BS 20 –
Morphological diagnosis
1. Splenitis, multifocal, mild to moderate with intralesional nematodes
2. Enteritis, intramuscular, multifocal, low numbers multifocal, low numbers

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).
Morphological diagnosis
1. Trematodiasis, intraocular, focal
2. Trematodiasis, pharyngeal, dermal encysted, multifocal

BS 22 – NAF

BS 23 -
Morphological diagnosis
1. Cestodes, intraluminal, intestinal
2. Coelomitis, granulomatous, focal, mild

BS 24 - NAF

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 intralesional nematode
5. Granuloma, renal interstitial, focal, small

BS 27 -
Morphological diagnosis
1. Enteritis, histiocytic, focal, mild
2. Coelomitis, granulomatous, focal, mild with intralesional nematode

BS 28 -
Morphological diagnosis
1. Branchitis, multifocal, mild

BS 29 –
Morphological diagnosis
1. Protozoan, interneuronal, focal

BS 30 –
Morphological diagnosis
1. Myxosporidiosis, pancreatic duct, multifocal cysts, moderate numbers
2. Pancreatitis, duct wall, multifocal

BS 31 - NAF

BS 32 - NAF

BS 33
Morphological diagnosis
1. Branchitis, multifocal, mild
2. Trichodinosis, branchial cavity, small numbers

BS 34
Morphological diagnosis
1. Hepatitis, multifocal, mild to moderate with intralesional nematodes

BS 35
Morphological diagnosis
1. Branchitis, regionally extensive, mild to moderate with inter-lamellar epithelial hyperplasia

BS 36
Morphological diagnosis
1. Branchitis, multifocal, mild

BS 37
Morphological diagnosis
1. Trematodiasis, retrobulbar, encysted, solitary

BS 38
Morphological diagnosis
1. Trichodinosis, branchial cavity, solitary

BS 39
Morphological diagnosis
1. Branchitis, multifocal, mild

BS 40 - NAF

BS 41
Morphological diagnosis
1. Granuloma, intraovarian, large, solitary

BS 42 - NAF

BS 43 - NAF

BS 44
Morphological diagnosis
1. Trichodinosis, branchial cavity, small numbers
2. Branchitis, multifocal, mild

BS 45
Morphological diagnosis
1. Branchitis, multifocal to regionally extensive, mild to moderate
2. Trematodiasis, intraocular, focal

BS 46
Morphological diagnosis
1. Branchitis, multifocal, mild

BS 47 - NAF

BS 48 - NAF

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 intralesional 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 intralesional nematodes

BS 53 - NAF

BS 54 - NAF

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
1. Hepatitis, multifocal to regionally extensive, with intralesional nematodes

BS 58 - NAF

BS 59
Morphological diagnosis.
1. Hepatitis, regionally extensive, severe with intralesional nematodes
2. Coelomitis, multifocal, moderate

BS 60
Morphological diagnosis.
1. Hepatitis, multifocal to regionally extensive, severe with intralesional nematodes

Table 2. Summary of notable lesions and organisms in the channel catfish (*Ictalurus punctatus* – CC) collected during the summer of 2006 (n=7) and the spring of 2007 (n=30) from Lake Winnipeg and surrounding waters.

Channel catfish Tissue/agent	Summer 2006 – Sixty fish (only seven caught)	Spring 2007 – Thirty fish
Gills	1. Monogenetic trematode (5/7) 2. Myxosporean (3/7) 3. *Trichodina* sp. *Caprinia* sp. (4/7) 4. Crustacean (1/7)	1. Monogenetic trematode (10/30) 2. Myxosporean (3/30) 3. *Trichodina* sp. *Caprinia* sp. (11/30) 4. Microsporean 1 (1/30) 5. Microsporean 2 (2/30)
Intestine/ peritoneum		1. Nematode (2/30) 2. Cestode (2/30)
Other	1. Myo/epicarditis (5/7)	1. Glial encephalitis (1/30) 2. Gill filament distortion (11/30)

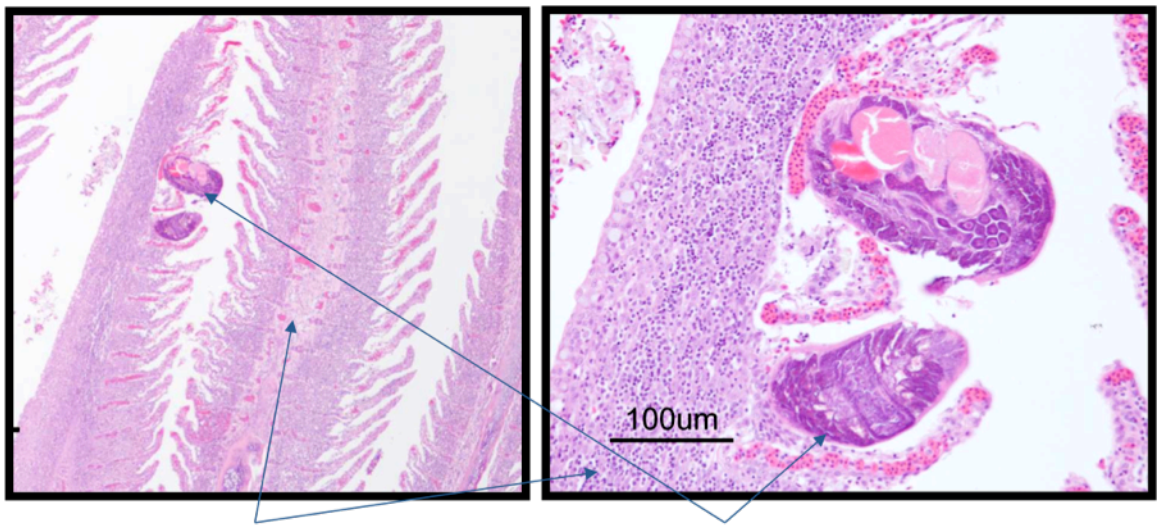
Summer
The sections consist of dissected tissues including skin, muscle, gill, heart, liver, spleen, pancreas, kidney, gastrointestinal tract (stomach and intestines), swim bladder, eye and ovary/testis.
Spring
The sections consist of dissected tissues including skin, muscle, gill, heart, liver, spleen,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

The 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.

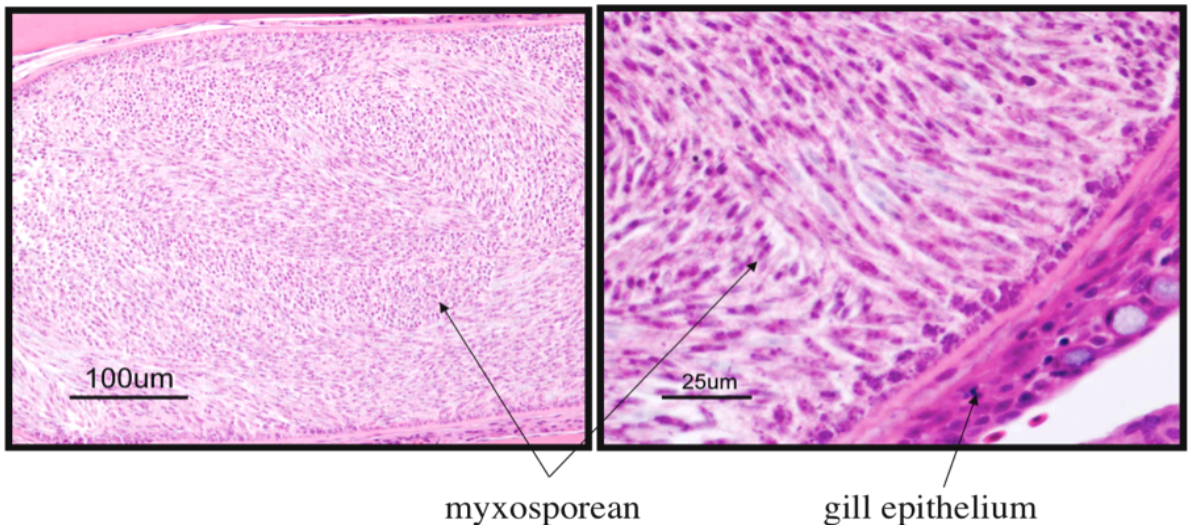
Lesions or organisms noted in both summer and spring.

Branchial trematodes - Branchitis, multifocal, mild to moderate with intralesional 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). Summer ---- 5 of 7 affected. Spring---- 10 of 30 affected.



Branchial myxosporeans - Branchitis, multifocal, with intralesional 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 400 µm, contain both immature multinucleate sporoplasm, primarily located at the periphery of the cysts, and maturing spores and is surrounded by an ~5-10 µm thick hyaline wall. The spores are fusiform in shape with a single polar capsule and are approximately 20 to 30 µm in length. The spores resemble a *Henneguya* sp. (Phylum Myxozoa, Class Myxosporea). Summer ---- 3 of 7 affected. Spring ---- 3 of 30 affected.



Branchial protozoan – Branchitis, multifocal, mild with intralesional ciliated protozoan
There are few to numerous ciliated protozoans that range in size from ~20-70 μm 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) Summer ---- 4 of 7 affected. (no photo)

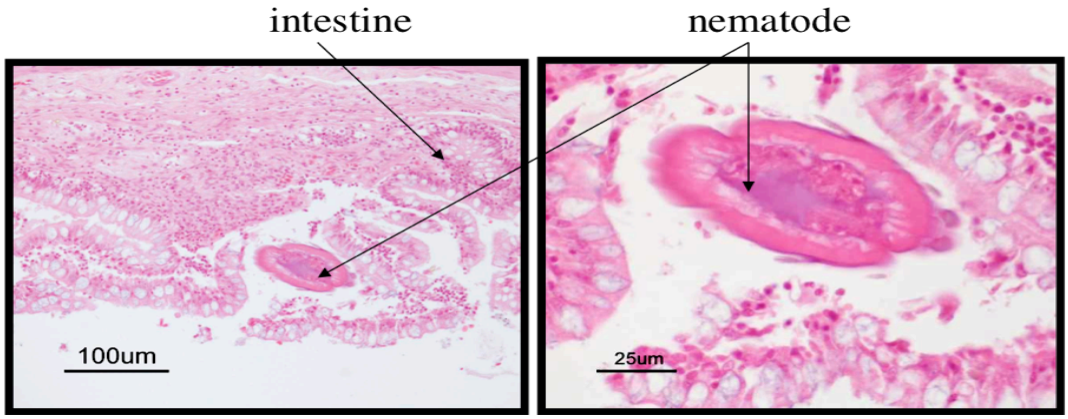
Branchial protozoan – Branchitis, multifocal, mild to moderate with intralesional protozoa (*Caprinia* and *Trichodina* species).
There are small numbers of protozoan organisms attached to lamellae of scattered filaments. The shape of one type of protozoan is variable but more often it is often elongate (45 to 90 μm) or sac-like 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*). There are also small to numerous ~25 μm 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. Spring ---- 11 of 30 affected.

Lesions or organisms only noted in summer.

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)

Lesions or organisms only noted in spring.

Gastrointestinal nematodes – Nematodes, luminal, intestinal.
There were few to moderate numbers of thin ~70-90 μm nematodes with an eosinophilic cuticle, thick muscular esophagus/intestine, reproductive tissue and lateral ridges (alae) on the cuticular surface. No host reaction was associated with the presence of these nematodes. (Phylum Nemathelminthes, Class Nematoda). ---- 2 of 30 affected.

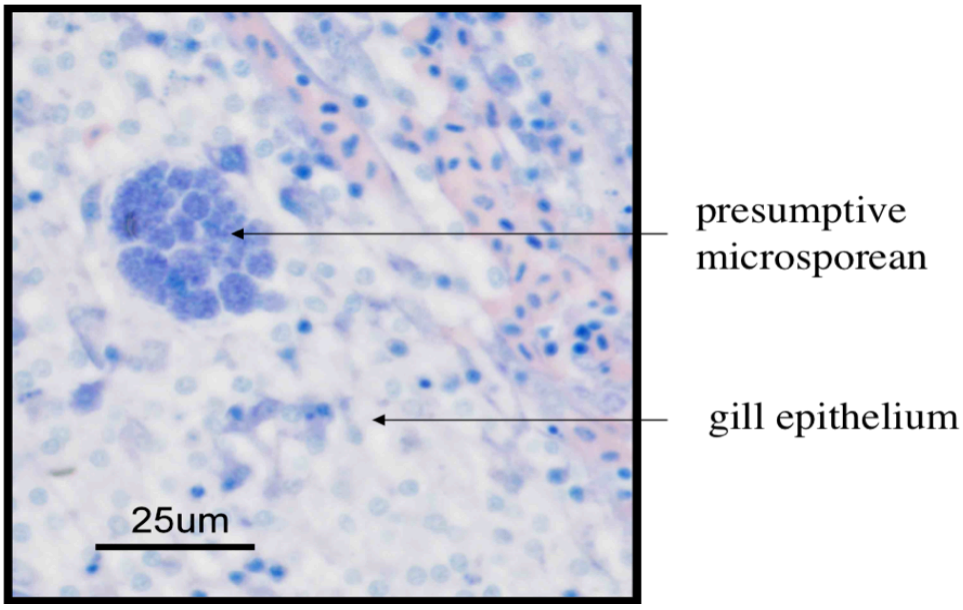


Intestinal cestodes – Cestode, luminal intestinal.
The intraluminal cestodes have calcareous corpuscles, are ~150 to 200 μm wide, and with regular folding (proglottids) sometimes apparent, and have a loose parenchymatous matrix and eosinophilic tegument. No gastrointestinal structures are visible. (Phylum Platyhelminthes, Class Cestoidea). ---- 2 of 30 affected.

Encephalitis, multifocal, mild.
Within various sections of the neuropil, there are multifocal accumulations of moderate numbers of glial cells surrounding vascular capillaries and viable neurons. ---- 1 of 30. (no photo)

Branchial filament microsporidians – Microsporidiosis, interfilamental, multifocal.
Replacing sections of cartilaginous core of single or multiple filaments, there are multiple ~ 60 – 120 μm accumulations of small ~ 1 μm in diameter, round, granular spores (Phylum Microspora, Family Microsporea). There is no tissue reaction surrounding these organisms. ---- 1 of 30 affected. (no photo)

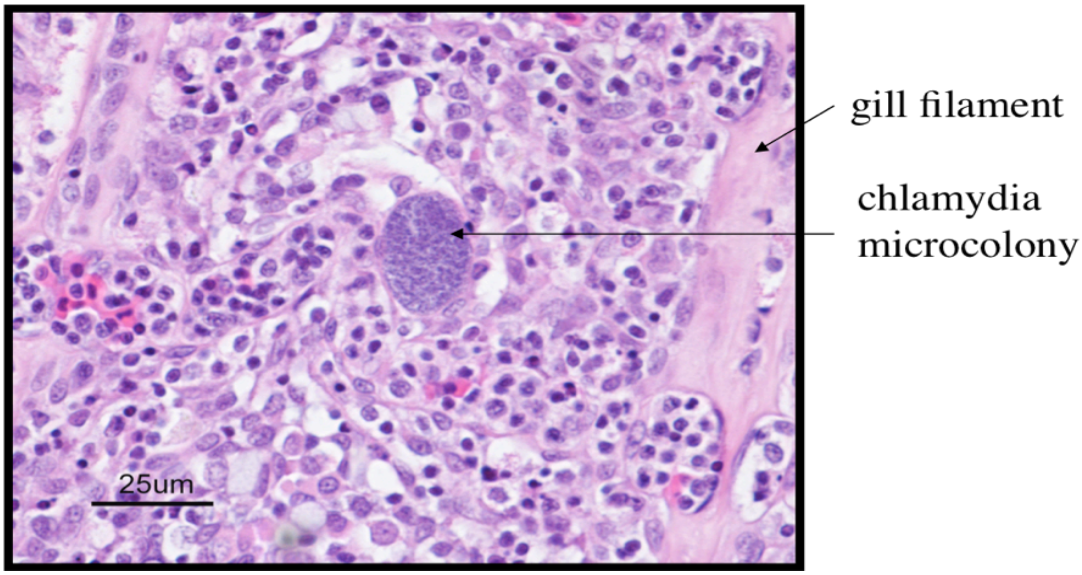
Branchial microsporidians – Branchitis, regionally extensive, moderate to marked with few intralesional microsporidian cysts.
On few filaments, there are moderate to marked accumulations of leukocytes filling and expanding regionally extensive sections of the vascular sinus and spilling over into the lamellar epithelium and interlamellar spaces. Within these areas there are single to several round to oval ~ 40-50 μm structures buried within the interlamellar epithelium, which have numerous ~ 2 μm in diameter, granular spores (Phylum Microspora, Family Microsporea) which are histomorphologically not similar to those microsporidians identified within brachial filaments. ---- 2 of 30 affected. Giemsa stain below.



Gill filament distortion – Chondrocyte dysplasia, multifocal, mild to moderate.

Along sections of scattered filaments, there is mild to moderate, focally extensive chondrocyte hyperplasia causing abnormal distortion of the filaments. There is no tissue reaction surrounding these foci. ---- 11 of 30 affected. (no photo)

Branchial epitheliocystis - Branchitis, multifocal, with interlamellar intraepithelial bacterial microcolonies (presumptive chlamydia-like bacteria).
There are single ~20-30 μm tightly-packed basophilic foci with a thin ~2 μm eosinophilic limiting membrane that delineates each microcolony. The microcolony is filled with ~1 μm basophilic bacteria. 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. ---- 1 of 30 affected.



Summaries of lesions or organisms noted in individual fish.

Summer

CC 1-
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate with a single monogenetic trematode
2. Myocarditis, lymphocytic, multifocal, mild

CC 2-
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate with few intralesional monogenetic trematodes
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 intralesional monogenetic trematodes
2. Branchitis, multifocal, with several intralesional myxosporean cysts
3. Myocarditis, lymphocytic, multifocal, mild
4. Branchitis, multifocal, mild with moderate intralesional ciliated protozoans
5. Branchial crustacea

CC 4-
Morphological diagnosis
1. Branchitis, multifocal, mild with intralesional ciliated protozoan
2. Epicarditis, lymphocytic, multifocal, mild

CC 5-
Morphological diagnosis
1. Branchitis, multifocal, with several intralesional myxosporean cysts
2. Branchitis, multifocal, mild with moderate intralesional ciliated protozoans

CC 6-
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate with few intralesional monogenetic trematodes
2. Branchitis, multifocal, with a single intralesional myxosporean cysts
3. Branchitis, multifocal, mild with few intralesional ciliated protozoans

CC 7-
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate with a single intralesional monogenetic trematode

Spring

CC 1-
Morphological diagnosis
1. Branchitis, multifocal, mild to severe with intralesional myxosporean cysts
2. Chondrocyte dysplasia, multifocal, moderate

CC 2-
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate with few intralesional trematodes
2. Branchitis, multifocal, mild to severe with intralesional myxosporean cysts

CC 3-
Morphological diagnosis
1. Nematodes, luminal, intestinal, few
2. Chondrocyte dysplasia, multifocal, mild

3. Branchitis, multifocal, mild to moderate with few intralesional trematodes

CC 4- NAF

CC 5-
Morphological diagnosis
1. Encephalitis, multifocal, mild
2. Chondrocyte dysplasia, focal, mild

CC 6-
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate
2. Nephritis, multifocal, mild with cellular debris

CC 7- NAF

CC 8 –
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate with intralesional protozoans

CC 9 – NAF
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate with intralesional protozoans

CC 10 –
Morphological diagnosis
1. Chondrocyte dysplasia, focal, mild

CC 11 – NAF

CC 12 –
Morphological diagnosis
1. Branchitis, multifocal, moderate with intralesional protozoans
2. Hepatitis, multifocal, mild
3. Chondrocyte dysplasia, focal, mild

CC 13 –
Morphological diagnosis
1. Branchitis, multifocal, moderate with intralesional protozoans
2. Cestode, intraluminal, intestinal

CC 14 –
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate with few intralesional trematodes, intralesional protozoans and microsporidian cysts

CC 15 –
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate with few intralesional monogenetic trematodes and protozoans
2. Myocarditis, focal, mild
3. Enteritis, multifocal to regionally extensive, lamina proprial, mild to moderate

CC 16 –
Morphological diagnosis
1. Branchitis, multifocal to regionally extensive, moderate with intralesional protozoans
2. Microsporidiosis, hepatic, encysted, focal
3. Chondrocyte dysplasia, focal, mild

CC 17 –
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate with few intralesional trematodes
2. Epicarditis, focal, mild

CC 18 –
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate with few intralesional protozoans
2. Branchitis, multifocal, mild to severe with intralesional myxosporean cysts
3. Microsporidiosis, interfilamental, multifocal
4. Chondrocyte dysplasia, focal, mild

CC 19 –
Morphological diagnosis
1. Branchitis, regionally extensive, moderate to marked with few intralesional microsporidian cysts
2. Branchitis, multifocal, mild to moderate with few intralesional trematodes
3. Dermatitis, necrotizing, focally extensive

CC 20 – NAF

CC 21 – NAF
Morphological diagnosis
1. Dermatitis, focally extensive, mild
2. Branchitis, regionally extensive, mild to moderate to marked with few intralesional protozoans and trematodes
3. Nephritis, multifocal, mild with tubular degeneration

CC 22 –
Morphological diagnosis
1. Branchitis, regionally extensive, moderate with few intralesional microsporidian cysts
2. Branchitis, multifocal, mild to moderate with few intralesional trematodes
3. Nematodes, luminal, intestinal, few

4. Chondrocyte dysplasia, focal, mild
5. Branchitis, multifocal, with interlamellar intraepithelial bacterial microcolonies

CC 23 – NAF

CC 24 –
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate with few intralesional protozoans
2. Cestodes, intraluminal, intestinal
3. Chondrocyte dysplasia, focal, mild

CC 25 –
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate with few intralesional trematodes
2. Chondrocyte dysplasia, focal, mild

CC 26 –
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate with few intralesional protozoans

CC 27 –
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate with few intralesional trematodes

CC 28 –
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate
2. Hepatitis and cholangiohepatitis, multifocal, mild
3. Chondrocyte dysplasia, focal, mild

CC 29 –
Morphological diagnosis
1. Hepatitis, focal, mild
2. Chondrocyte dysplasia, focal, mild

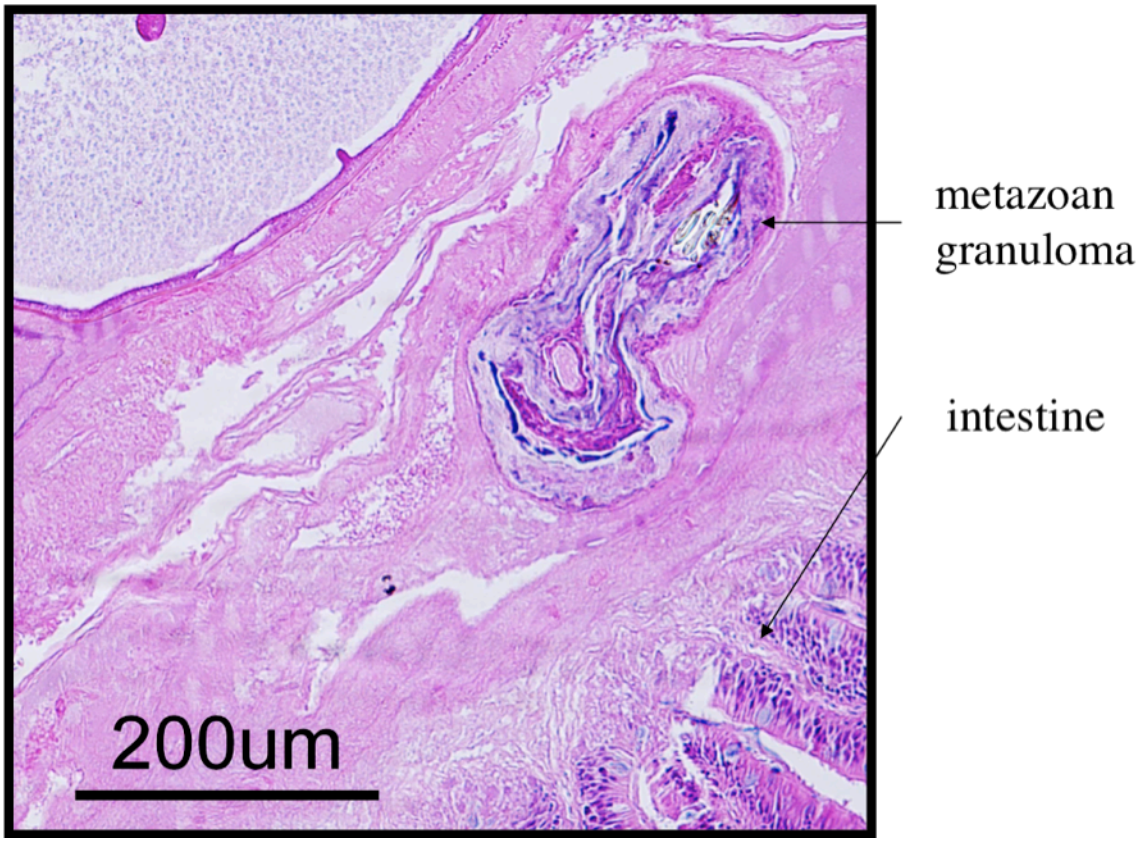
CC 30 –
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate with few intralesional protozoans

Table 3. Summary of notable lesions and organisms in the emerald shiner (*Notropis atherinoides* – ES) collected during the summer of 2006 (n=60) and the spring of 2007 (n=30) from Lake Winnipeg and surrounding waters.

Emerald shiner Tissue/agent	Summer 2006 – Sixty fish	Spring 2007 – Thirty fish
Gills	1. Myxosporean, likely *Myxobolus* sp. (32/60) 2. Monogenetic trematode (2/60)	1. Myxosporean, likely *Myxobolus* sp. (4/30)
Intestine/ Peritoneum/ Pancreas	1. Cestode (44/60) 2. Apicomplexan (28/60)	1. Cestode (2/30) 2. Trematodes (2/30) 3. Cestode (pancreas) (1/30) 3. Nematode (pancreas) (1/30) 4. Acanthocephalan (1/30)
Skeletal muscle and skin	1. Trematode (24/60) 2. Myxosporean (4/60)	1. Trematode (1/30) 2. Dermal trematode (1/30)
Kidney		1. Myxosporean (1/30)
Heart and pericardium		1. Myxosporean, likely *Myxobolus* sp. (3/30)
Liver and bile ducts		1. Metazoan (1/30)
Other	1. Focal myositis (6/60) 2. Lens trematode, likely *Diplostomulum* sp. (1/60)	1. Numerous granulomatous lesions in varied organs

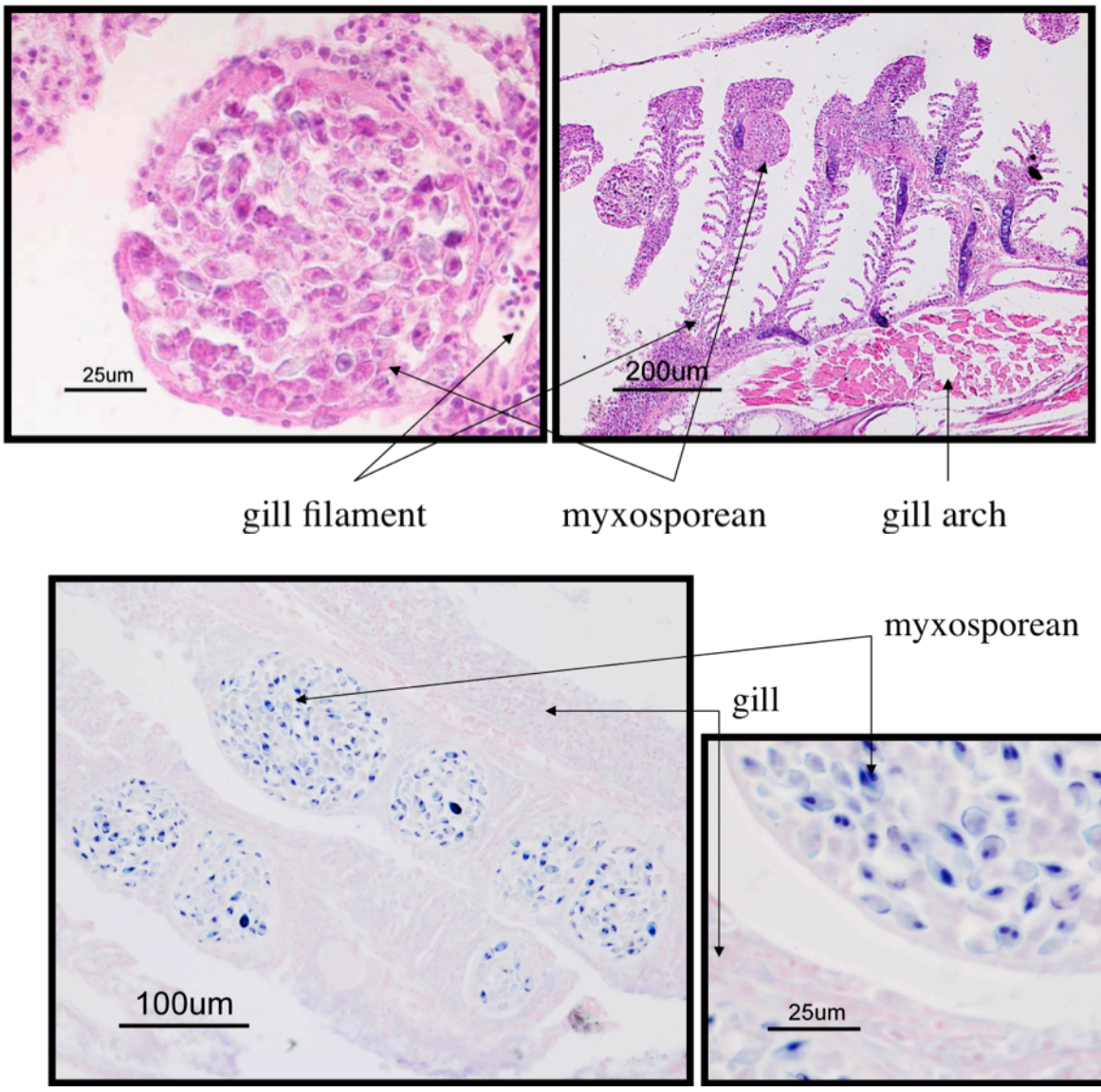
The sections of this species consists of multiple sections of whole fish including skin, gill, heart, liver, spleen, pancreas, head kidney, caudal kidney, gastrointestinal tract (stomach and intestine), thymus, swim bladder, brain, spinal cord, eye and ovary/testis. Not all of the tissues listed above were present on each slide examined.

Granulomatous lesions were common and were found in numerous tissues in this species of fish. A minority of these granulomatous lesions contained remnants of metazoan parasites.

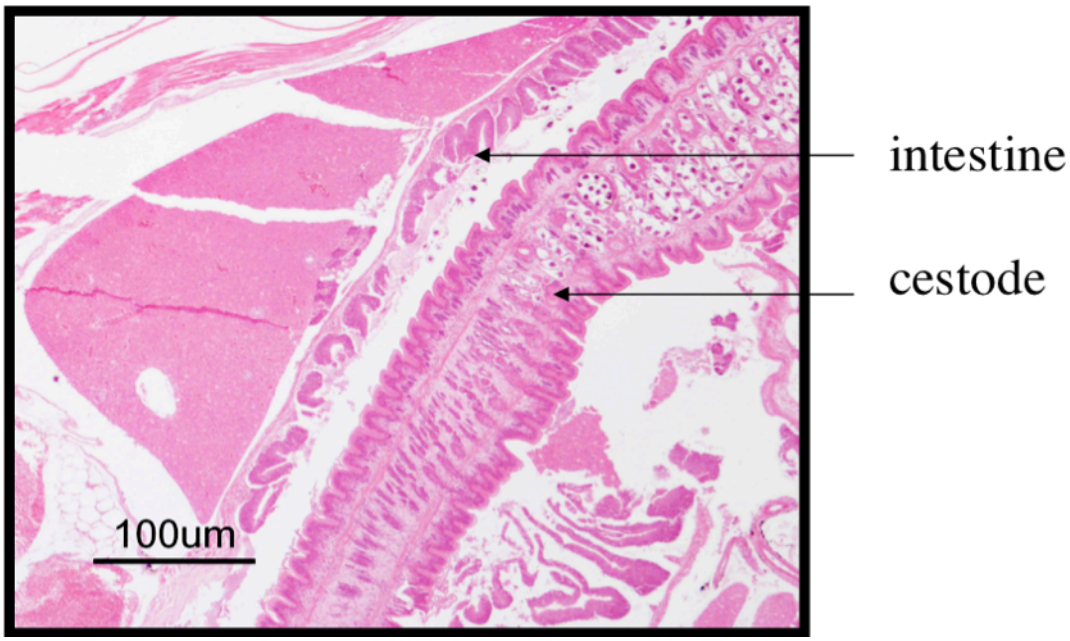


Lesions or organisms noted in both summer and spring.

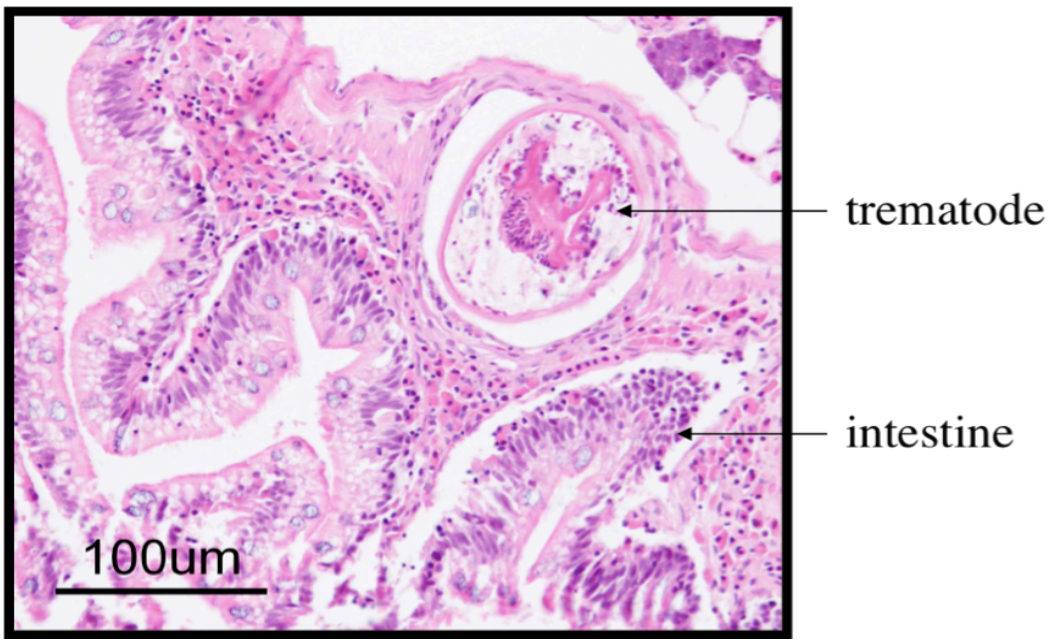
Branchial myxosporeans - Branchitis, multifocal to diffuse, with intralesional myxosporean cysts
Within various sections of gill, there are solitary to multiple myxosporean cysts between lamellae and surrounded by multifocal to regionally extensive epithelial hyperplasia admixed within increased numbers 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 12 to 15 µm in length. There is multifocal to diffuse accumulations of macrophages, lymphocytes, and eosinophilic granular cells surrounding the cysts. (Phylum Myxozoa, Class Myxosporea, Family Myxobolidae, presumptive *Myxobolus* sp.) Summer ---- 32 of 60 affected. Spring ---- 4 of 30 affected. Giemsa stain; lower pictures below.



Intestinal cestodes – Cestodiasis, intraluminal, 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). Summer ----44 of 60 affected. Spring ---- 2 of 30 affected.

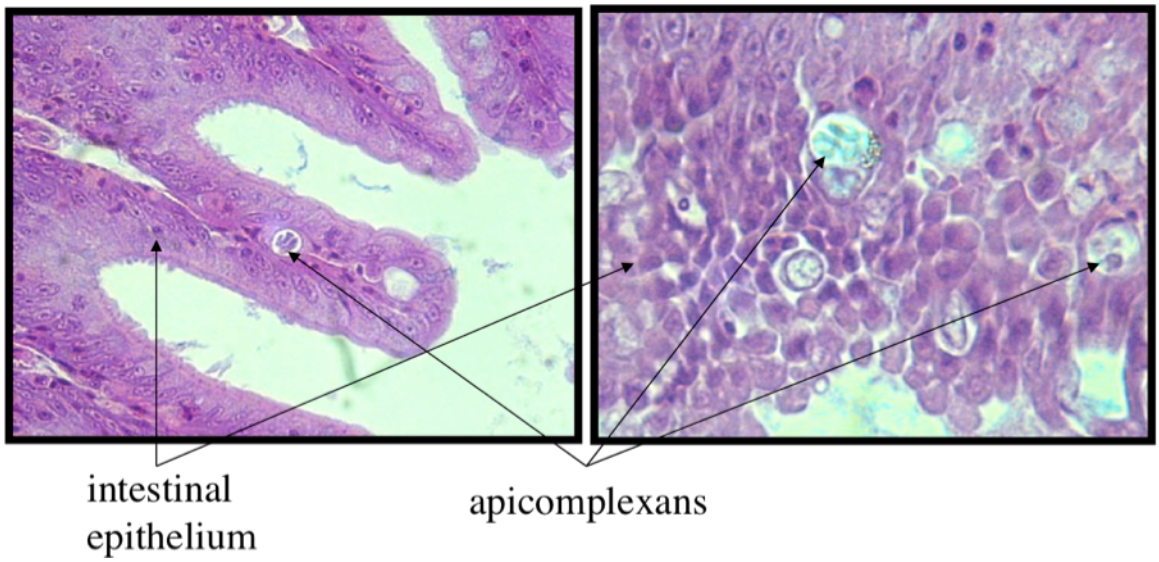


Intramuscular trematodes – Trematodiasis, intramuscular, encysted or Granuloma, intramuscular, parasitic.
There is a single metazoan organism migrating through the body wall musculature and penetrating the abdominal wall. The metazoan is characterized by a non-segmented thin tegument supported by row 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 intralesional trematodes are present in other sections examined. Summer ---- 24 of 60 affected. Spring ---- 1 of 30 affected.

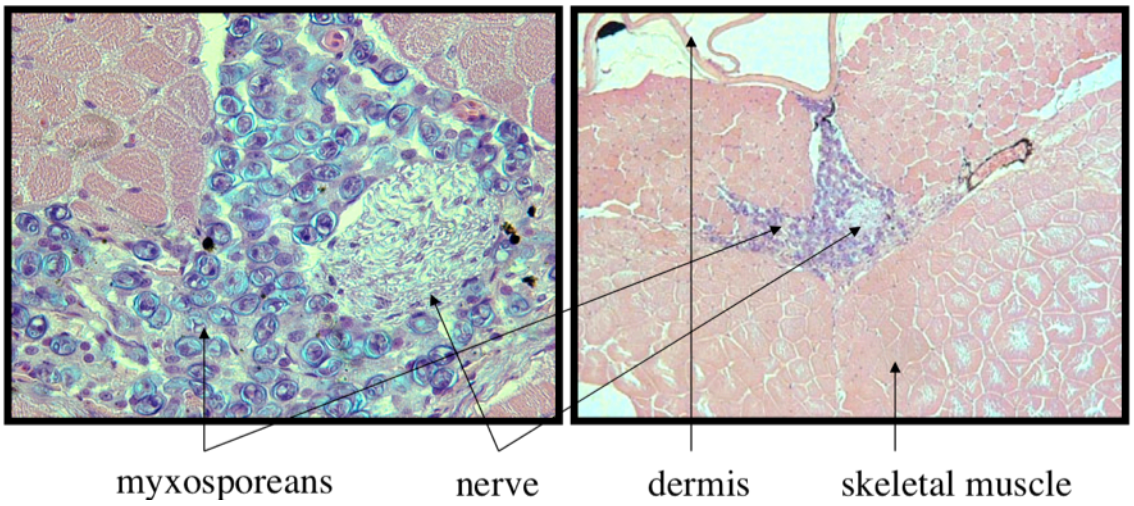


Lesions or organisms only noted in summer.

Intestinal apicomplexans - Enteritis, mild, multifocal with intralesional, 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 macrogamonts that range in size from ~10 – 20 μm) observed are similar to those of a coccidian apicomplexan (Phylum Apicomplexa, Subclass Coccidia, Family Eimeriidae or Calyptosporidae, presumptive). In still other areas there is mild erosion of the epithelial lining within limited areas of inflammation and accumulation of sporulated oocysts. ---- 28 of 60 affected.

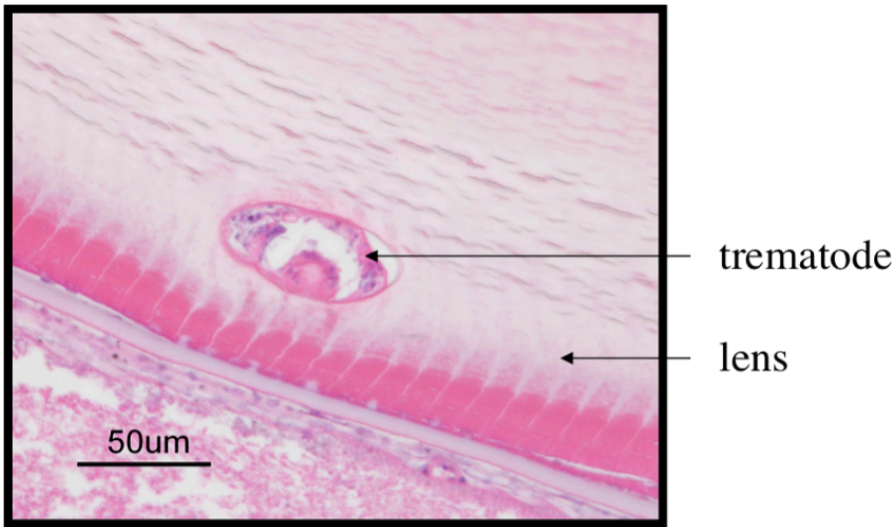


Perineural and muscular myxosporeans – Perineuritis and myositis, focal to multifocal, mild to moderate with intralesional myxosporeans.
Expanding spaces between muscle fibers and extending into the perineural space surrounding the nerve fascicle of the lateral line, there are moderate accumulations 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.



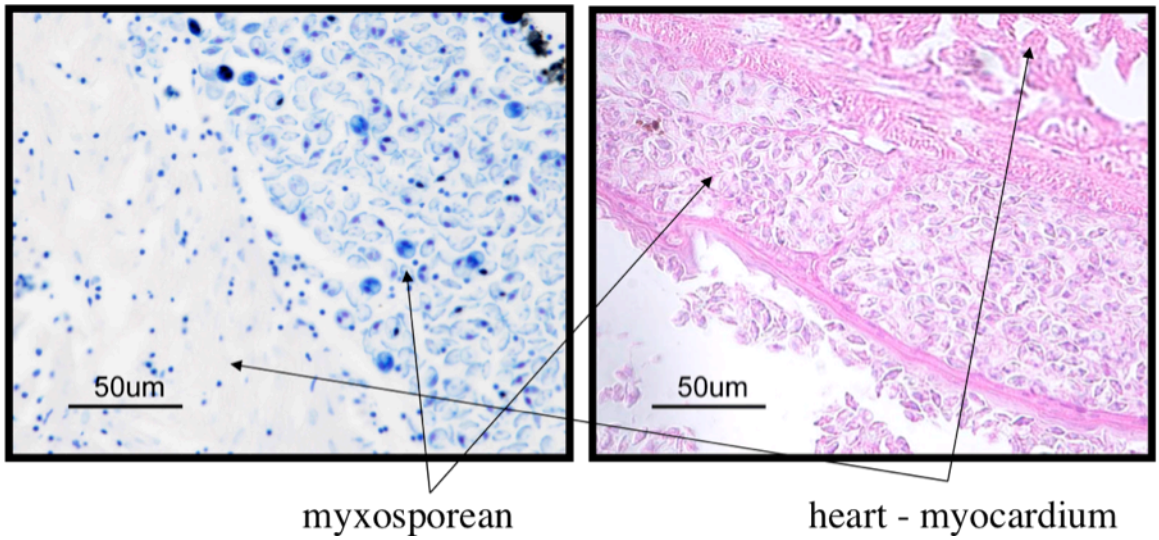
Branchial trematodes - – Branchitis, multifocal, mild to moderate, intralesional trematodes Between lamellae of filaments there are moderate accumulations of macrophages and lymphocytes expanding lamellae surrounding tubular metazoan organism that possess scleritinized hooks and tubular organs embedded within a loose reticular parenchyma (Phylum Platyhelminthes, Class Trematoda). ---- 2 of 60 affected. (no photo)

Eye fluke – Trematodiasis, intralenticular. Likely *Diplostomulum* sp. possibly *D. spathaceum*. Since a key for *Diplostomulum* indicates that a larval trematode in the eye and more specifically in the lens is likely this species. Embedded under the cuboidal epithelium of the lens, there are sections of a trematode that is approximately 40 by 60 µm characterized by thin eosinophilic tegument and that lacks a body cavity and calcareous corpuscles. There may be a pseudosucker present. (Phylum Platyhelmintheses, Class Trematoda, Order Strigeidida, likely Genus *Diplostomulum*). ---- 1 of 60 affected.

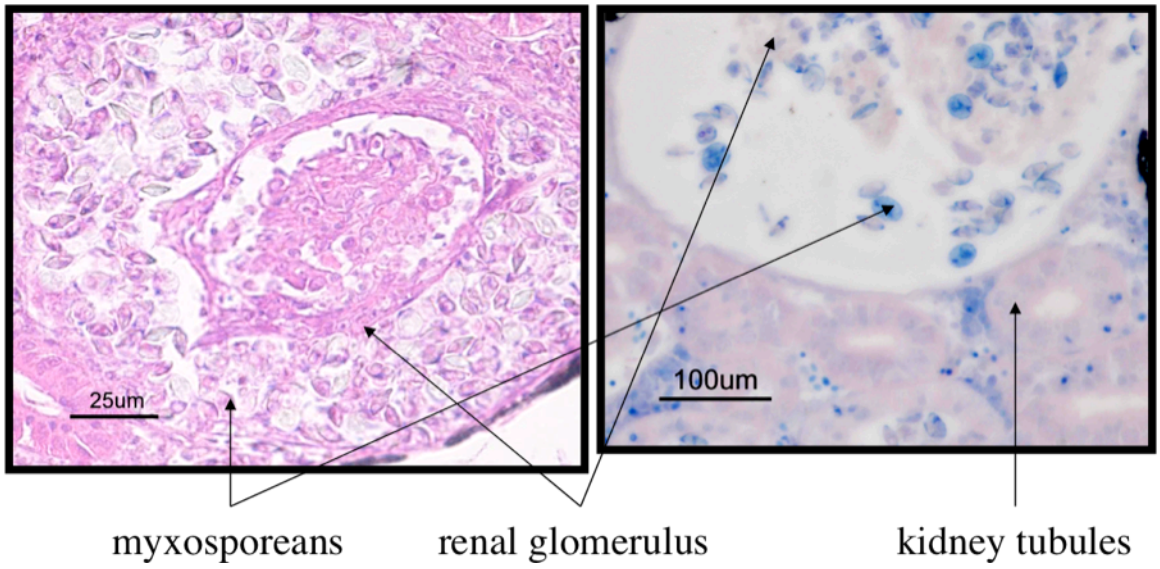


Lesions or organisms only noted in spring.

Pericardial myxosporeans – Myxosporidiosis, focal to regionally extensive, pericardial, moderate to large numbers.
Expanding spaces between connective tissue of the pericardium, there are moderate, multifocal accumulations of mature myxosporean spores. In muscle lesions, the myxosporeans are 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, Family Myxobolidae, presumptive *Myxobolus* sp.). ---- 3 of 30 affected. Giemsa stain at left, below.



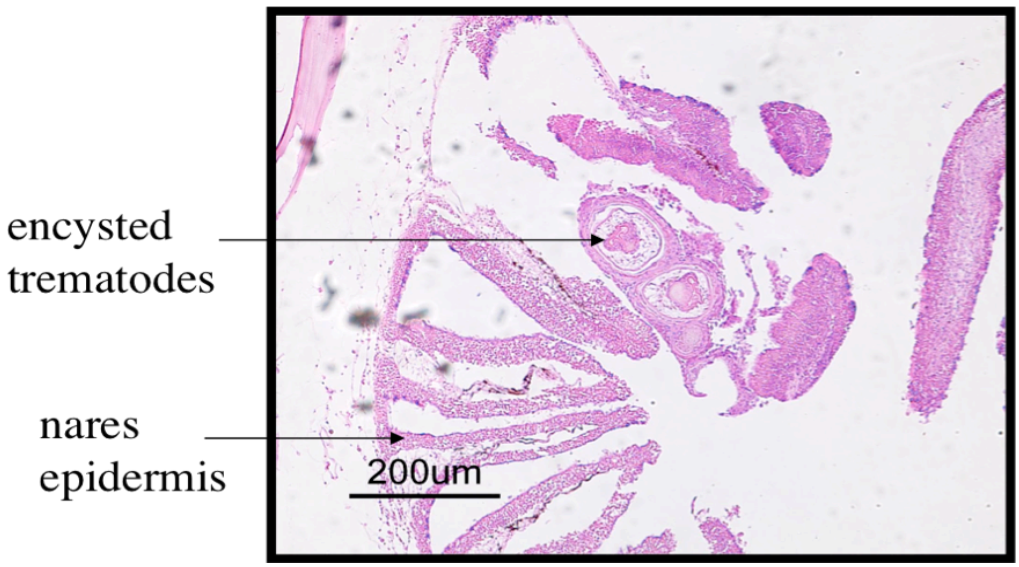
Renal myxosporeans - Myxosporidiosis, renal, interstitial, multifocal.
Within the interstitium of the caudal kidney, there are multifocal accumulations of mature myxosporean spores with similar morphology as those identified within gill, heart and muscle tissue. There is little to no host reaction surrounding these organisms (Phylum Myxozoa, Class Myxosporea). ---- 1of 30 affected. Giemsa stain at right, below.



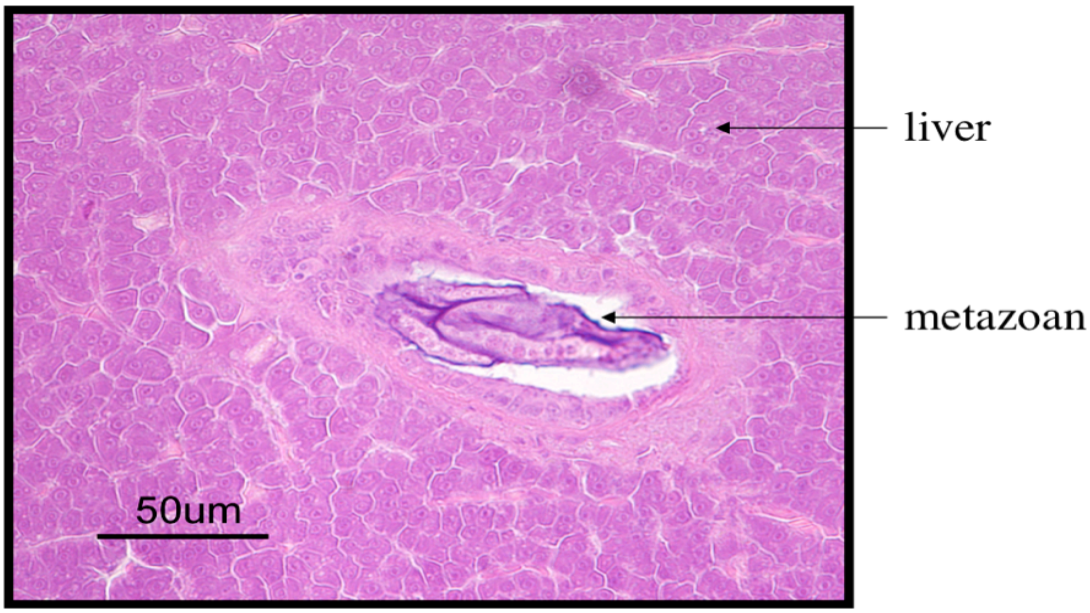
Pancreatic cestodiasis – Cestode, pancreatic, encysted.
Cross section of a large (100-200 μm) metazoan parasite is encysted in the pancreas. The metazoan possess an eosinophilic tegument, a lack of digestive tract and numerous round to ovoid, basophilic, calcium bodies (calcareous corpuscles) embedded within a loose paranchymatous matrix (larval cestode) (Phylum Platyhelminthes, Class Cestoidea). ----1 of 30 affected. (no photo)

Mural intestinal trematodiasis – Mural enteritis and peritonitis, solitary to multifocal, with intralesional trematodes.
Encysted within longitudinal muscle of the gut there are focal and multifocal cross-section of a trematode parasite characterized by thin tegument surrounding a layer of sub-tegumental cells and a loose parenchyma (Phylum Playthelminthes, Class Trematoda) and surrounded by multiple layers of plump and attenuated macrophages (cyst wall), and lower numbers of leukocytes. ---- 2 of 30 affected. (no photo)

Dermal trematodiasis – Trematodes, intradermal, encysted, multifocal.
Embedded within the epidermis of the nares, there are multifocal cross-sections of a metazoan parasite characterized by thin tegument surrounding a layer of sub-tegumental cells and a loose parenchyma and an absence of calcareous corpuscles (Phylum Playthelminthes, Class Trematoda). The trematodes are surrounded by multiple layers of plump and attenuated macrophages (cyst wall), and lower numbers of leukocytes. ---- 1 of 30 affected.

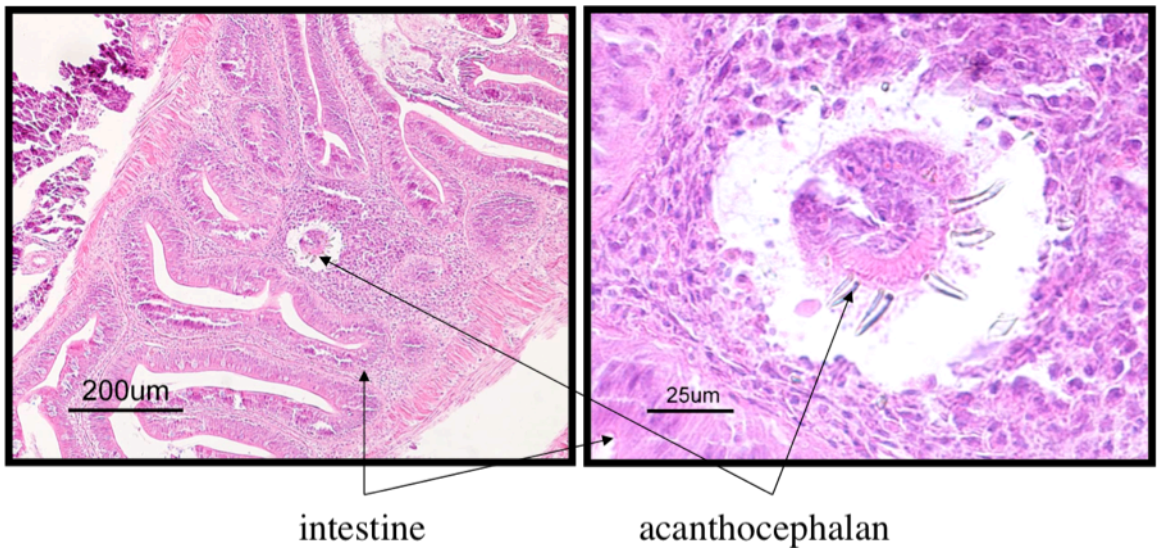


Bile duct metazoans – Metazoans, bile ducts, multifocal, low numbers.
Within the lumen of low numbers of large bile ducts, there are poorly preserved cross-sections of a metazoan parasite. In one section, the parasite is attached to the duct wall by a sucker-like structure not dissimilar to that of a trematode (Phylum Playthelminthes, Class Trematoda). Little to no host inflammatory reaction is associated with these metazoans. ---- 1 of 30 affected.



Pancreatic nematodiasis – Nematode, peritoneal, serosal, pancreatic, encysted.
Attached to and sometimes embedded within the serosal surface of the intestine, there are focal to multifocal cross-sections of a small and large encysted metazoan parasite. Similar encysted metazoans are replacing sections of pancreas. The metazoans are characterized by a thin eosinophilic cuticle, pseudocoelom and muscular pharynx. (Platyhelminthes, Class Nematoda). ---- 1 of 30 affected. (no photo)

Intestinal acanthocephalan – Acanthocephalan, intestinal, solitary.
Embedded with the intestinal mucosa there is a single cross-section of a metazoan parasite surrounded by minimal accumulations of host leukocytes. The metazoan is characterized by the presence of numerous spiny, scleritinized hooks lining a thick eosinophilic cuticle (proboscis of an acanthocephalan) (Phylum Acanthocephala). ---- 1 of 30 affected.



Summaries of lesions or organisms noted in individual fish.

Summer.

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 chitinous exoskeleton and striated muscle; these are presumed 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

2. Stomatitis, oro-pharyngeal, mild to moderate with epithelial hyperplasia, erosion, and ulceration.
3. Cestodiasis, intraluminal,
4. Branchitis, multifocal to regionally extensive, with intralesional myxosporean cysts.
5. Myxosporidiosis, intramuscular, focal

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 intralesional myxosporean cysts.
2. Stomatitis, regionally extensive epithelial hyperplasia with erosion, ulceration and filamentous bacteria.
3. Cestodiasis, intraluminal, intestinal

ES 3- NAF

ES 4 –
Morphological diagnosis

1. Branchitis, multifocal, intralesional myxosporean cysts
2. Cestodiasis, intraluminal, intestinal

ES 5 –
Morphological diagnosis

1. Cestodiasis, intraluminal, intestinal
2. Enteritis, mild, multifocal with intralesional accumulations of an intracellular apicomplexans
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 intra-cytoplasmic 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, intraluminal, intestinal
2. Branchitis, mild to moderate, diffuse.
3. Myositis, granulomatous, multifocal (Note: lesion similar to those surrounding encysted intramuscular trematodes)

ES 7 –
Morphological diagnosis

1. Cestodiasis, intraluminal, intestinal
2. Branchitis, mild, regionally extensive.
3. Myositis, focal, granulomatous (Note: lesion similar to encysted intramuscular trematodes)

ES 8 –
Morphological diagnosis

1. Branchitis, diffuse, with intralesional myxosporean cysts.
2. Myositis, multifocal, mild to moderate with intralesional myxosporeans
3. Enteritis, mild, multifocal with intralesional accumulations of an intracellular protozoan organism
4. Granuloma, focal, parasitic

ES 9 –
Morphological diagnosis

1. Branchitis, regionally extensive, with few small intralesional myxosporean cysts.

ES 10 –
Morphological diagnosis

1. Cestodiasis, intraluminal, intestinal

2. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans
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 intralesional myxosporean cysts
2. Cestodiasis, intraluminal, intestinal
3. Trematodiasis, intramuscular to peritoneal migration
4. Granuloma, intramuscular, encysted, parasitic.

ES 12 – NAF

ES 13 –
Morphological diagnosis
1. Cestodiasis, intraluminal, intestinal
2. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans
3. Branchitis, mild, multifocal.

ES 14 –
Morphological diagnosis
1. Branchitis, multifocal with intralesional myxosporean cysts
2. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

ES 15 –
Morphological diagnosis
1. Branchitis, regionally extensive with intralesional myxosporeans.
2. Cestodiasis, intraluminal, intestinal
3. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

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
1. Cestodiasis, intraluminal, intestinal
2. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans
3. Branchitis, mild, focal.

ES 17–
Morphological diagnosis

1. Dermatitis, locally extensive, ulcerative with mild, multifocal myositis and myodegeneration.
2. Branchitis, mild to moderate, multifocal.
4. Cestodiasis, intraluminal, intestinal
5. Granuloma, small, intramuscular, parasitic.
6. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

ES 18 –
Morphological diagnosis
1. Granuloma, small, intramuscular (epaxial), parasitic.
2. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

ES 19 –
Morphological diagnosis
1. Myositis, mild, focal (lateral line), histiocytic.
2. Branchitis, mild, multifocal.
3. Cestodiasis, intraluminal, intestinal
4. Granuloma, intramuscular (tail), metazoan type.
5. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

ES 20 –
Morphological diagnosis
1. Myositis, mild, focal (lateral line), histiocytic.
2. Branchitis, regionally extensive with intralesional myxosporean cysts.
3. Cestodiasis, intraluminal, intestinal

ES 21 –
1. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

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, intraluminal, intestinal
3. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans
4. Steatitis, focal, histiocytic.

ES 23 –
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
1. Branchitis, regionally extensive with intralesional myxosporean cysts
2. Retro bulbar myxosporean cysts
3. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

ES 24 –
Morphological diagnosis

1. Stomatitis, oral-pharyngeal, multifocal epithelial hyperplasia, erosion and ulceration.
2. Cestodiasis, intraluminal, intestinal
3. Branchitis, regionally extensive with intralesional myxosporean cysts

ES 25 –
Morphological diagnosis

1. Cestodiasis, intraluminal, intestinal
2. Branchitis, mild to moderate, multifocal to regionally extensive, with intralesional remnants of a metazoan organism.
3. Trematodiasis, larval, multifocal, intramuscular, encysted
4. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

ES 26 –
Morphological diagnosis

1. Branchitis, regionally extensive with intralesional myxosporean cysts

ES 27 –
Morphological diagnosis

1. Branchitis, regionally extensive with intralesional myxosporean cysts
2. Branchitis, mild, focal, with intralesional remnants of a single metazoan organism.
3. Cestodiasis, intraluminal, intestinal
4. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

ES 28 –
Morphological diagnosis

1. Cestodiasis, gastrointestinal, adult
2. Branchitis, multifocal, with intralesional myxosporean cysts
3. Trematodiasis, larval, intramuscular, encysted

ES 29 –
Morphological diagnosis

1. Cestodiasis, intraluminal, intestinal
2. Branchitis, mild, multifocal.

ES 30 –
Morphological diagnosis

1. Cestodiasis, intraluminal, intestinal
2. Branchitis, multifocal, with intralesional myxosporean cysts
3. Trematodiasis, larval, intramuscular, encysted
4. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

ES 31 –
Morphological diagnosis

1. Cestodiasis, intraluminal, intestinal
2. Branchitis, multifocal, with intralesional myxosporean cysts
3. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

ES 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.
Morphological diagnosis
1. Branchitis, multifocal, with intralesional myxosporean cysts
2. Granuloma, multifocal, retro bulbar, parasitic.

ES 33 –
Morphological diagnosis
1. Branchitis, multifocal, with intralesional myxosporean cysts
2. Cestodiasis, intraluminal, intestinal
3. Trematodiasis, larval, intramuscular, encysted

ES 34 –
Morphological diagnosis
1. Cestodiasis, intraluminal, intestinal
2. Branchitis, mild to moderate, multifocal intralesional trematodes
3. Trematodiasis, larval, focal, intramuscular, encysted

ES 35 - NSF

ES 36 –
Morphological diagnosis
1. Cestodiasis, intraluminal, intestinal
2. Trematodiasis, larval, focal, intramuscular, encysted
3. Granuloma, focal, intramuscular, parasitic.

ES 37 –
Morphological diagnosis
1. Cestodiasis, intraluminal, intestinal
2. Trematodiasis, larval, focal, intramuscular, encysted
3. Branchitis, mild to moderate, multifocal intralesional trematodes

ES 38 –
Morphological diagnosis
1. Cestodiasis, intraluminal, intestinal
2. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans
3. Branchitis, mild to moderate, focal.

ES 39 –

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, intraluminal, intestinal
2. Trematodiasis, focal, intramuscular, encysted
3. Myositis, mild to moderate, multifocal, histiocytic

ES 41 –

Morphological diagnosis

1. Cestodiasis, intraluminal, intestinal
2. Branchitis, multifocal, with intralesional myxosporean cysts
3. Myositis, mild, focal, histiocytic.

ES 42 –

Morphological diagnosis

1. Cestodiasis, intraluminal, intestinal
2. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans
3. Cellulitis, focal, periosteal, histiocytic.

ES 43 –

Morphological diagnosis

1. Cestodiasis, intraluminal, intestinal
2. Branchitis, multifocal, with intralesional myxosporean cysts
3. Granuloma, focal, intramuscular, parasitic.

ES 44 –

Morphological diagnosis

1. Cestodiasis, intraluminal, intestinal
2. Branchitis, multifocal, with intralesional myxosporean cysts
3. Granuloma, focal, intramuscular, parasitic.
4. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

ES 45 –

Morphological diagnosis

1. Cestodiasis, intraluminal, intestinal
2. Branchitis, multifocal, with intralesional myxosporeans
3. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

ES 46 –

Morphological diagnosis

1. Cestodiasis, intraluminal, intestinal

2. Branchitis, multifocal, with intralesional myxosporeans

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
1. Cestodiasis, intraluminal, intestinal
2. Branchitis, multifocal, with intralesional myxosporeans
3. Perineuritis and myositis, focal to multifocal, mild to moderate with intralesional myxosporeans
4. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

ES 48 –
Morphological diagnosis
1. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

ES 49 –
Morphological diagnosis
1. Cestodiasis, intraluminal, intestinal
2. Branchitis, multifocal, with intralesional myxosporeans

ES 50 –
Morphological diagnosis
1. Cestodiasis, intraluminal, intestinal
2. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

ES 51 –
Morphological diagnosis
1. Cestodiasis, intraluminal, intestinal
2. Branchitis, multifocal, with intralesional myxosporeans
3. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

ES 52 –
Morphological diagnosis
1. Cestodiasis, intraluminal, intestinal
2. Branchitis, multifocal, with intralesional myxosporeans
3. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

ES 53 –
Morphological diagnosis
1. Cestodiasis, intraluminal, intestinal
2. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans
3. Branchitis, mild, multifocal.

ES 54 –
Morphological diagnosis

4. Cestodiasis, intraluminal, intestinal
5. Branchitis, multifocal, with intralesional myxosporeans
6. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

ES 55 –
Morphological diagnosis

1. Cestodiasis, intraluminal, intestinal
2. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans
3. Granuloma, focal, intramuscular, parasitic.
4. Branchitis, mild, multifocal.

ES 56 –
Morphological diagnosis

1. Branchitis, mild, multifocal, intralesional myxosporean cysts
2. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans
3. Trematodiasis, multifocal, intramuscular, encysted

ES 57 –
Morphological diagnosis

1. Cestodiasis, intraluminal, intestinal
2. Granuloma, multifocal , intramuscular, intra-coelimic, parasitic.
3. Branchitis, multifocal, with intralesional myxosporeans
4. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

ES 58 –
Morphological diagnosis

1. Stomatitis, moderate, regionally extensive, with erosion and ulceration.
2. Branchitis, multifocal, with intralesional myxosporeans
3. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

ES 59 –
Morphological diagnosis.

1. Cestodiasis, intraluminal, intestinal
2. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans
3. Trematodiasis, multifocal, intramuscular, encysted

ES 60 –
Morphological diagnosis.

1. Cestodiasis, intraluminal, intestinal
2. Branchitis, mild, multifocal.
3. Enteritis, mild, multifocal with intralesional, intracellular apicomplexans

Spring

ES 1 –
Morphological diagnosis
1. Trematodes, intradermal, encysted, multifocal
2. Cestodes, intraluminal, intestinal
3. Enteritis, focal with intralesional encysted trematode

ES 2 –
Morphological diagnosis
1. Branchitis, multifocal, with intralesional myxosporean cysts

ES 3 –
1. Branchitis, multifocal, mild

ES 4 –
Morphological diagnosis
1. Branchitis, multifocal with intralesional myxosporean cysts
2. Dermatitis, multifocal to focally extensive, mild to moderate

ES 5 –
Morphological diagnosis
1. Branchitis, multifocal, mild
2. Trematode, hepatic, encysted, focal
3. Granuloma, parasitic, serosal, multifocal

ES 6 –
Morphological diagnosis
1. Dermatitis, focal, moderate
2. Branchitis, multifocal, mild
3. Trematodes, intramuscular, encysted, focal
4. Myositis, regionally extensive with myofiber degeneration and necrosis

ES 7 - NAF

ES 8 –
Morphological diagnosis
1. Branchitis, multifocal, mild
2. Myxosporidiosis, multifocal, pericardial, moderate numbers

ES 9 –
Morphological diagnosis
1. Stomatitis, regionally extensive, mild to moderate
2. Cestode, larval, pancreatic, encysted
3. Acanthocephalan, intestinal, solitary

ES 10 –
Morphological diagnosis

1. Nematode, larval, pancreatic, encysted, multifocal
2. Branchitis, multifocal, with intralesional myxosporean cysts
3. Granuloma, parasitic, intramuscular, focal

ES 11 –
Morphological diagnosis
1. Metazoans, bile ducts, multifocal, low numbers
2. Granulomas, parasitic, peritoneal, multifocal

ES 12 – NAF

ES 13 – NAF

ES 14 –
Morphological diagnosis
1. Myxosporidiosis, renal, interstitial, multifocal
2. Nephrocalcinosis, multifocal, mild
3. Granulomas, parasitic, serosal, multifocal

ES 15 –
Morphological diagnosis
1. Branchitis, multifocal, mild
2. Myxosporidiosis, multifocal, pericardial, moderate numbers

ES 16 –
Morphological diagnosis
1. Branchitis, multifocal, mild

ES 17 – NAF

ES 18
Morphological diagnosis
1. Pericarditis, granulomatous, multifocal
2. Cestode, intraluminal, intestinal

ES 19 –
Morphological diagnosis
1. Branchitis, multifocal, mild

ES 20 - NAF

ES 21 –
Morphological diagnosis
1. Pericarditis, granulomatous, multifocal
2. Trematode, retrobulbar, intramuscular, encysted, focal

ES 22 –
Morphological diagnosis
1. Branchitis, multifocal, mild
2. Granuloma, parasitic, intramuscular, focal with intralesional calcification

ES 23 –
Morphological diagnosis
1. Myxosporidiosis, multifocal, pericardial, moderate to large numbers
2. Granulomas, parasitic, endomeningial, multifocal
3. Granuloma, parasitic, intramuscular, multifocal

ES 24 –
Morphological diagnosis
1. Granulomas, parasitic, mural and serosal, multifocal

ES 25 - NAF

ES 26 - NAF

ES 27 –
Morphological diagnosis
1. Branchitis, multifocal with intralesional myxosporean cysts

ES 28 –
Morphological diagnosis
1. Granuloma, retrobulbar, intramuscular, encysted, focal

ES 29 –
Morphological diagnosis
1. Branchitis, multifocal, mild

ES 30 –
Morphological diagnosis
1. Branchitis, multifocal, mild
2. Enteritis and peritonitis, multifocal, with intralesional encysted trematodes

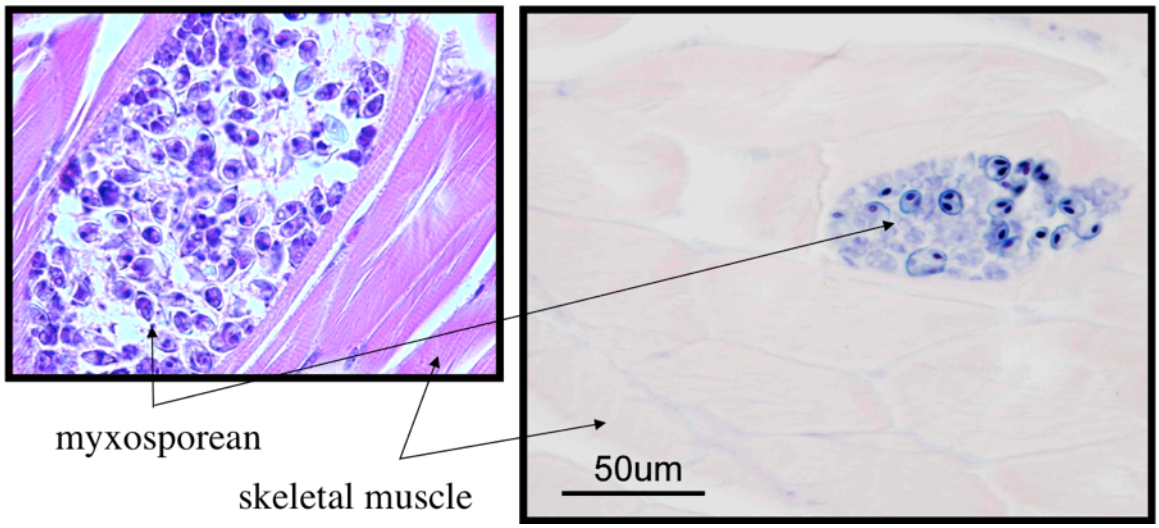
Table 4. Summary of notable lesions and organisms in the fathead minnow (*Pimephales promelas* – FHM) collected during the summer of 2006 (n=60) and the spring of 2007 (n=30) from Lake Winnipeg and surrounding waters.

Fathead minnow Tissue/agent	Summer 2006 – Sixty fish	Spring 2007 – Thirty fish
Gills	1. Myxosporean (5/60) 2. Epitheliocystis (1/60) 3. Monogenetic trematode (9/60)	1. *Trichodina* sp. (1/30)
Intestine/ peritoneum		1. Myxosporean (1/30) 2. Peritoneal trematode (14/30) 3. Peritoneal nematode (3/30) 4. Intestinal cestode (4/30)
Skeletal muscle and skin	1. Myxosporean, likely *Myxobolus* sp. (38/60) 2. Trematode (15/60)	1. Myxosporean, likely *Myxobolus* sp. (2/30) 2. Trematode (17/30)
Brain and meninges	1. Meningeal trematodes (31/60)	1. Meningeal trematodes (6/30)
Liver and bile ducts	1. Nematodes (12/60)	
Kidney	1. Myxosporean (5/60) 2. Myxosporean plasmodia (2/60)	
Heart	1. Myxosporean plasmodia (5/60) 2. Myxosporean, pericardial, likely *Myxobolus* sp. (1/60)	
Other	1. Thymic myxosporean, likely *Myxobolus* sp. (2/60) 2. skin – monogenetic trematode (1/60)	1. Pancreatic nematode (see peritoneum above)

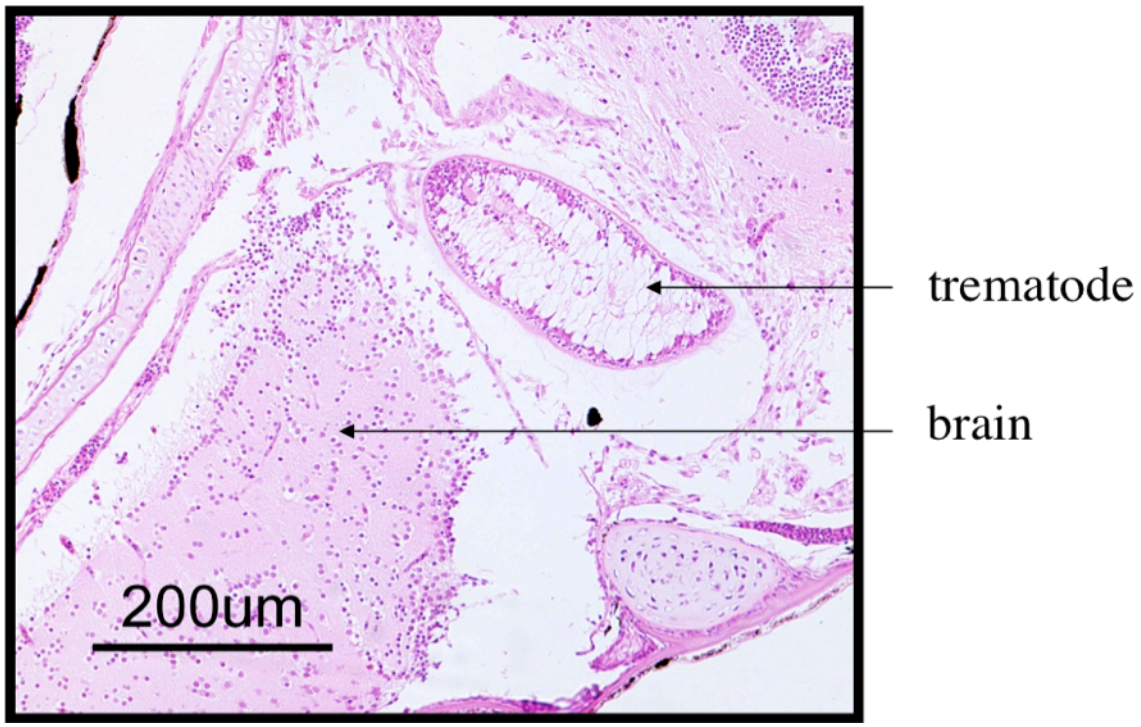
Each slide consists of multiple sections of whole fish including skin, gill, heart, liver, spleen, pancreas, head kidney, caudal kidney, gastrointestinal tract (stomach and intestine), thymus, swim bladder, brain, spinal cord, eye and ovary/testis. Not all of the tissues listed above were present on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

Lesions or organisms found in both spring and summer.

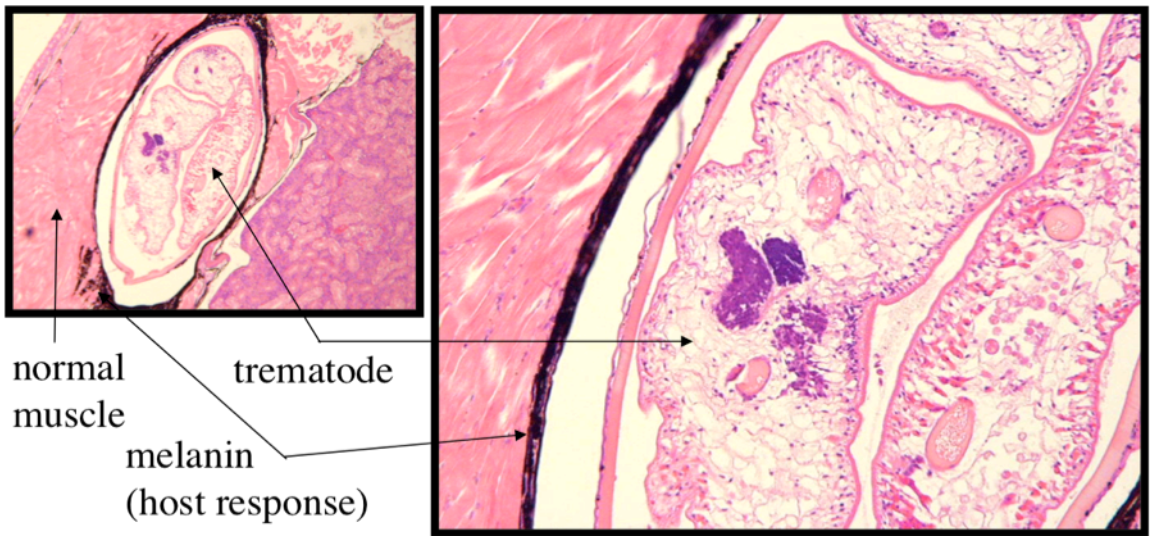
Intramuscular myxosporidiosis. Myxosporidiosis, intramuscular, focal to multifocal. Replacing sections of skeletal myofibres, there are single to several myxosporean cysts (ranging is size from 100 to 200 μm) consisting of both immature multinucleate sporoplasts and maturing spores (approx 7 x 15 μm). 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, Family Myxobolidae, presumptive *Myxobolus* sp.). Summer ----- 38 of 60 affected. Spring ---- 2 of 30 affected. Giemsa stain at right, below.

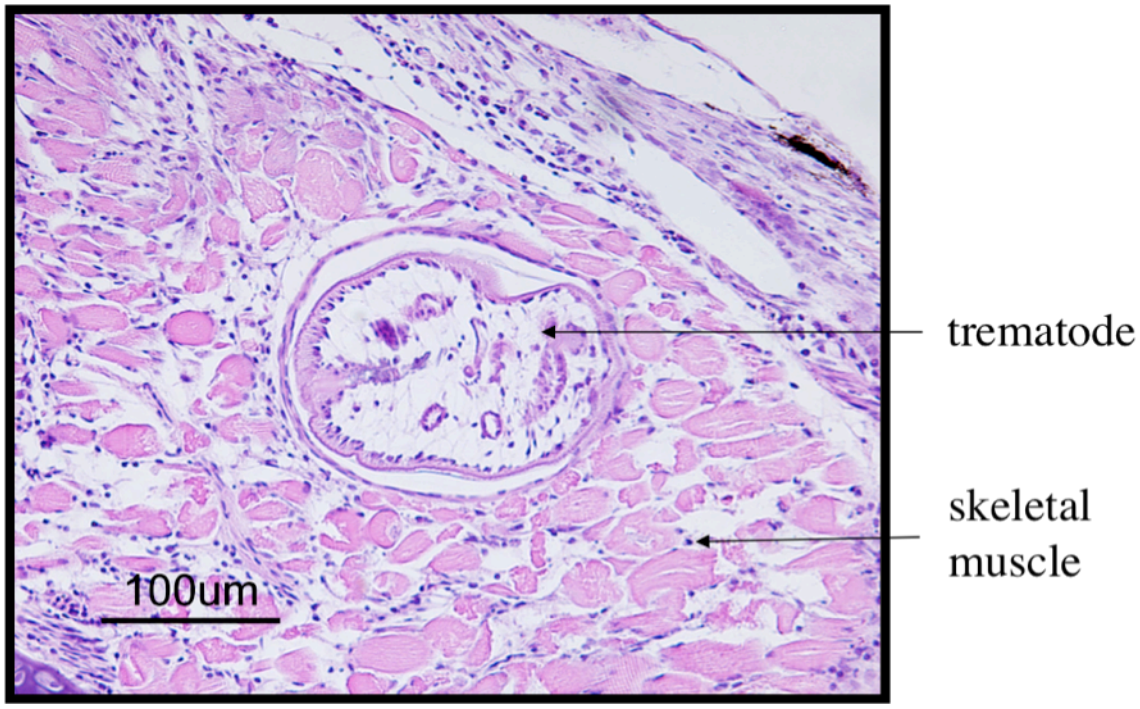


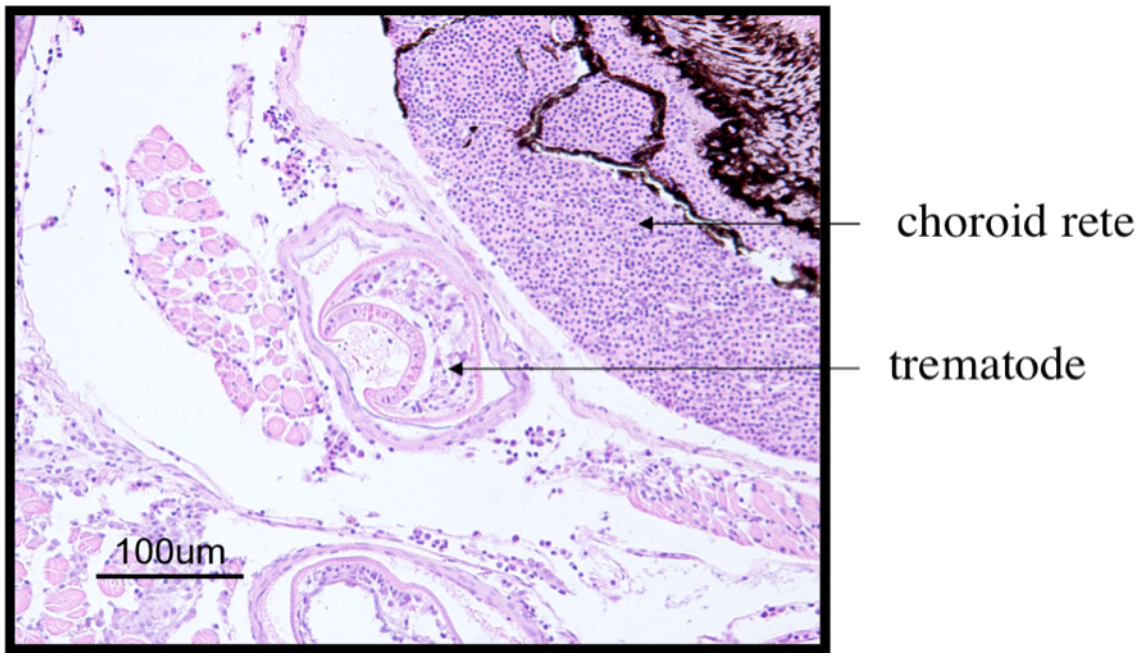
Endomeningial trematodiasis: Trematodiasis, endomeningial, encysted, focal and multifocal. Cross sections of trematode organisms ranging in size from 50 to 200 μm in diameter are expanding endomeningial spaces surrounding multiple sections of the brain. The trematodes are encysted by single to few layers of flattened macrophages and characterized by a thin eosinophilic tegument and a parenchymatous matrix with no calcareous corpuscles. (Phylum Platyhelmintheses, Class Trematoda). 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 intralesional trematodes were present within the semicircular canal of small numbers of fish.
Summer ----- 31 of 60 affected. Spring ---- 6 of 30 affected.



Intramuscular trematodiasis: Myositis, multifocal, small and large with intralesional trematodes.
Single to multiple cross sections (200 to 500 µm) 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 variable multiple layers of plump and attenuated macrophages (cyst wall), and a subset also have a variable number of melanin-laden macrophages in the cyst wall. These two morphologically-distinct trematodes are likely different species but were not separated here. A third morphological type was noted rarely in the retrobulbar musculature. (Phylum Platyhelminthes, Class Trematoda) Summer ----- 15 of 60 affected. Spring ---- 17 of 30 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.

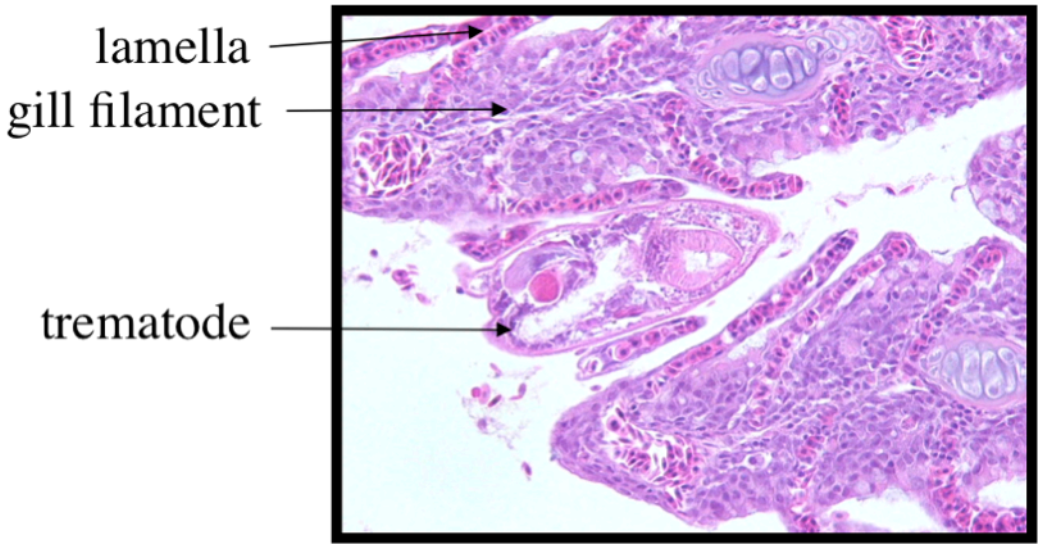

normal
muscle
trematode
melanin
(host response)


trematode
skeletal
muscle
100um

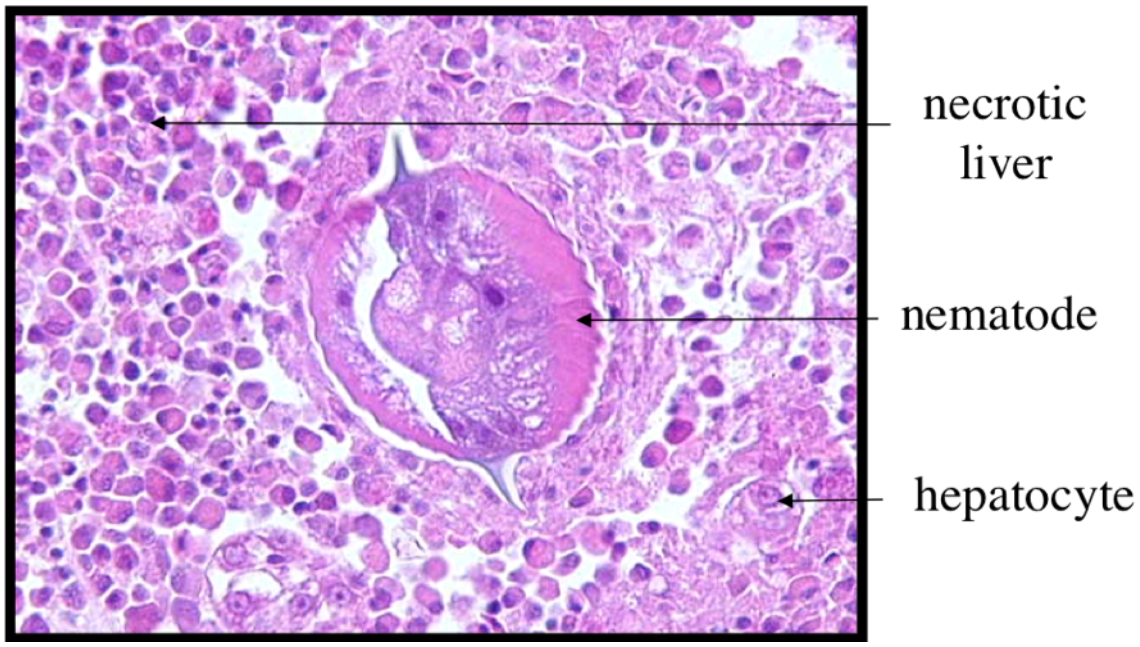


Lesions or organisms only noted in summer.

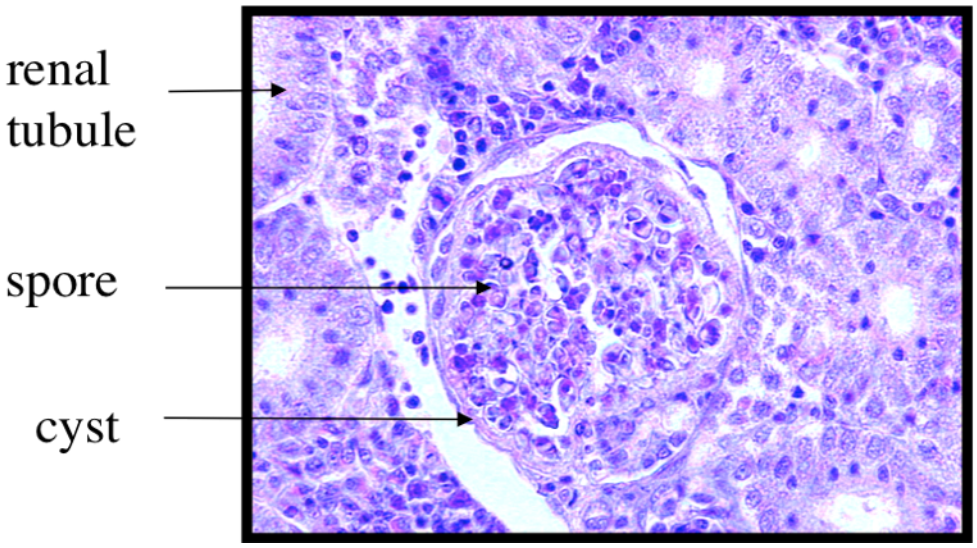
Branchial trematodiasis: Branchitis, multifocal to regionally extensive, mild to moderate, with intralesional trematodes, likely a monogenetic trematode.
Within several fish, cross sections of trematodes within the branchial cavity are associated mild to moderate, multifocal to regionally extensive accumulations of macrophages and lesser numbers of eosinophils. The trematodes, ranging is size from 100 to 175 µm 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.



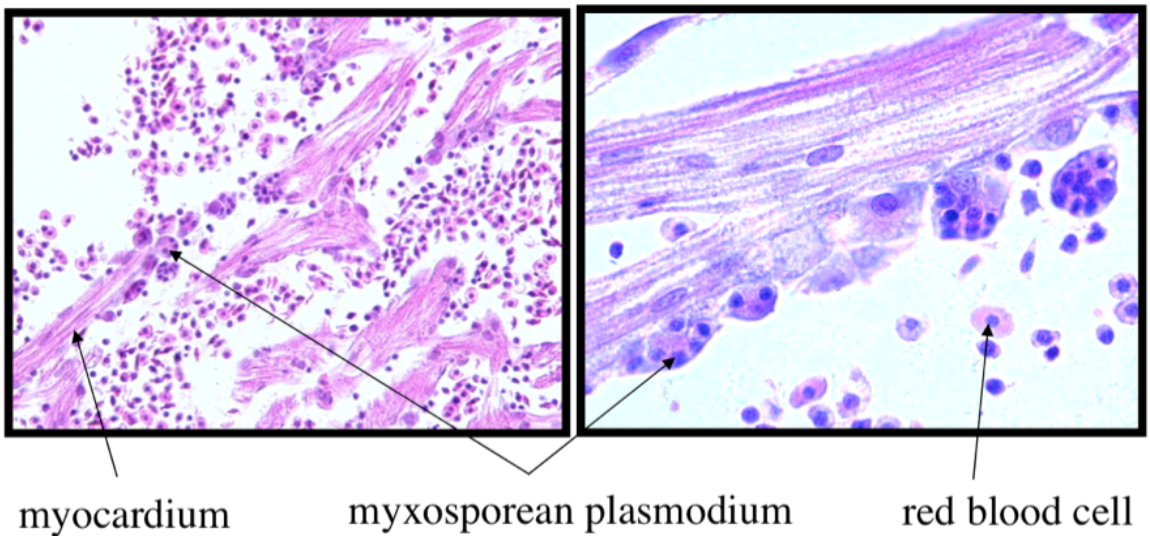
Hepatic and extra-hepatic nematodiasis. Hepatitis, multifocal to regionally extensive, mild to moderate, with intralesional nematodes and/or; Coelomitis, multifocal to regionally extensive, mild to moderate, with intralesional trematodes**.**
Multiple sections of histomorphologically dissimilar nematodes 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 to 120 μm 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 200 μm) 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.

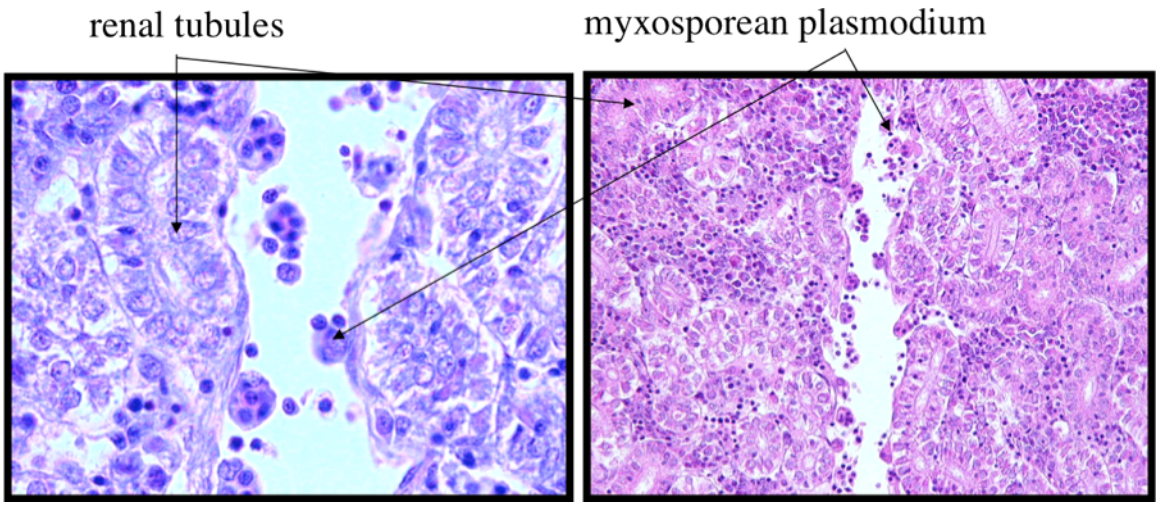


Renal myxosporidiosis. Myxosporidiosis**,** multifocal, mild.
Within the interstitium, there is a small myxosporean cyst (70 to 95 μm in diameter) consisting of both immature multinucleate sporoplasm and maturing spores. The spores are ellipsoid to oval (valvular view) in shape and are histomorphologically 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.



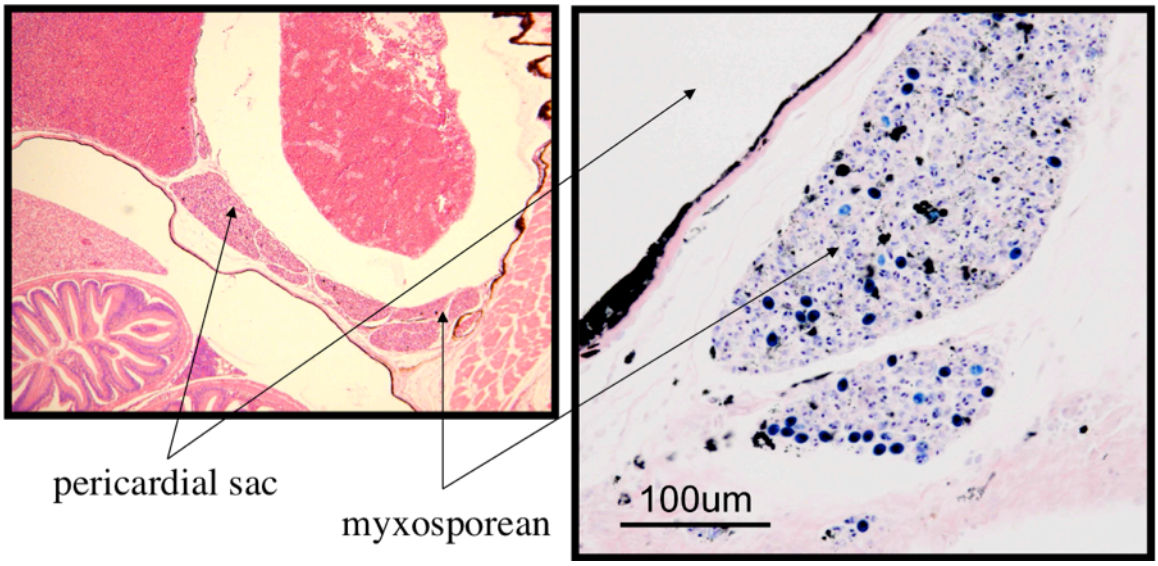
Myocardial and Renal intravascular myxosporidiosis. Myxosporidiosis**,** multifocal, myocardial, nephric, inter-vascular.
Heart - Attached and abutted against sections of the endocardium, there are small to moderate numbers of multinucleate plasmodium (15 to 40 µm in diameter). Within some sections the 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).



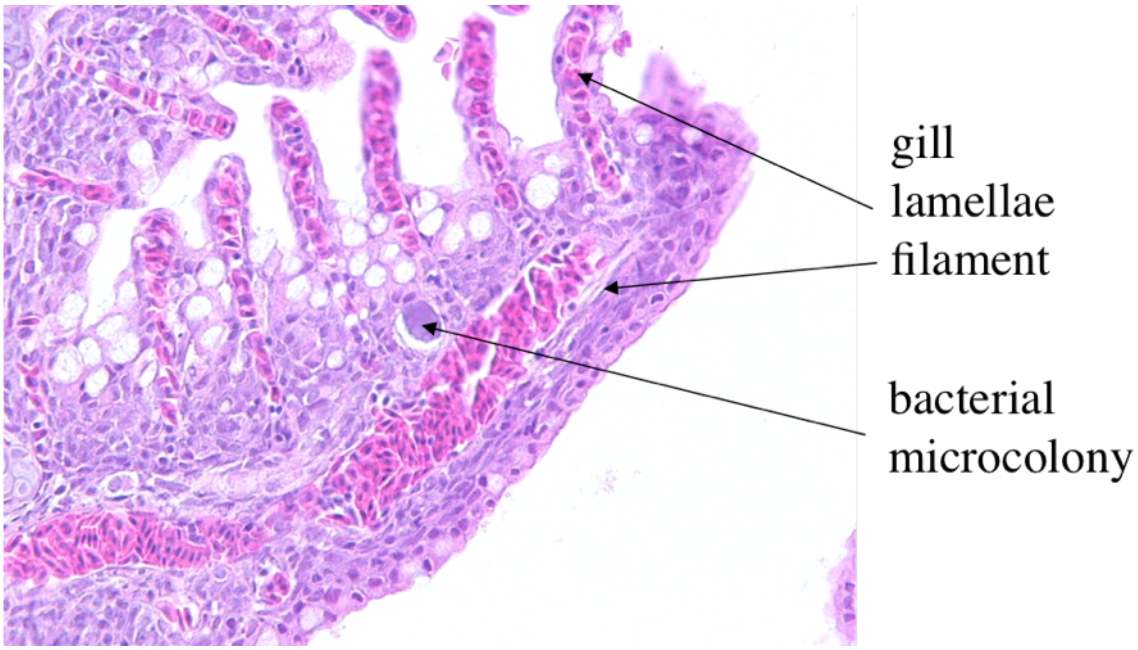


Branchial myxosporidiosis. Branchitis, multifocal, mild to moderate with intralesional myxsporeans.
Between lamellae on scattered filaments, there are multifocal myxosporean cysts (60 to 100 µm in diameter) consisting of both immature multinucleate sporoplasm and maturing spores. Small numbers of macrophages, 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. (no photo)

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 µm in length and 7 µm at the widest diameter. Little to no inflammatory cells surrounds the accumulation of spores. (Phylum Myxozoa, Class Myxosporea, Family Myxobolidae, presumptive *Myxobolus* sp.) ----- 1 of 60 affected. Giemsa stain at right, below.



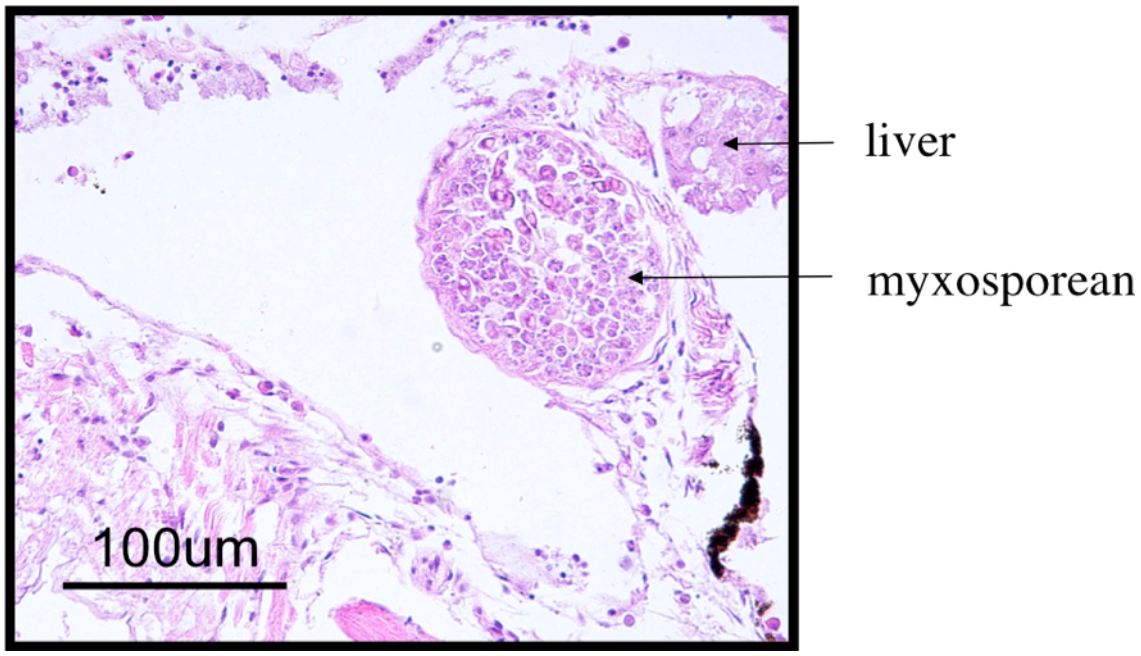
Branchial epitheliocystis. Epitheliocystis, focal.
A single focus (~ 20 µm in diameter) of amorphous basophilic chlamydia-like organisms is present between lamellae of a single filament and is surrounded by small numbers of plump macrophages and lesser numbers of lymphocytes. (chlamydia-like organism, presumptive) ----- 1 of 60 affected.



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 11 µm in length and 6-7 µm in diameter. (Phylum Myxozoa, Class Myxosporea, Family Myxobolidae, presumptive *Myxobolus* sp.) ----- 2 of 60 affected. (no photo)

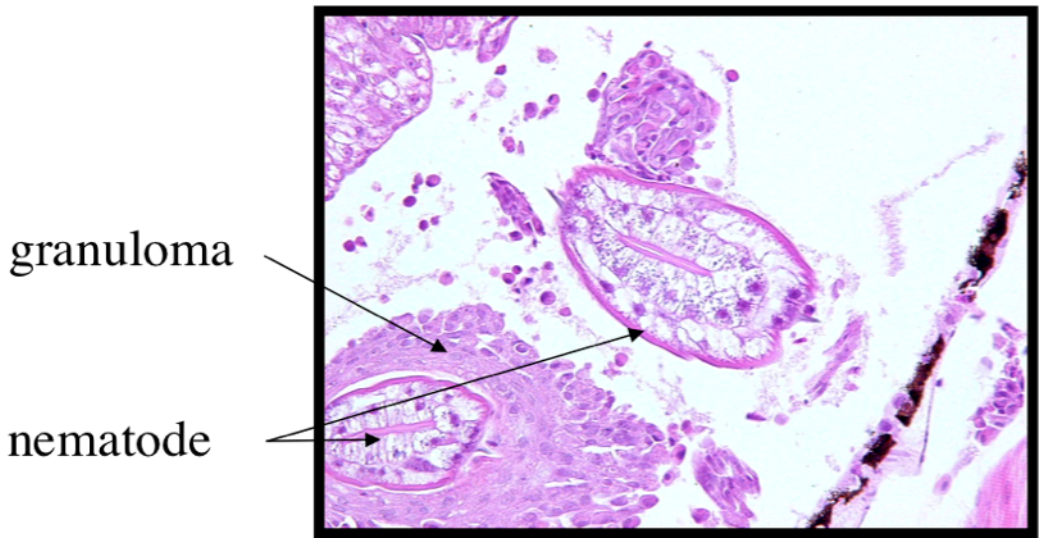
Lesions or organisms only noted in spring.

Peritoneal myxosporidiosis - Myxosporidiosis**,** focal, low to moderate numbers.
Expanding space between the connective tissue of the peritoneum, there is a single myxosporean cyst (100 to 125 µm 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 cysts. ---- 1 of 30 affected.



Peritoneal trematodiasis - Coelomitis, granulomatous, multifocal, mild to moderate, with intralesional trematodes**.**
Focal or multifocal cross sections of trematodes are present within the peritoneal cavity of several fish. 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 attenuated macrophages (cyst wall) (Phylum Platyhelminthes, Class Trematoda). ----- 14 of 30 affected. (no photo)

Pancreatic and peritoneal nematodiasis. Pancreatitis and/or coelomitis, granulomatous, multifocal, mild to moderate, with intralesional nematodes**.**
Multiple cross-sections of larval nematodes are within the peritoneal cavity or embedded within pancreatic tissue. The nematodes (~20 to 30 μm) 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 (Phylum Nemathelminthes, Class Nematoda). ----- 3 of 30 affected.



Intestinal cestodiasis – Cestodes, intraluminal, intestinal.
Sections of a cestode parasite are present within multiple sites along the digestive tract. The cestodes possess 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). ---- 4 of 30 affected. (no photo)

Branchial trichodinosis – Branchitis, multifocal, with lamellar *Trichodina*.
There are small numbers of ~25 μm dorsoventrally flattened protozoans with a chitinous endoskeleton (denticular ring) and ring of cilia (*Trichodina* sp.) (Phylum Ciliophora, Class Litostomatea, Family Trichodina). ---- 1 of 30 affected. (no photo)

Summaries of lesions or organisms noted in individual fish.

Summer

FHM 1 –
Morphological diagnosis
1. Myxosporidiosis, intramuscular, focal

FHM 2 –
Morphological diagnosis
1. Branchitis, multifocal, mild with intralesional trematode.
2. Trematodiasis, endomeningial, semicircular canal, intramuscular

FHM 3 –
Morphological diagnosis
1. Branchitis, multifocal, mild.
2. Trematodiasis, endomeningial, focal

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. Coelomitis, parasitic, granulomatous, multifocal, mild
2. Hepatitis, multifocal, mild

FHM 5 -
Morphological diagnosis

1. Branchitis, regionally extensive, mild
2. Trematodiasis, intramuscular, solitary
3. Myxosporidiosis, intramuscular, multifocal

FHM 6 –
Morphological diagnosis

1. Hepatitis, multifocal to regionally extensive, moderate with intralesional nematodes.
2. Coelomitis, multifocal, moderate with intralesional nematodes.
3. Nematodiasis, intra-hepatic, intra-peritoneal, low numbers
4. Myxosporidiosis, intramuscular, multifocal

FHM 7 –
Morphological diagnosis

1. Branchitis, regionally extensive, mild with an intralesional trematode
2. Trematodiasis, endomeningial, encysted

FHM 8 –
Morphological diagnosis

1. Branchitis, multifocal, mild with an intralesional trematode
2. Trematodiasis, endomeningial, encysted, multifocal

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
2. Branchitis, regionally extensive, mild with intralesional protozoa (Trichodina: presumptive)
3. Hepatitis, multifocal, mild to moderate
4. Granuloma, parasitic, intramuscular, multifocal, small and large.

FHM 10
Morphological diagnosis

1. Myxosporidiosis, intramuscular, multifocal
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
2. Branchitis, regionally extensive, mild with an intralesional protozoa
3. Dermatitis, mild, focal
4. Nephritis, mild focal

FHM 12 –
Morphological diagnosis

1. Myxosporidiosis, intramuscular, intra-nephric, intravascular, multifocal, moderate numbers

FHM 13 –
Morphological diagnosis

1. Trematodiasis, endomeningial, focal
2. Myxosporidiosis, intramuscular, intravascular, multifocal
3. Hepatitis, multifocal, mild to moderate.
4. Coelomitis, focal, mild to moderate.

FHM 14 –
Morphological diagnosis

1. Trematodiasis, endomeningial, focal

FHM 15 –
Morphological diagnosis

1. Branchitis, regionally extensive, moderate
2. Trematodiasis, endomeningial and peritoneal

FHM 16 -
Morphological diagnosis

1. Trematodiasis, endomeningial, solitary
2. Myxosporidiosis, intramuscular, intravascular, multifocal

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

2. Enteritis, multifocal with intralesional enterocyte degeneration and necrosis / unknown intra-cytoplasmic organism
3. Endomeningitis, parasitic, focal

FHM 18 –
Morphological diagnosis
1. Myxosporidiosis, thymic, intramuscular, multifocal
2. Trematodiasis, endomeningial, intramuscular, multifocal
3. Protozoan, branchial arch, multifocal, low numbers (Presumptive Phylum Myxozoa, Class Myxosporea).

FHM 19
Morphological diagnosis
1. Branchitis, multifocal, moderate with scattered intralesional necrosis, cellular degeneration and trematodes
2. Coelomitis, multifocal mild to moderate with intralesional nematodes
3. Myxosporidiosis, intramuscular, multifocal

FHM 20 –
Morphological diagnosis
1. Myxosporidiosis, intramuscular, multifocal
2. Endomeningitis, parasitic, focal

FHM 21–
Morphological diagnosis
1. Myxosporidiosis, intramuscular, nephric, branchial, multifocal
2. Nephritis, interstitial, multifocal with intralesional immature multinucleate plasmodium and maturing spores
3. Branchitis, multifocal, mild to moderate intralesional 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
3. Endomeningitis, parasitic, multifocal

FHM 24 -
Morphological diagnosis
1. Branchitis, multifocal, mild
2. Myxosporidiosis, intramuscular, focal

FHM 25 -
Morphological diagnosis

1. Branchitis, multifocal to regionally extensive, mild
2. Myxosporidiosis, intramuscular, multifocal
3. Granuloma, parasitic, encysted, intramuscular, focal
4. Trematodiasis, endomeningial, multifocal

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
3. Trematodiasis, endomeningial, focal

FHM 28 -
Morphological diagnosis

1. Granuloma, parasitic, encysted, intramuscular, focal
2. Myxosporidiosis, nephric, interstitial, intravascular multifocal
3. Trematodiasis, endomeningial, focal

FHM 29 –
Morphological diagnosis

1. Granuloma, parasitic, encysted, intramuscular, focal
2. Myxosporidiosis, intramuscular, multifocal
3. Trematodiasis, endomeningial, multifocal
4. Coelomitis, multifocal, mild to moderate with intralesional nematodes
5. Hepatitis, focally extensive, mild to moderate
6. Branchitis, multifocal, mild

FHM 30 –
Morphological diagnosis

1. Trematodiasis, endomeningial, multifocal
2. Myxosporidiosis, intramuscular, multifocal
3. Branchitis, multifocal, mild with intra-branchial trematode

FHM 31
Morphological diagnosis

1. Myxosporidiosis, intramuscular, multifocal
2. Trematodiasis, endomeningial, multifocal

FHM 32

Gastrointestinal tract – 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. Coelomitis, multifocal, mild with pancreatic degeneration
3. Trematodiasis, endomeningial, multifocal
4. Branchitis, regionally extensive, moderate with intralesional cellular degeneration and solitary intra-branchial trematode
5. Myositis, histiocytic and granulocytic, focal large
6. Myxosporidiosis, nephric, focal

FHM 33
Morphological diagnosis

1. Myxosporidiosis, intramuscular, multifocal
2. Branchitis, multifocal, mild

FHM 34
Morphological diagnosis

1. Myositis, histiocytic, multifocal
2. Dermatitis, histiocytic, mild
3. Branchitis, multifocal, mild to moderate with intralesional myxosporean

FHM 35 - NSF

FHM 36
Morphological diagnosis

1. Myxosporidiosis, intramuscular, multifocal
2. Coelomitis, multifocal, mild to moderate with intralesional nematodes

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
2. Myositis, histiocytic, focal, mild
3. Myxosporidiosis, intramuscular, multifocal
4. Branchitis, regionally extensive to diffuse, mild to moderate

FHM 38
Morphological diagnosis

1. Epicarditis, focal, mild

2. Myxosporidiosis, intramuscular, focal
3. Branchitis, multifocal, mild with intralesional myxosporean

FHM 39
Morphological diagnosis
1. Branchitis, regionally extensive, moderate
2. Trematodiasis, endomeningial, multifocal
3. Myositis, histiocytic, focal, moderate
4. Myxosporidiosis, intramuscular, multifocal
5. Granuloma, parasitic, focal, large

FHM 40
Morphological diagnosis
1. Myxosporidiosis, intramuscular, multifocal
2. Coelomitis, multifocal, mild to moderate with intralesional nematodes
3. Hepatitis, multifocal, mild
4. Branchitis, multifocal, mild with intralesional trematode

FHM 41
Morphological diagnosis
1. Myxosporidiosis, intramuscular, pericardial, intravascular, meningial, perineural, nephric, multifocal
2. Trematodiasis, endomeningial, multifocal

FHM 42
Morphological diagnosis
1. Myxosporidiosis, intramuscular, multifocal

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
2. Granuloma, parasitic, intramuscular, solitary.

FHM 47
Morphological diagnosis

1. Trematodiasis, endomeningial, multifocal

FHM 48
Morphological diagnosis

1. Myxosporidiosis, intramuscular, multifocal
2. Endomeningitis, parasitic, multifocal
3. Epitheliocystis, focal

FHM 49
Morphological diagnosis

1. Hepatitis, focal, mild to moderate with intralesional nematode
2. Trematodiasis, endomeningial, multifocal
3. Myxosporidiosis, intramuscular, multifocal
4. Nephritis, granulomatous, interstitial, focal

FHM 50
Morphological diagnosis

1. Myxosporidiosis, intramuscular, multifocal

FHM 51
Morphological diagnosis

1. Myxosporidiosis, intramuscular, multifocal

FHM 52
Morphological diagnosis

1. Enteritis, regionally extensive, moderate with enterocyte degeneration and necrosis
2. Branchitis, multifocal, mild to moderate
3. Myxosporidiosis, intramuscular, multifocal

FHM 53
Morphological diagnosis

1. Myxosporidiosis, intramuscular, multifocal
2. Granuloma, parasitic, intramuscular, multifocal
3. Branchitis, diffuse, mild

FHM 54
Morphological diagnosis

1. Hepatitis, multifocal, mild
2. Myxosporidiosis, intramuscular, multifocal
3. Trematodiasis, endomeningial, multifocal

FHM 55
Morphological diagnosis

1. Myxosporidiosis, intramuscular, multifocal
2. Trematodiasis, endomeningial, multifocal

FHM 56
Morphological diagnosis
1. Trematodiasis, endomeningial, intramuscular, retrobulbar, multifocal
2. Myxosporidiosis, thymic

FHM 57
Morphological diagnosis
1. Trematodiasis, endomeningial, multifocal
2. Branchitis, multifocal to regionally extensive, mild, with intralesional trichodinid protozoan
3. Myxosporidiosis, intramuscular, focal
4. Dermatitis, focal

FHM 58
Morphological diagnosis
1. Endomeningitis, parasitic, multifocal
2. Granuloma, parasitic, intramuscular, multifocal
3. Myxosporidiosis, intramuscular, focal

FHM 59 - NSF

FHM 60
Morphological diagnosis.
1. Trematodiasis, intramuscular, endomeningial, multifocal
2. Branchitis, multifocal, mild
3. Myositis, multifocal, mild

Spring

FHM 1 –
Morphological diagnosis
1. Trematodiasis, endomeningial, encysted, focal
2. Myositis, focal, with intralesional trematode

FHM 2 –
Morphological diagnosis
1. Coelomitis, granulomatous, multifocal, mild to moderate, with intralesional larval nematode

FHM 3 –
Morphological diagnosis
1. Myositis, multifocal, with intralesional trematodes

FHM 4 –

Morphological diagnosis
1. Trematodiasis, endomeningial, encysted, focal
2. Myositis, retro-bulbar, focal, with intralesional trematode

FHM 5 –
Morphological diagnosis
1. Myositis, retro-bulbar, focal, with intralesional trematode

FHM 6 –
Morphological diagnosis
1. Pancreatitis, granulomatous, multifocal, moderate, with intralesional larval nematode

FHM 7 – NAF

FHM 8 – NAF

FHM 9 -
Morphological diagnosis
1. Cestodes, intraluminal, intestinal
2. Trematodiasis, endomeningial, encysted, focal
3. Myositis, retro-bulbar, focal, with intralesional trematode

FHM 10 -
Morphological diagnosis
1. Cestodes, intraluminal, intestinal
2. Trematodiasis, choridal rete, encysted, focal
3. Myositis, multifocal, with intralesional trematode
4. Myxosporidiosis, *intramuscular*, multifocal

FHM 11 – NAF

FHM 12 –
Morphological diagnosis
1. Branchitis, multifocal, mild with intralesional *Trichodina*
2. Myositis, retro-bulbar, focal, with intralesional trematode
3. Myositis, multifocal, with intralesional trematode

FHM 13 –
Morphological diagnosis
1. Myositis, multifocal, with intralesional trematode

FHM 14 –
Morphological diagnosis
1. Myositis, multifocal, with intralesional trematode

FHM 15 – NAF

FHM 16 -
Morphological diagnosis
1. Myositis, multifocal, with intralesional trematode
2. Myositis, retro-bulbar, focal, with intralesional trematode
3. Pancreatitis, granulomatous, focal moderate, with intralesional larval nematode

FHM 17 –
Morphological diagnosis
1. Myositis, multifocal, with intralesional trematode
2. Myositis, retro-bulbar, focal, with intralesional trematode

FHM 18 –
Morphological diagnosis
1. Myositis, retro-bulbar, focal, with intralesional trematode

FHM 19 – NAF

FHM 20 –
Morphological diagnosis
1. Trematodiasis, endomeningial, encysted, focal

FHM 21–
Morphological diagnosis
1. Coelomitis, granulomatous, focal, mild to moderate, with intralesional larval nematode

FHM 22 – NAF

FHM 23 -
Morphological diagnosis
1. Cestodes, intraluminal, intestinal
2. Coelomitis, granulomatous, focal, mild to moderate, with intralesional trematode

FHM 24 -
Morphological diagnosis
1. Myositis, focal, with intralesional trematode

FHM 25 -
Morphological diagnosis
1. Cestode, intraluminal, intestinal
2. Trematodiasis, endomeningial, encysted, focal
3. Myositis, multifocal, with intralesional trematode

FHM 26 –
Morphological diagnosis
1. Myositis, multifocal, with intralesional trematode

2. Myxosporidiosis**,** focal, low to moderate numbers

FHM 27 -
Morphological diagnosis
1. Cestodes, intraluminal, intestinal
2. Trematodiasis, endomeningial, encysted, multifocal
3. Myositis, retro-bulbar, focal, with intralesional trematode

FHM 28 -
Morphological diagnosis
1. Cestode, intraluminal, intestinal
2. Myositis, multifocal, with intralesional trematode

FHM 29 – NAF

FHM 30 –
Morphological diagnosis
1. Myositis, multifocal, with intralesional trematode

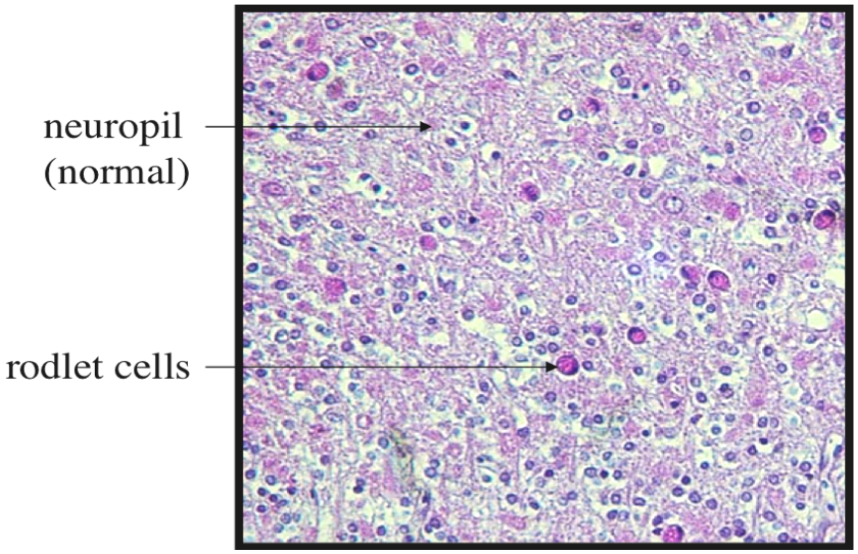
Table 5. Summary of notable lesions and organisms in the goldeye (*Hiodon allosoides* – G) collected during the summer of 2006 (n=60) and the spring of 2007 (n=30) from Lake Winnipeg and surrounding waters.

Goldeye Tissue/agent	Summer 2006 – Sixty fish	Spring 2007 – Thirty fish
Gills	1. *Trichodina* sp. (2/60)	
Stomach/ Intestine/ peritoneum	1. Intestinal cestode (26/60) 2. Gastric nematodes (3/60) 3. Capsular hepatitis/splenitis (21/60) 4. Intestinal trematode (5/60)	1. Intestinal cestode (13/30) 2. Gastric nematodes (8/30)
Liver and bile ducts		1. Trematode (1/60)
Heart	1. Myo/epicarditis (7/60)	1. Epicardial trematode (1/30)
Other	1. Branchitis	1. Branchitis 2. Encephalitis

Tissue sections were predominantly oriented as transverse sections through the musculature and body cavity, with longitudinal sections through the head and as individual tissues for coelomic 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.

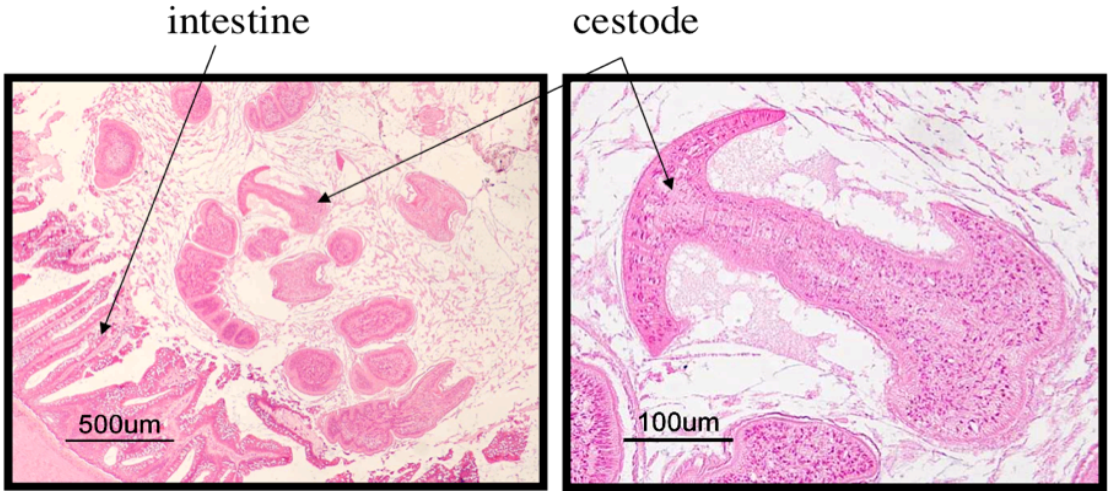
Relative to many of the other species examined in this survey goldeye has relatively few notable lesions. A common lesion that was not systematically enumerated was a mild to moderate lymphohistiocytic cholangiohepatitis/pancreatitis (inflammatory cells primarily oriented around either bile ducts or 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)**.**

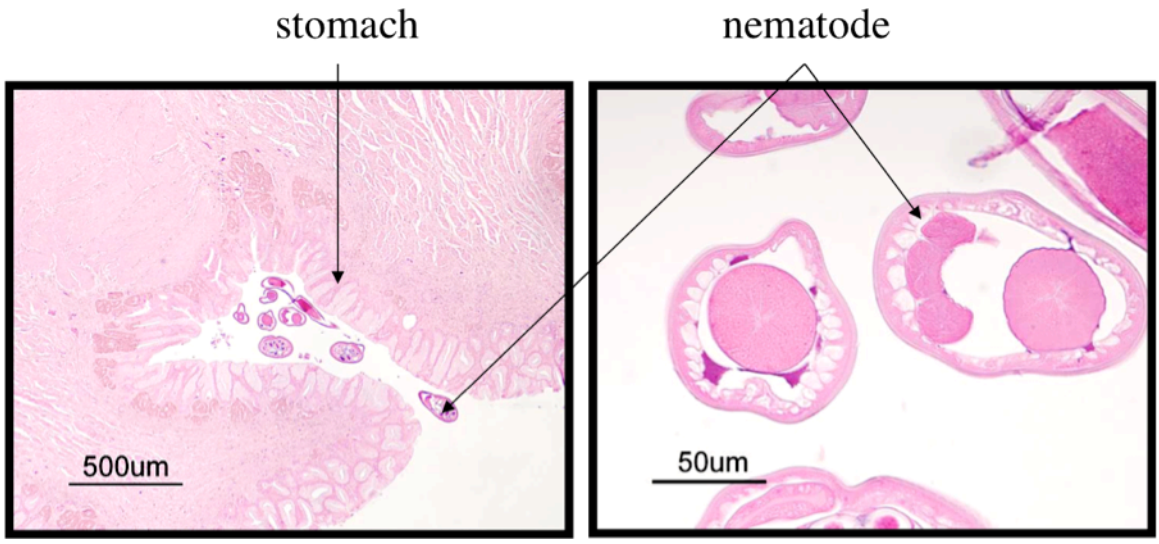


Lesions or organisms noted in both summer and spring.

Intestinal cestodes – Cestode, luminal intestinal. These have calcareous corpuscles, are ~150 to 200 μm 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). Summer ---- 26 of 60 affected. Spring ---- 13 of 30 affected.



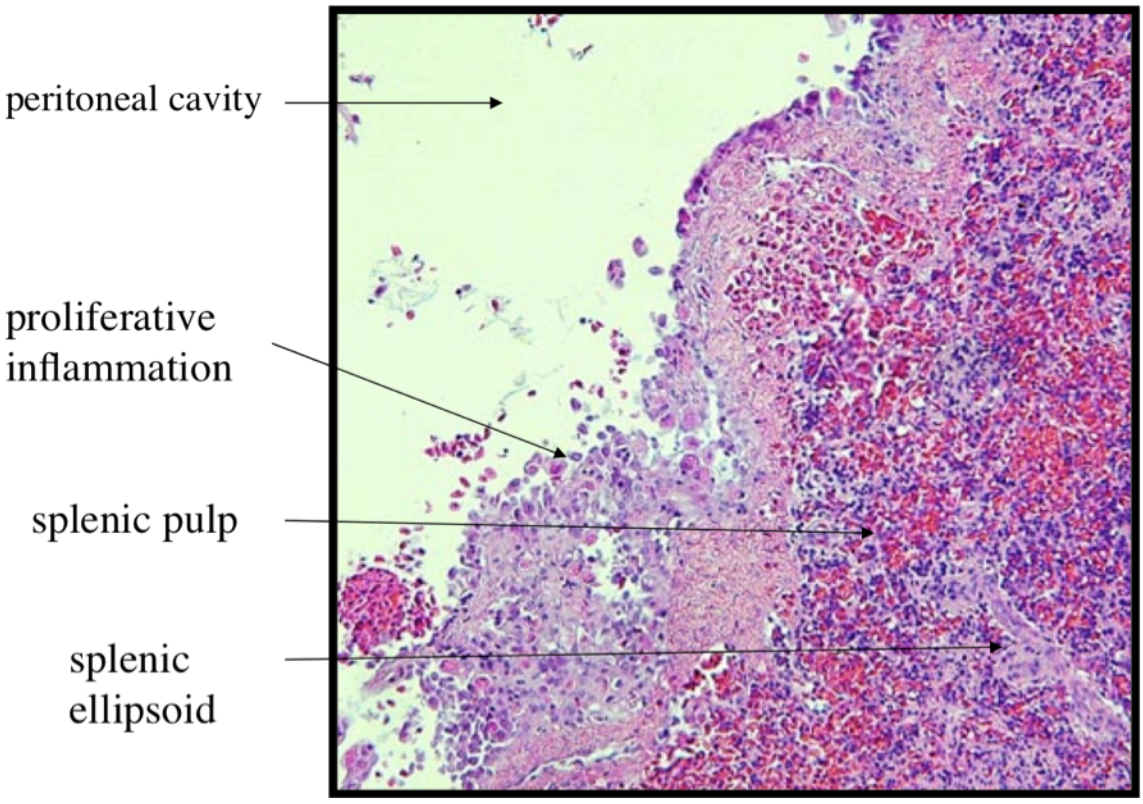
Gastric nematodes – Nematodes, few to moderate, gastric, luminal.
There were few to moderate numbers of thin ~70 to 90 µm nematodes between folds and are characterized by an eosinophilic cuticle, thick muscular esophagus/intestine and reproductive tissue. No host reaction was associated with the presence of these nematodes. (Phylum Nemathelminthes, Class Nematoda). Summer ---- 3of 60 affected. Spring ---- 8 of 30 affected.



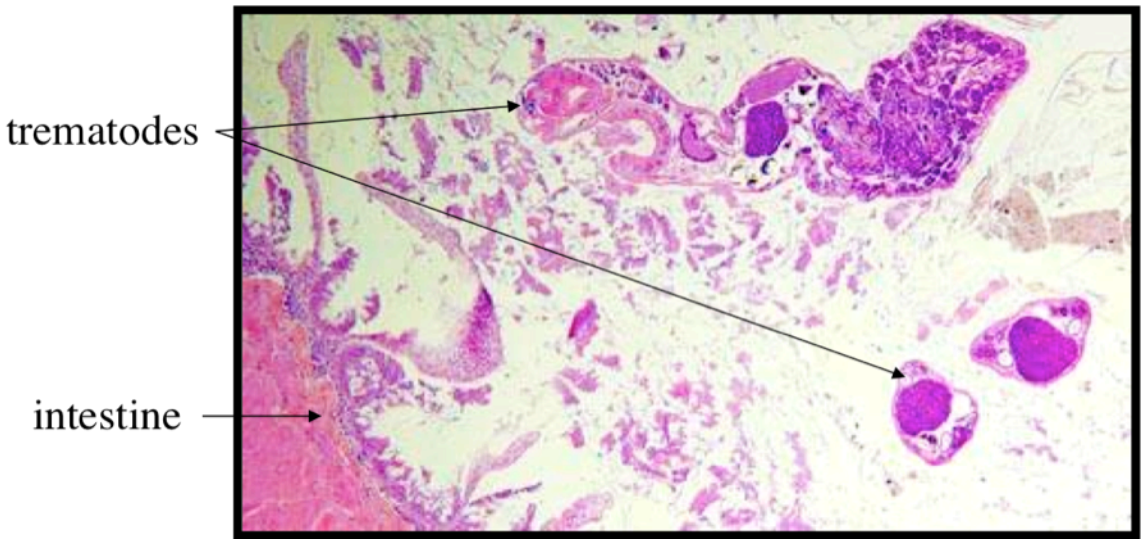
Branchitis – This is common to majority of fish. The interlamellar and to a lesser extent, lamellar epithelium has varied but often moderate numbers of eosinophilic granular cells present. Mild multifocal epithelial hyperplasia may also be present. In the vast majority of cases there are no organisms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

Lesions or organisms only noted in summer.

Capsulitis/coelomitis, 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 coelomic organs and in coelomic 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 coelomic metazoans or other agent. ---- 21 of 60 affected.



Intestinal trematodes – Trematode, luminal intestinal. There are typically few visible in any one section. They are ~150 to 250 μm 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.



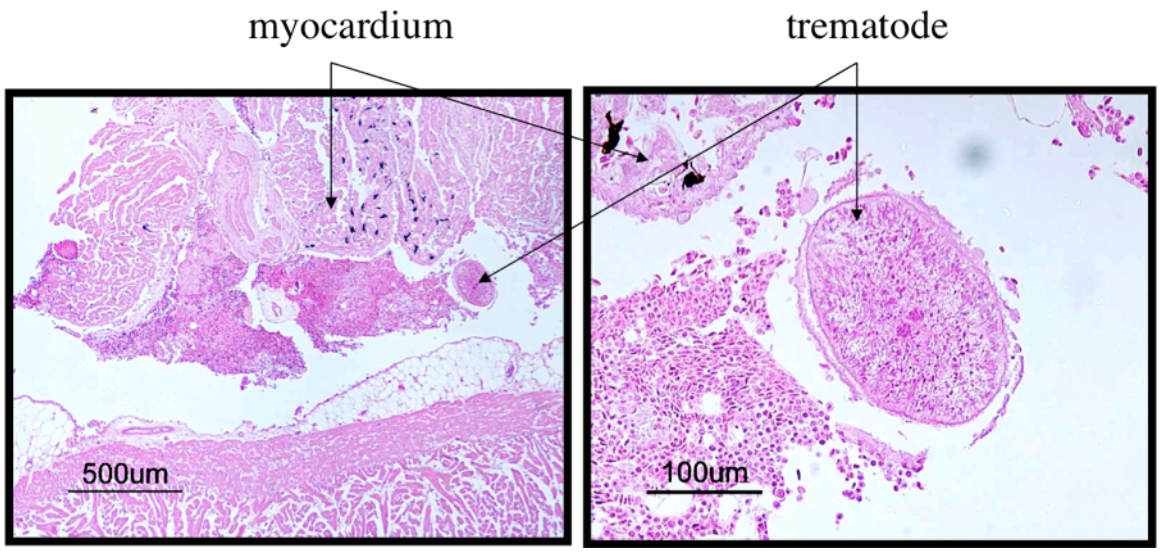
***Trichodina* sp**. few, lamellar.
There were very small numbers of ~20 μm protozoans that were dorsoventrally flattened, had cilia and a denticular ring. The tissue reaction (branchitis described above) noted for these fish

did not seem to be associated with the presence of these organisms. (Phylum Ciliophora, Class Litostomatea, Family Trichodina) (no photo) ---- 2 of 60 affected. (no photo)

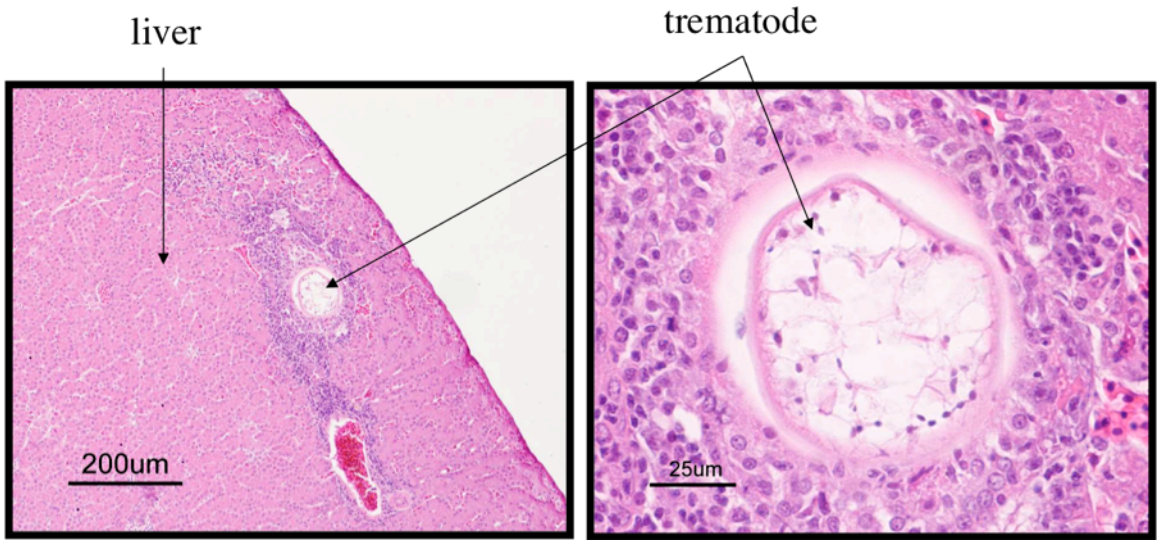
Myo/epicarditis, mild, focal, lymphohistiocytic.
Few fish had focal accumulations of small to moderate numbers of lymphocytes with fewer macrophages, either within the myocardium (less common) or within and expanding the epicardium. No organisms were associated with these lesions. Small, histiocyte-rich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

Lesions or organisms only noted in spring.

Cardiac notch (epicardial) trematode – Epicarditis, regionally extensive, moderate with intralesional trematode.
Within sections of a single fish, there are moderate numbers of plump amphiphilic macrophages and lesser numbers of rodlet cells expanding sections of the epicardium between the atrial myocardium and the bulbus arteriosus. Within this foci of inflammatory cells there is a single cross section of a metazoan with a tegument and parenchymatous matrix but no calcareous corpuscles or intestine or cuticle (trematode, presumptive) (Phylum Platyhelmintheses, Class Trematoda). ---- 1 of 30 affected.



Hepatic trematodes - Trematodiasis, hepatic, focal.
There is a single parasitic granuloma embedded within the parenchyma. Within this granuloma, there is a single cross-section of a trematode parasite characterized by a thin eosinophilic tegument, lack of body cavity and a parenchymatous body matrix. (Phylum Platyhelmintheses, Class Trematoda). ---- 1 of 30 affected.



Encephalitis - focal, mild to moderate.
Within sections of the neuropil, there are multifocal accumulations of moderate numbers of glial cells surrounding vascular capillaries and viable neurons. ---- 2 of 30 affected. (no photo)

Summaries of lesions or organisms noted in individual fish.

Summer

G 1-
Morphological diagnosis
1. Cestode, luminal intestinal, few
2. Trematode, luminal intestinal, few

G 2-
Morphological diagnosis
1. Cestode, luminal intestinal, few

G 3-
Morphological diagnosis
1. Cestode, luminal intestinal, numerous

G 4 – NAF

G 5-
There is a small focus of macrophages within coelomic connective tissue. There are no organisms present.
Morphological diagnosis
1. *Trichodina* sp., few, lamellar
2. Cestode, luminal intestinal, few
3. Coelomitis, focal, histiocytic

G 6 – 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

G 8 – NAF

G 9 -
Morphological diagnosis
1. Cestode, luminal intestinal, numerous
2. Capsulitis/coelomitis, 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
2. Capsulitis/coelomitis, splenic/hepatic, mild to moderate, proliferative
3. Glomeruli, diffuse, basement membrane thickening

G 14 -
Morphological diagnosis
1. Trematode, luminal intestinal, few
2. Capsulitis/coelomitis, splenic/hepatic, mild to moderate, proliferative

G 15 – NAF

G 16 -
Morphological diagnosis
1. Capsulitis/coelomitis, splenic/hepatic, mild to moderate, proliferative

G 17 -
Morphological diagnosis
1. Nematodes, few to moderate, gastric, luminal

G 18 -

Liver – There is a single phenotypically distinct ~150 μm, basophilic focus.
Morphological diagnosis
1. Capsulitis/coelomitis, 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
2. Myo/epicarditis, mild, focal, lymphohistiocytic

G 21 -
Morphological diagnosis
1. Cestode, luminal intestinal, numerous

G 22 -
Morphological diagnosis
1. Cestode, luminal intestinal, few

G 23 -
Morphological diagnosis
1. Capsulitis/coelomitis, splenic/hepatic, mild, proliferative

G 24 -
Morphological diagnosis
1. Capsulitis/coelomitis, splenic/hepatic, mild, proliferative

G 25 -
Morphological diagnosis
1. Capsulitis/coelomitis, splenic/hepatic, mild, proliferative
2. Cestode, luminal intestinal, few

G 26 – NAF

G 27 -
Morphological diagnosis
1. Trematode, luminal intestinal, moderate

G 28 – NAF

G 29 -
Morphological diagnosis
1. Cestode, luminal intestinal, few

G 30 -
Morphological diagnosis
1. Cestode, luminal intestinal, few
2. Capsulitis/coelomitis, splenic/hepatic, moderate, proliferative

G 31-
Morphological diagnosis
1. Myo/epicarditis, mild, focal, lymphohistiocytic

G 32 -
Morphological diagnosis
1. Cestode, luminal intestinal, numerous

G 33 -
Morphological diagnosis
1. Trematode, luminal intestinal, moderate
2. Capsulitis/coelomitis, splenic/hepatic, moderate, proliferative

G 34 -
Morphological diagnosis
1. Capsulitis/coelomitis, splenic/hepatic, mild, proliferative
2. Cestode, luminal intestinal, few to moderate

G 35 -
Morphological diagnosis
1. Capsulitis/coelomitis, splenic/hepatic, mild, proliferative
2. Myo/epicarditis, mild, focal, lymphohistiocytic

G 36 -
Liver – There is a single phenotypically distinct ~150 μm, roughly round, eosinophilic focus (liver on G37 – mislabeled).
Morphological diagnosis
1. Cestode, luminal intestinal, few
2. Capsulitis/coelomitis, 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
2. Capsulitis/coelomitis, splenic/hepatic, mild, proliferative

G 40 -
Morphological diagnosis
1. Cestode, luminal intestinal, few
2. Capsulitis/coelomitis, splenic/hepatic, mild, proliferative

G 41 -
Morphological diagnosis
1. Capsulitis/coelomitis, splenic/hepatic, moderate, proliferative
2. Cestode, luminal intestinal, moderate

G 42 -
Morphological diagnosis
1. *Trichodina* sp., few, lamellar
2. Cestode, luminal intestinal, moderate

G 43 -
Morphological diagnosis
1. Capsulitis/coelomitis, 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

G 45 -
Morphological diagnosis
1. Capsulitis/coelomitis, splenic/hepatic, moderate, proliferative

G 46 -
Morphological diagnosis
1. Cestode, luminal intestinal, numerous

G 47 -
Morphological diagnosis
1. Cestode, luminal intestinal, few to moderate

G 48 -
Morphological diagnosis
1. Trematode, luminal intestinal, few

G 49 -
Morphological diagnosis
1. Capsulitis/coelomitis, splenic/hepatic, mild, proliferative

G 50 – NAF

G 51 – NAF

G 52 -
Morphological diagnosis
1. Capsulitis/coelomitis, splenic/hepatic, mild, proliferative

G 53 -
Morphological diagnosis
1. Capsulitis/coelomitis, splenic/hepatic, mild, proliferative
2. Nematodes, few to moderate, gastric, luminal

G 54 -
Morphological diagnosis
1. Cestode, luminal intestinal, numerous
2. Nematodes, few to moderate, gastric, luminal

G 55 -
Morphological diagnosis
1. Cestode, luminal intestinal, few

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/coelomitis, splenic/hepatic, moderate to severe, proliferative
3. Cestode, luminal intestinal, few to moderate

G 59 -
Morphological diagnosis
1. Cestode, luminal intestinal, moderate

G 60 -
Morphological diagnosis
1. Myo/epicarditis, mild, focal, lymphohistiocytic

Spring

G 1- NAF

G 2 - NAF

G 3-
Morphological diagnosis
1. Branchitis, multifocal, mild
2. Hepatitis, focal with intralesional encysted trematode
3. Cestode, luminal intestinal, focal

G 4 –
Morphological diagnosis
1. Granuloma, epicardial, focal, small

G 5- NAF

G 6 – NAF
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate

G 7- NAF

G 8 –
Morphological diagnosis
1. Cestode, luminal intestinal, numerous
2. Nematode, luminal gastric, moderate numbers

G 9 -
Morphological diagnosis
1. Cestode, luminal intestinal, numerous

G 10 –
Morphological diagnosis
1. Nematode, luminal gastric, moderate numbers
2. Encephalitis, multifocal, mild to moderate

G 11 –
Morphological diagnosis
1. Cestode, luminal intestinal, numerous

G 12 – NAF

G 13 -
Morphological diagnosis
1. Nematode, luminal gastric, moderate numbers

G 14 -

Morphological diagnosis
1. Nematode, luminal gastric, moderate numbers

G 15 –
Morphological diagnosis
1. Nematode, luminal gastric, moderate numbers

G 16 -
Morphological diagnosis
1. Encephalitis, focal, mild

G 17 -
Morphological diagnosis
1. Cestode, luminal intestinal, numerous

G 18 -
Morphological diagnosis
1. Cestode, luminal intestinal, numerous

G 19 -
Morphological diagnosis
1. Nematode, luminal gastric, moderate numbers

G 20 -
Morphological diagnosis
1. Cestode, luminal intestinal, numerous

G 21 -
Morphological diagnosis
1. Cestode, luminal intestinal, numerous

G 22 -
Morphological diagnosis
1. Cestode, luminal intestinal, few

G 23 -
Morphological diagnosis
1. Cestode, luminal intestinal, numerous

G 24 -
Morphological diagnosis
1. Cestode, luminal intestinal, numerous

G 25 -
Morphological diagnosis
1. Cestode, luminal intestinal, numerous

2. Epicarditis, regionally extensive, moderate with intralesional trematode

G 26 –
Morphological diagnosis
1. Nematode, luminal gastric, moderate numbers

G 27 -
Morphological diagnosis
1. Cestode, luminal intestinal, numerous
2. Hepatitis, focal, mild

G 28 –
Morphological diagnosis
1. Nematode, luminal gastric, moderate numbers

G 29 -
Morphological diagnosis
1. Cestode, luminal intestinal, numerous
2. Epicarditis, focal, mild

G 30 - NAF

Table 6. Summary of notable lesions and organisms in the Northern pike (*Esox lucius*) collected during the summer of 2006 (n=60) and the spring of 2007 (n=30) from Lake Winnipeg and surrounding waters.

Northern pike (*Esox lucius*) Tissue/agent	Summer 2006 – Sixty fish	Spring 2007 – Thirty fish
Gills	1. Monogenetic trematode (7/60) 2. Protozoa (6/60)	
Stomach/ Intestine/ peritoneum	1. Intestinal cestodes (19/60) 2. Gastrointestinal nematodes (3/60)	1. Intestinal cestodes (6/30) 2. Steatitis (1/30)
Skeletal muscle and skin		1. Dermatitis/ lymphocytic (1/30) – lymphoma?
Brain and meninges		1. Meningeal trematode (1/30)
Liver and bile ducts	1. Cholangiohepatitis	
Kidney	1. Tubular myxosporean (10/60) 2. Interstitial myxosporean (7/60)	1. Nephritis. Lymphocytic (1/30)
Other	1. Stomach, necrotizing wound	1. Retrobulbar trematode (1/30)

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.

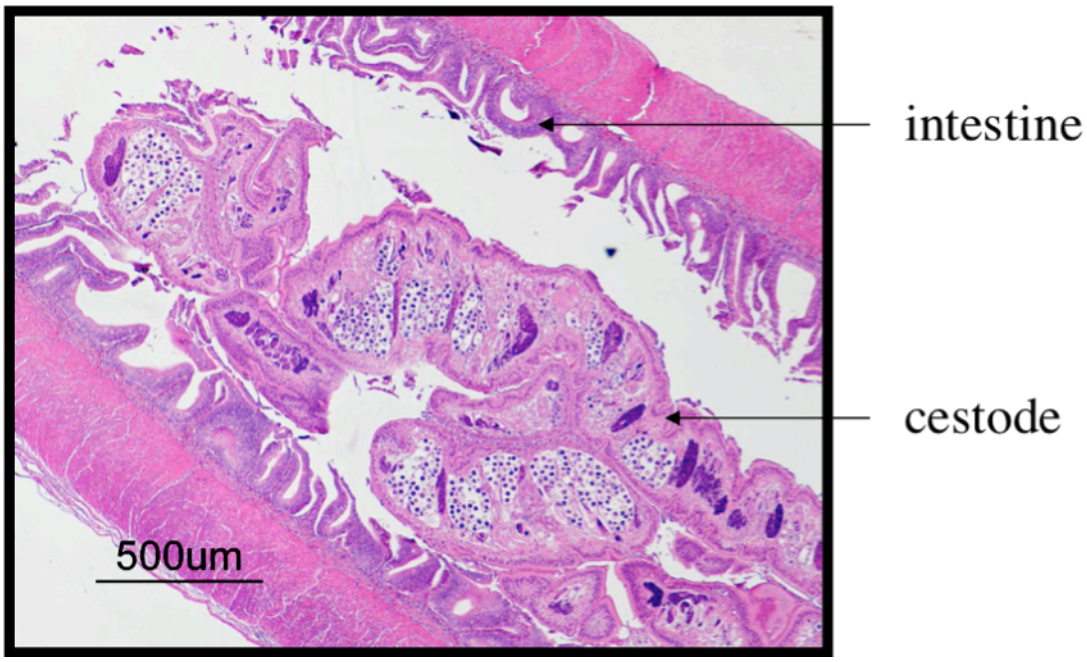
Occasionally these fish have heart lesions ranging from focal splitting with outpouching of the compact layer (resembling an aneurysm), to equivocal to mild multifocal myocardial hypereosinophilia often with pyknotic myo- and endocardial nuclei (mild myocardial degeneration/necrosis), or focal areas 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 characterized by 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.

The most notable feature of the comparison between sampling times is that apart from the intestinal cestodes, there are no lesions noted apart from the above mentioned, that were present both times.

Lesions or organisms noted in both summer and spring.

Intestinal cestodes – Cestodes, intraluminal, 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. Summer ---- 19 of 60 affected. Spring ---- 6 of 30 affected.



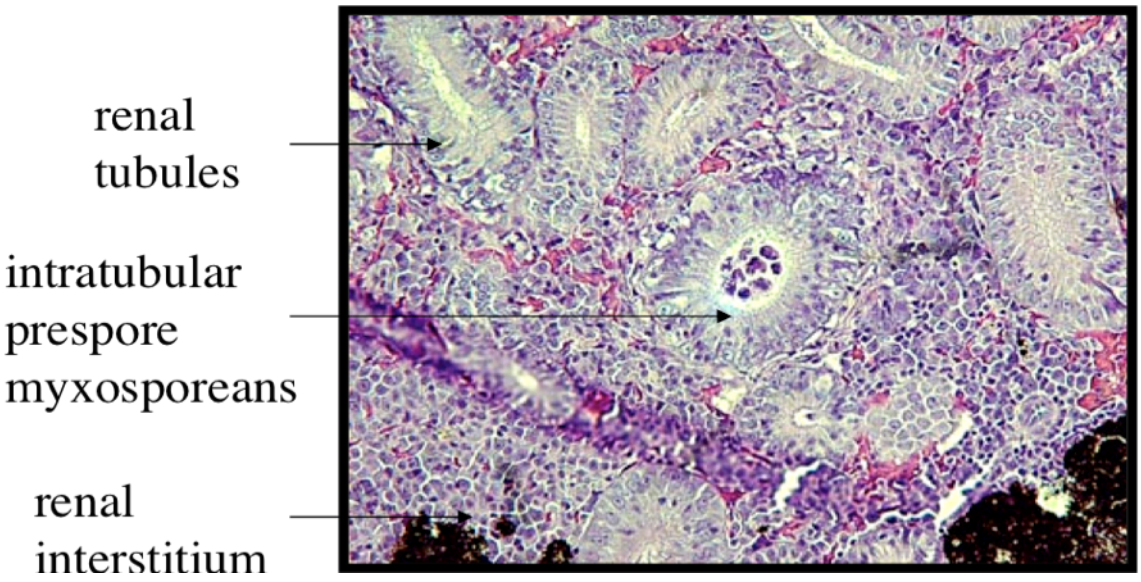
Lesions or organisms only noted in summer.

Branchial trematodes - Branchitis, multifocal, mild to moderate with intralesional 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 (haptor) and a loose reticular parenchyma and thin tegument (Phylum Platyhelminthes, Class Trematoda). ---- 7 of 60 affected. (no photo)

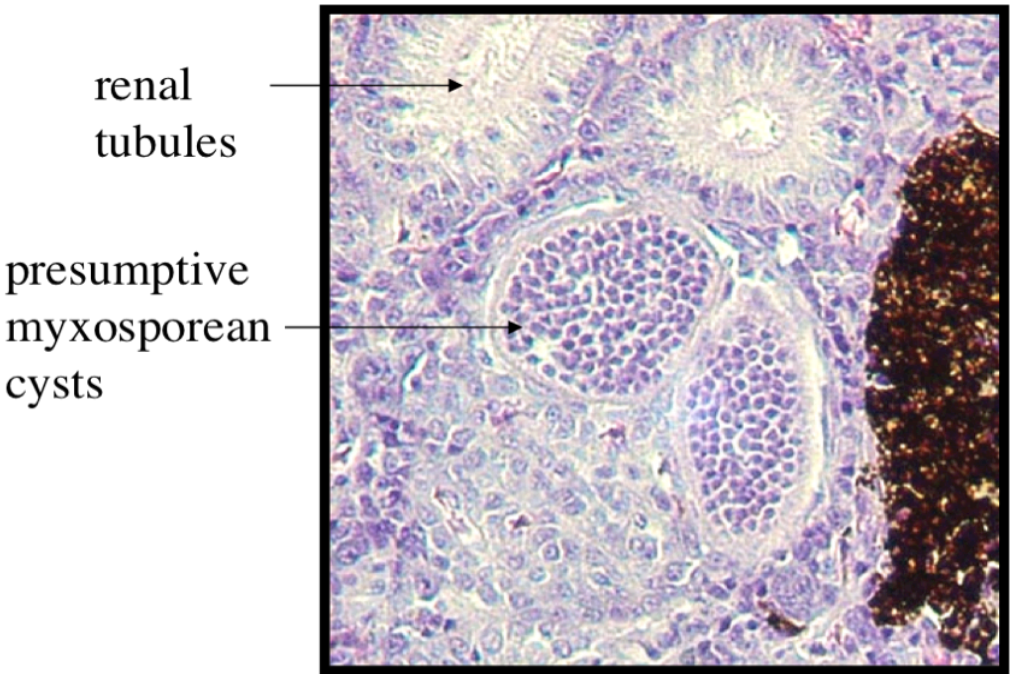
Branchial protozoan – Protozoa (*Trichodina* sp.), few, multifocal.
There were very small numbers of ~20 µm protozoans that were dorsoventrally flattened, had cilia and a denticular ring. (Phylum Ciliophora, Class Litostomatea, Family Trichodina). ---- 2 of 60 affected. (no photo)

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 to 90 µm) or sac-like 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. (no photo)

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 (~15 µm in diameter) organisms. (Phylum Myxozoa, Class Myxosporea) ---- 10 of 60 affected.



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



Intestinal/gastric nematodes – Nematodes, intraluminal, intestinal
There are a few cross sections of a metazoan organism that is ~200 µm in diameter, with a thick eosinophilic cuticle, lateral alae and chords, an intestine and reproductive organs. In one fish (NP

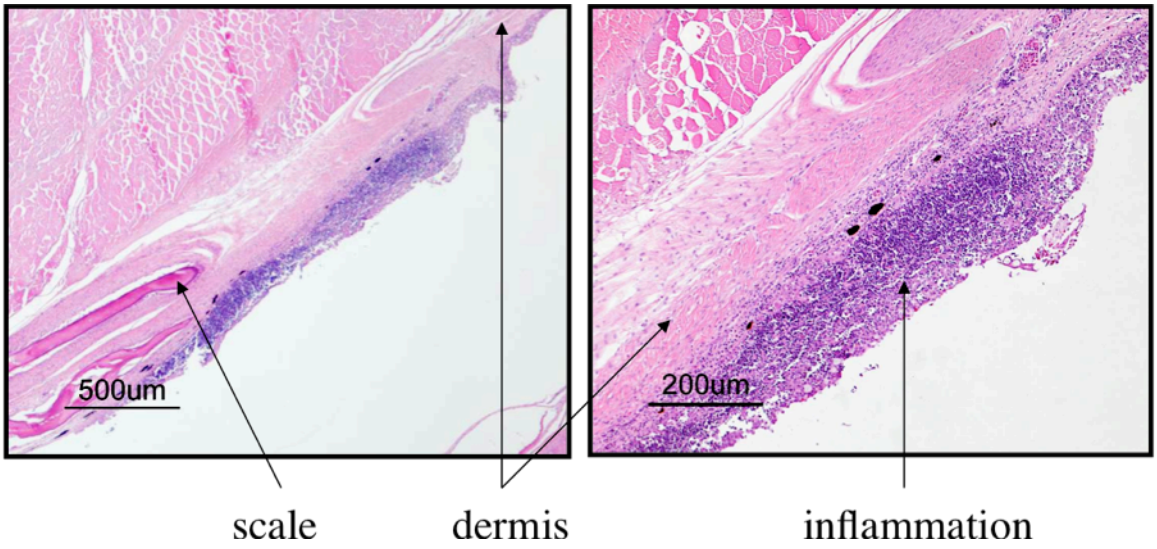
30), the nematodes contain numerous ~25 x 35 μm eggs. (Phylum Nemathelminthes, Class Nematoda) ---- 3 of 60 affected. (no photo)

Lesions or organisms only noted in spring.

Coelomic fat inflammation – Steatitis, histiocytic and granulocytic, focally extensive, mild to moderate.
Within a section of coelomic fat attached to the serosal surface of intestine, there are moderate numbers of plump amphophilc macrophages and lesser numbers of eosinophilic granular cells filling spaces between adipocytes and surrounding variably sized blood vessels. In some sections the macrophages are arranged in loosely packed concentric circles surrounding viable and degenerate macrophages and cellular debris. ---- 1 of 30 affected.

Retrobulbar encysted trematode – Trematodiasis, intramuscular, retrobulbar, encysted.
Encysted by few to several layers of thin attenuated macrophages, there are multiple cross sections of a trematode parasite embedded within skeletal muscle surrounding the cartilage of the eye. The trematodes are characterized by a thin tegument surrounding a layer of sub-tegumental cells and loose paranchymatous matrix. ---- 1 of 30 affected. (no photo)

Dermatitis, lymphocytic, focally extensive, moderate to marked.
Within one section of skin, there are large numbers of lymphocytes and lesser numbers of macrophages replacing and expanding spaces between epithelial cells. In some areas there is almost complete replacement of the epithelial cells by trafficking lymphocytes. ---- 1 of 30 affected.



Interstitial nephritis – Nephritis, interstitial, histiocytic and lymphocytic, multifocal, moderate. Within the interstitium of the caudal kidney, there are multifocal accumulations of moderate numbers of macrophages and lymphocytes expanding the space between viable and degenerate tubules. In some sections, similar populations of cells surround multiple glomeruli. ---- 1 of 30 affected. (no photo)

Branchitis – Branchitis, filamental, focally extensive, mild to moderate.
On a single filament, there is a single focally extensive accumulation of plump, amphophilic macrophages expanding the space between the connective tissue and surrounding and filling vasculature sinuses. No organism (s) are identified in sections examined. ---- 1 of 30 affected. (no photo)

Endomeningial trematodiasis: Trematodiasis, endomeningial, encysted, focal.
A single cross section of trematode organism is expanding the endomeningial space surrounding a single section 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 30 affected. (no photo)

Summaries of lesions or organisms noted in individual fish.

Summer

NP 1 - NAF

NP2 –
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, few

NP 3 – NAF

NP4 -
Morphological diagnosis
1. Myxosporidiosis, renal intratubular

NP 5 –
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, few

NP 6 – NAF

NP 7 –
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, moderate

NP 8 -
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, moderate

NP 9 -
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, moderate

NP 10- NAF

NP 11- NAF

NP 12 – NAF

NP 13 –
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, few
2. Myxosporidiosis, renal intratubular

NP 14 –
Stomach – There is a focal, transmural necrotizing lesion, approximately 200 μm 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 hypereosinophilic 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

NP 15 – NAF

NP 16 – NAF

NP 17 – NAF

NP 18 –
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, few

NP 19 –
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, few
2. Nematodes, intraluminal, intestinal, few

NP 20 –
Morphological diagnosis
1. Branchial *Trichodina* sp., few, lamellar

NP 21 -
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, few

NP 22 – NAF

NP 23 - NAF

NP 24 –
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, few

NP 25 –
Morphological diagnosis
1. Branchitis, multifocal, mild, with intralesional monogenetic trematodes

NP 26 – NAF

NP 27 – NAF

NP 28 –
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, few
2. Myxosporidiosis, renal intratubular

NP 29 -
No discernible anatomic location – There is a large ~300 x 500 μm 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

NP 30 –
Morphological diagnosis
1. Nematodes, intraluminal, intestinal

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
2. Myxosporidiosis**,** multifocal, few, renal interstitial

NP 33 – NAF

NP 34 –
Morphological diagnosis
1. Protozoa (*Caprinia* sp.), branchial, mild
2. Myxosporidiosis**,** multifocal, few, renal interstitial

NP 35 - NAF

NP 36 – NAF

NP 37 – NAF

NP 38 –
Morphological diagnosis
1. Protozoa (*Caprinia* sp.), branchial, mild

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, intraluminal, intestinal, few

NP 45 - NAF

NP 46 –
Morphological diagnosis
1. Myxosporidiosis, renal intratubular

NP 47 –
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, few
2. Myxosporidiosis, renal intratubular

NP 48 –
Morphological diagnosis
1. Myxosporidiosis**,** multifocal, moderate, renal interstitial

NP 49 –NAF

NP 50 –
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, few
2. Branchitis, multifocal, mild to moderate with intralesional monogenetic trematodes

NP 51 –
Morphological diagnosis
1. Branchitis, multifocal, mild, with intralesional monogenetic trematodes
2. Myxosporidiosis, renal intratubular

NP 52 –
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, few
2. Branchitis, multifocal, mild to moderate with intralesional monogenetic trematodes

NP 53 –
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate with intralesional monogenetic trematodes
2. Myxosporidiosis**,** multifocal, moderate, renal interstitial

NP 54 –
Morphological diagnosis

1. Cestodes, intraluminal, intestinal, few

NP 55 –
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate with intralesional monogenetic trematodes

NP 56a –
Morphological diagnosis
1. Nematode, intraluminal, gastric, single. This fish also had a mild focal histiocytic-rich gastritis.

NP 56b – NAF

NP 57 –
Morphological diagnosis
1. Myxosporidiosis, renal intratubular
2. Myxosporidiosis**,** multifocal, few, renal interstitial

NP 58 –
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, few

NP 59 –
Morphological diagnosis
1. Branchitis, multifocal, mild, with intralesional monogenetic trematodes
2. Myxosporidiosis, renal intratubular
3. Myxosporidiosis**,** multifocal, moderate, renal interstitial

NP 60 –
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, few
2. Myxosporidiosis, renal intratubular

Spring

NP 1 – NAF

NP2 –
Morphological diagnosis
1. Branchitis, multifocal, mild

NP 3 – NAF

NP4 -
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, few

2. Hepatitis, focal, mild

NP 5 –
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, few
2. Dermatitis, lymphocytic, focally extensive, moderate

NP 6 –
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, few

NP 7 –NAF

NP 8 -
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, moderate

NP 9- NAF

NP 10- NAF

NP 11- NAF

NP 12 – No fish number 12

NP 13 –
Morphological diagnosis
1. Trematodiasis, intramuscular, retrobulbar, encysted
2. Trematodiasis, endomeningial, encysted, focal

NP 14 – NAF

NP 15 – NAF

NP 16 – NAF

NP 17 – NAF

NP 18 – NAF

NP 19 – NAF

NP 20 – NAF

NP 21 - NAF

NP 22 – NAF

NP 23 -
Morphological diagnosis
1. Branchitis, lamellar, multifocal, mild
2. Branchitis, filamental, focally extensive, mild to moderate
3. Nephritis, interstitial, histiocytic and lymphocytic, multifocal, moderate

NP 24 –
Morphological diagnosis
1. Steatitis, histiocytic and granulocytic, focally extensive, moderate

NP 25 – NAF

NP 26 – NAF

NP 27 –
Morphological diagnosis
1. Branchitis, lamellar, multifocal, mild

NP 28 –NAF

NP 29-
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, moderate

NP 30 –
Morphological diagnosis
1. Cestodes, intraluminal, intestinal, moderate

Table 7. Summary of notable lesions and organisms in the sauger (*Sander canadensis* – S) collected during the summer of 2006 (n=60) and the spring of 2007 (n=30) from Lake Winnipeg and surrounding waters.

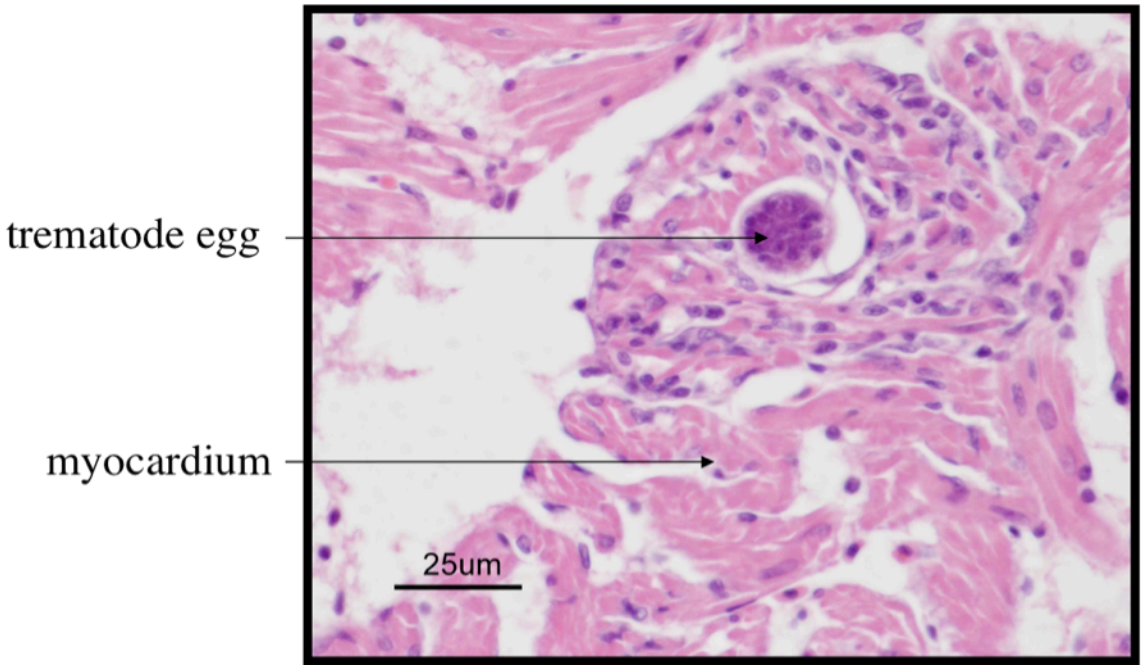
Sauger (*Sander canadensis*) Tissue/agent	Summer 2006 – Sixty fish	Spring 2007 – Thirty fish
Gills	1. Trematode egg (11/60) 2. *Trichodina* sp. (1/60) 3. Crustacean (25/60)	1. Trematode egg (2/30) 2. Monogentic trematode (1/30)
Stomach/ Intestine/ peritoneum	1. Intestinal cestode (25/60) 2. Mural intestinal trematode (11/60) 3. Intestinal nematode (1/60)	1. Intestinal cestode (8/30) 2. Mural intestinal trematode (10/30) 3. Apicomplexan (2/30)
Brain and meninges	1. Meningeal trematode (1/60)	
Kidney	1. Tubular protozoan (1/60)	1. Myxosporean (2/30)
Heart	1. Myocarditis (25/60) 2. Proliferative endocarditis (6/60) with suspect *Sanguinicola* sp. (2/60)	1. Myocarditis (3/30) 2. Proliferative endocarditis (6/30) with suspect *Sanguinicola* sp. (1/60)
Other	1. Granulomas, numerous tissues	

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.

Lesions or organisms noted in both summer and spring.

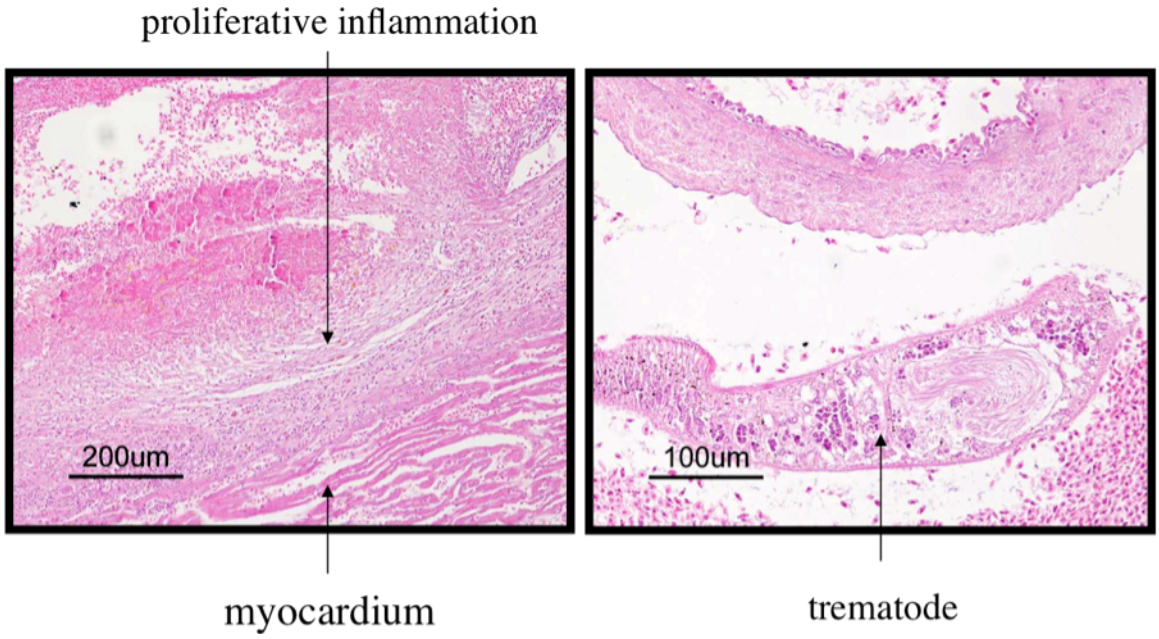
Intracardial trematode 'complex'
Intravascular metazoan 'eggs' – Gill, intravascular foreign body. Trematode egg, presumptive. There are few to numerous intravascular multicellular bodies that expand the lamellar pillar cell channel. The highly basophilic cells form a round ~10-15 μm 'egg' packet that appears to be without a case. These same bodies are rarely (once S54) seen intimately associated with the multifocal myocardial lesions described below. Summer ----11 of 60 affected. Spring---- 2 of 30 affected. For a photo see the walleye section.

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. Summer ----- 25 of 60 affected. Spring ----- 3 of 30 affected.

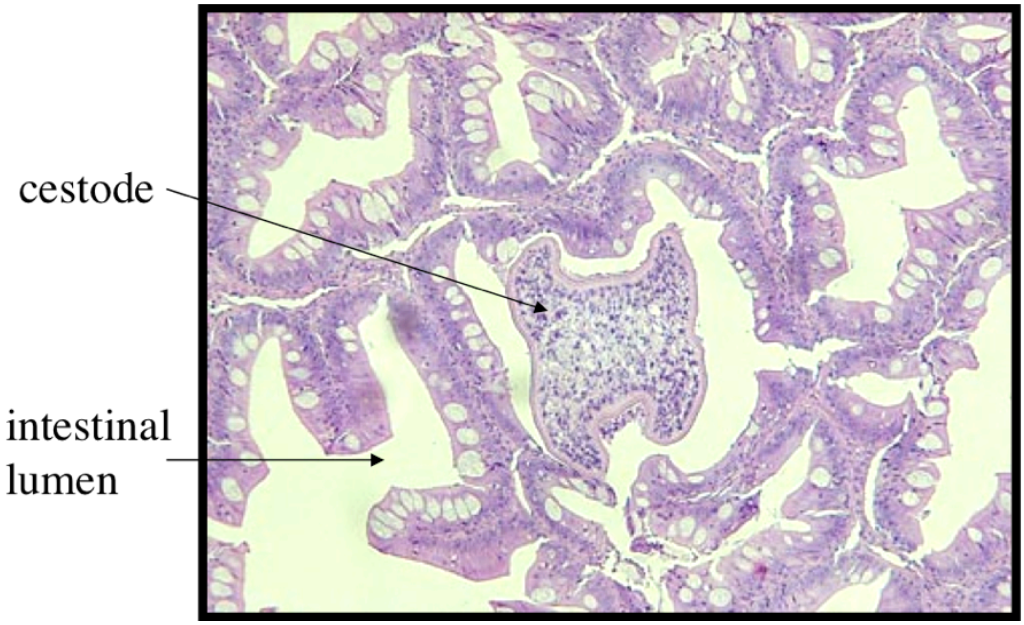


Proliferative endocarditis - Endocarditis, proliferative, focally extensive
There is a focally extensive hyperplastic plaque of hypertrophied endocardial cells subtended by fibrous hyperplasia. This is occasionally up to 50-100 μm thick and is typically found in the intraventricular lumen near the atrioventricular valves. The hyperplasia occasionally affects the ventricular side of the valves. There is cellular debris, fibrin and cellular thrombi, and rarely portions of a ~100 x 1200 μm metazoan with a tegument and parenchymatous matrix but no

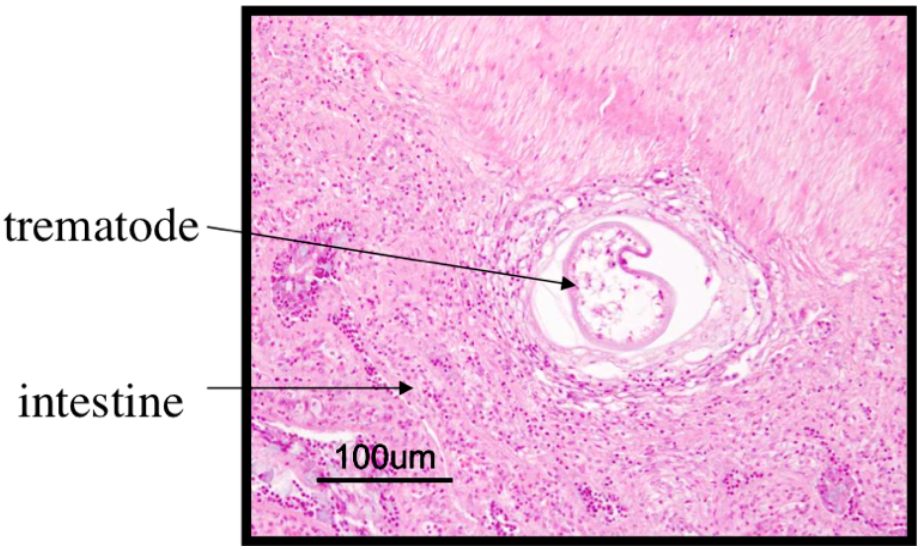
calcareous corpuscles or intestine (trematode, presumptive). Summer ---- 6 of 60 affected, with a single intralesional trematode ---- 2 of 60 affected. Spring ---- 6 of 30 with proliferative endocarditis but no trematodes seen.



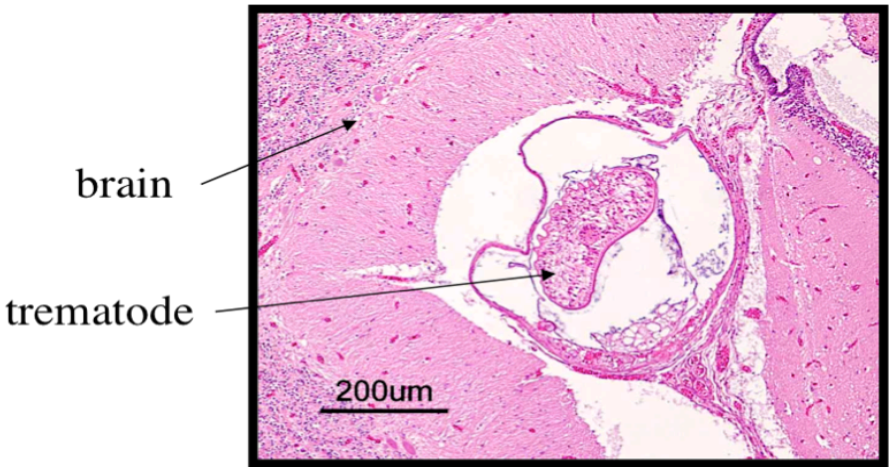
Intestinal cestodes – Cestode, luminal intestinal.
Within the lumen of the intestine there are multiple cross-sections of a cestode parasite. The cestodes are ~150 to 200 μm wide and have a parenchymatous matrix and tegument and occasionally have calcareous corpuscles (Phylum Platyhelminthes, Class Cestoidea). Summer ---- 25 of 60 affected. Spring ---- 8 of 30 affected.



Mural intestinal trematodiasis – Mural enteritis, multifocal, with intralesional trematodes. Single to multiple cross sections (100 to 500 µm) 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 coelomic connective tissue (Phylum Platyhelminthes, Class Trematoda). Summer ----- 11 of 60 affected. Spring ----- 10 of 30 affected.

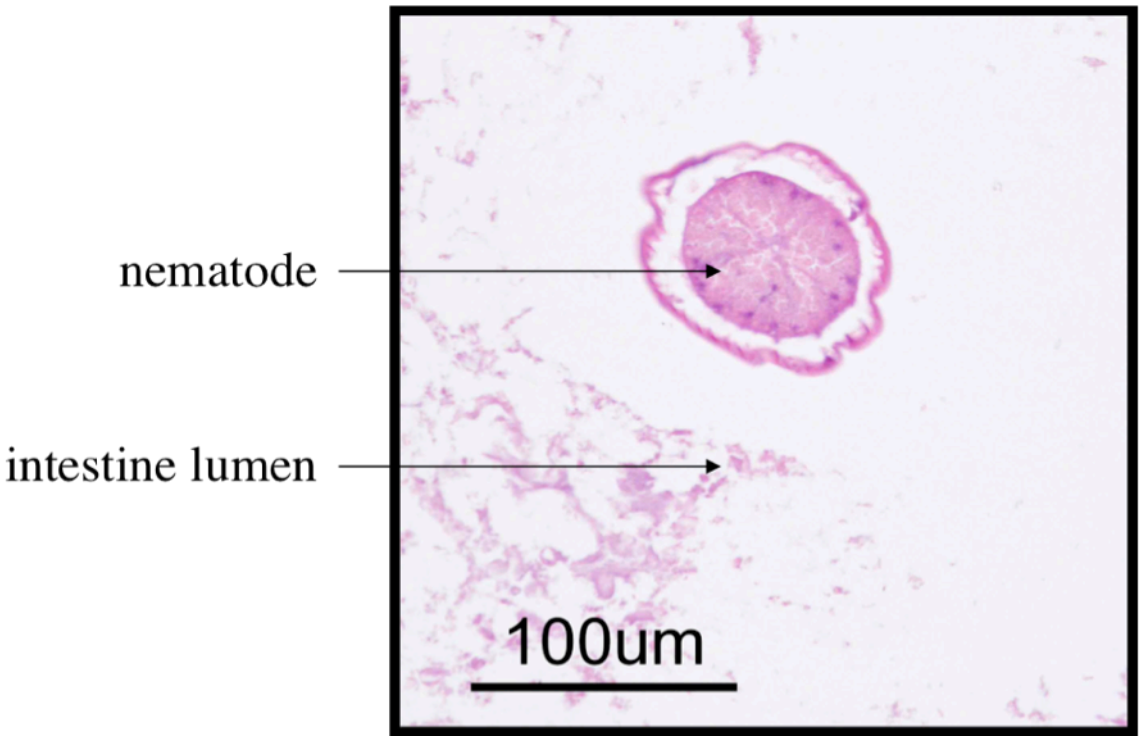


Endomeningial trematodiasis - Trematode, endomeningial, encysted, focal
A single cross sections of a metazoan that is ~ 150 µm in diameter is present within the ventricle of the brain. The trematode is encysted by single to few layers of flattened macrophages and characterized by a thin eosinophilic tegument and a parenchymatous matrix with no calcareous corpuscles (Phylum Platyhelmintheses, Class Trematoda). Summer ----- 1 of 60 affected. Spring----- 1 of 30 affected.



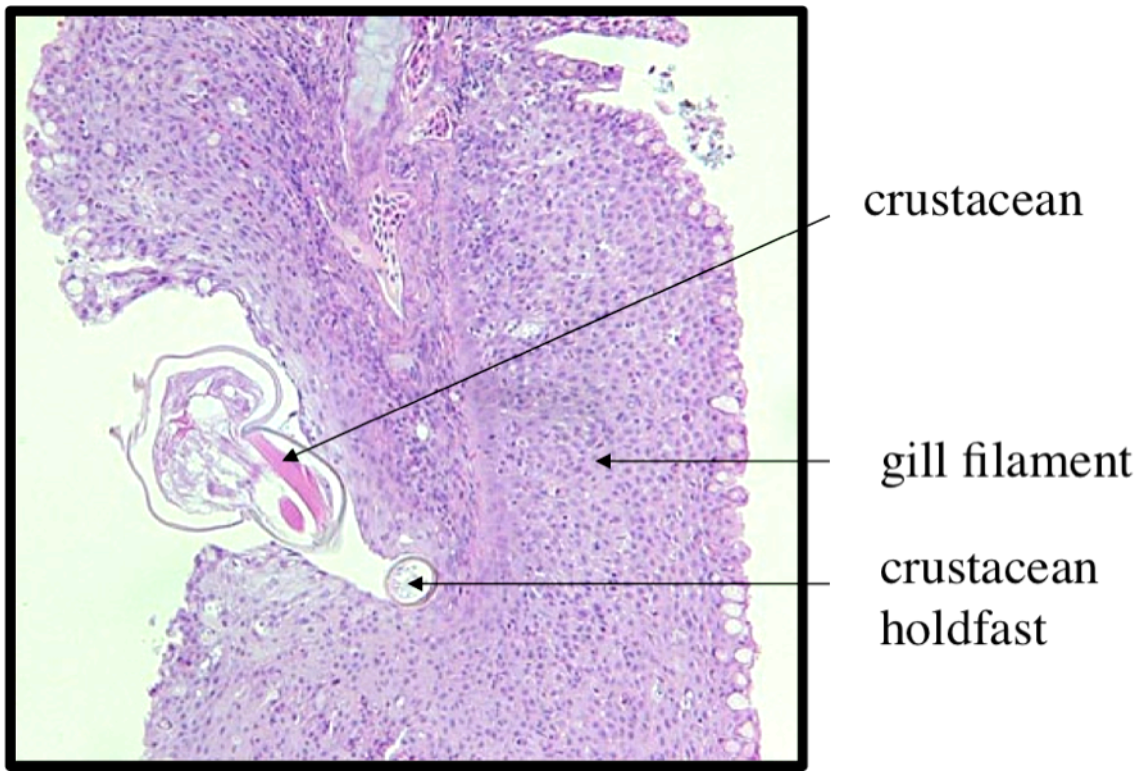
Lesions or organisms only noted in summer.

Intestinal nematodes – Nematodes, few, intestinal, luminal.
There were few to moderate numbers of thin ~100 μm 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.



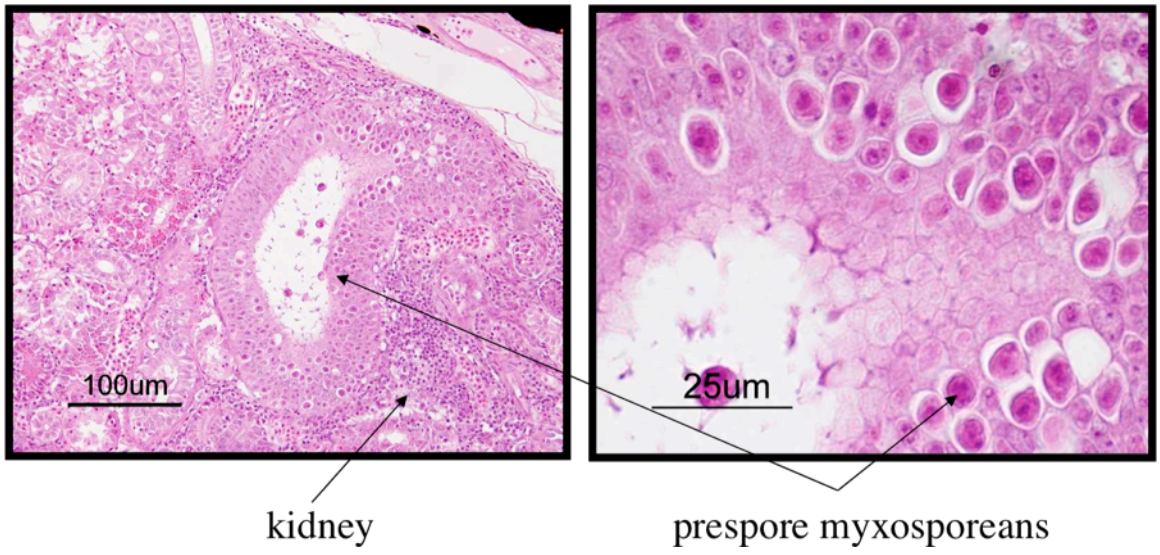
Branchial protozoan - *Trichodina* sp., few, lamellar.
There were very small numbers of ~20 μm protozoans that were dorsoventrally flattened, had cilia and a denticular ring. No tissue reaction was associated with the presence of these organisms (Phylum Ciliophora, Class Litostomatea, Family Trichodina). ---- 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 ~400 x 700 μm 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). This is possibly an *Ergasilus* sp. ---- 25 of 60 affected.

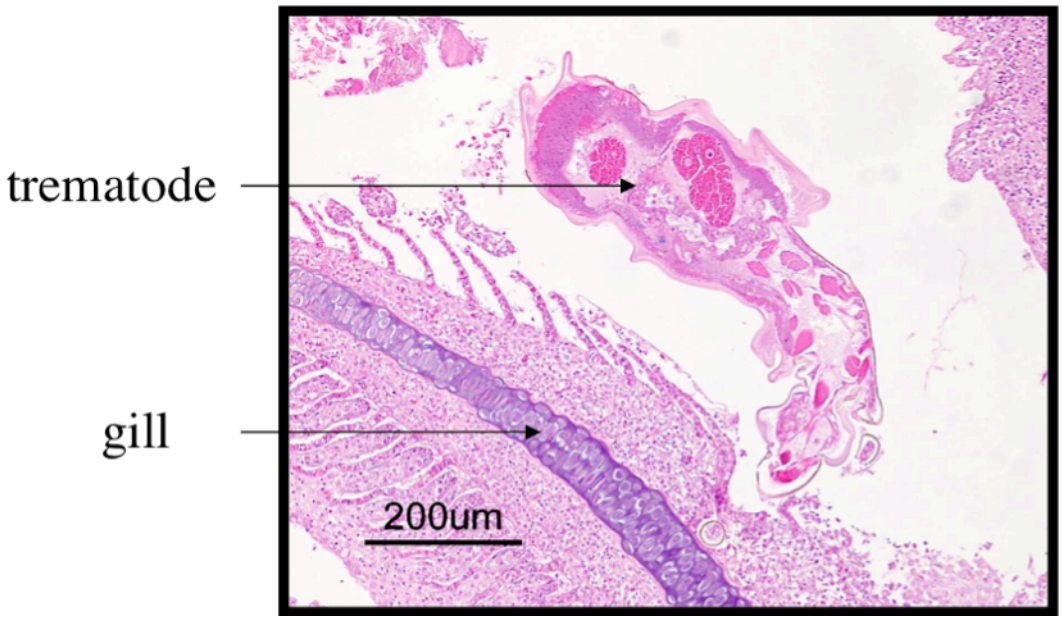


Lesions or organisms only noted in spring.

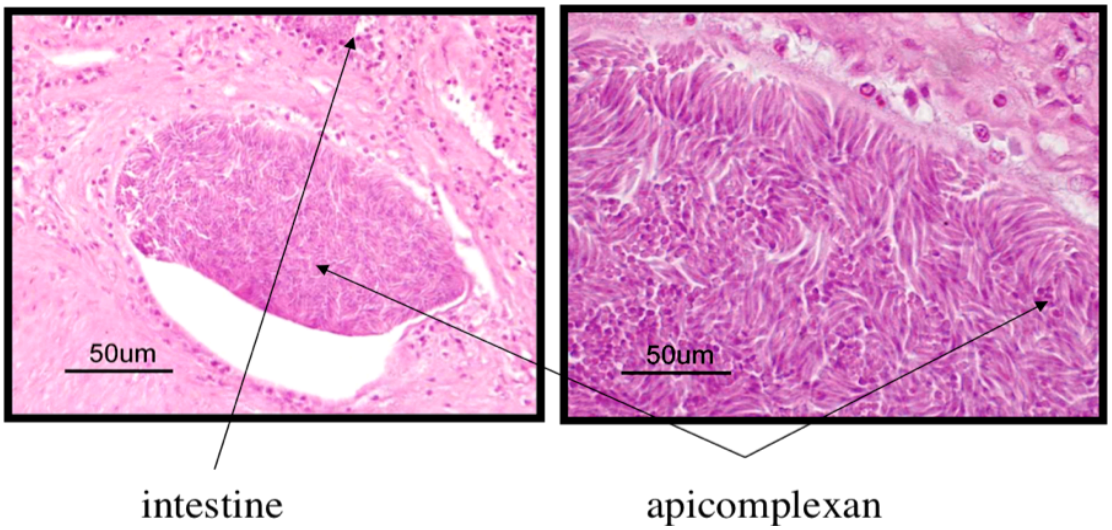
Renal myxosporidiosis – Myxosporidiosis, renal, multifocal.
Large numbers of 7 to 10 μm bi-nucleate sporoplasm are within and replacing epithelial cells of scattered tubules. In some sections, similar multinucleate plasmodium are within the lumen of affected tubules. There is mild to moderate numbers of leukocytes accumulating within the interstitium and surrounding affected tubules. (Phylum Myxozoa, Class Myxosporea). ---- 2 of 30 affected.



Branchial trematodiasis – Branchitis, focally extensive, moderate with intralesional trematode. There is a single large (200 x 900 μm) trematode attached to a section of a single filament. The trematode is characterized by a thin eosinophilic tegument, a parenchymatous matrix with no calcareous corpuscles and scleritinzed hooks which are attached to the branchial filament. (Phylum Platyhelmintheses, Class Trematoda). This is likely a monogenean trematode as there was a haptor-like structure buried in gill tissue. ---- 1 of 30 affected.



Submucosal protozoal cysts – Enteritis, submucosal, focal with intralesional apicomplexan. Within sections of intestinal submucosa, there large numbers of closely packed, 10 to 12 μm in length, pyriform protozoan organisms. There are small numbers of macrophages and lesser numbers of lymphocytes surrounding (cyst). The organisms appear to be within a greatly hypertrophied host cell (possibly merozoites within a mature shizont). (Phylum Apicomplexa, Subclass Coccidia). ---- 2 of 30 affected.



Summaries of lesions or organisms noted in individual fish.

Summer

S 1-
Morphological diagnosis
1. Branchitis, distal, focally extensive, hyperplastic, moderate with intralesional crustaceans

S 2-
Morphological diagnosis
1. Branchitis, distal, focally extensive, hyperplastic, moderate
2. Cestode, luminal intestinal, few

S 3-
Morphological diagnosis
1. Branchitis, distal, focally extensive, hyperplastic, moderate with intralesional crustaceans

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

S 6-
Morphological diagnosis
1. Cestode, luminal intestinal, few

S 7-
Morphological diagnosis
1. Branchitis, distal, focally extensive, hyperplastic, moderate with intralesional crustaceans
2. Cestode, luminal intestinal, moderate

S 8-
Morphological diagnosis
1. Endocarditis, proliferative, focally extensive, moderate with a single intralesional trematode
2. Myocarditis, multifocal, necrotizing, mild

S 9- NAF

S 10-
Morphological diagnosis
1. Mural enteritis, multifocal, with intralesional trematodes, moderate

2. Cestode, luminal intestinal, few

S 11-
Morphological diagnosis
1. Myocarditis, multifocal, necrotizing, mild

S 12-
Morphological diagnosis
1. Cestode, luminal intestinal, moderate
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

S 14-
Morphological diagnosis
1. Myocarditis, multifocal, necrotizing, mild
2. Cestode, luminal intestinal, moderate
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

S 16-
Morphological diagnosis
1. Branchitis, distal, focally extensive, hyperplastic, mild with intralesional crustaceans
2. Mural enteritis, multifocal, with intralesional trematodes, few

S 17-
Morphological diagnosis
1. Myocarditis, multifocal, necrotizing, mild
2. Cestode, luminal intestinal, few
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
2. Myocarditis, multifocal, necrotizing, mild
3. Mural enteritis, multifocal, with intralesional trematodes, moderate
4. Branchitis, distal, focally extensive, hyperplastic, moderate with intralesional crustaceans

S 20-
Morphological diagnosis
1. Gill, intravascular foreign body
2. Cestode, luminal intestinal, few

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
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-
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.
Morphological diagnosis
1. Nematodes, few, intestinal, luminal
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

3. Granulomas, intestinal, focal

S 27- NAF

S 28-
Connective tissue – There is a large ~ 500 μm 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
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
3. Granulomas, intestinal, multifocal

S 30-
Morphological diagnosis
1. Cestode, luminal intestinal, numerous
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
5. *Trichodina* sp., few, lamellar

S 32-
Morphological diagnosis
1. Cestode, luminal intestinal, few

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
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

S 36-
Morphological diagnosis
1. Myocarditis, multifocal, necrotizing, mild
2. Mural enteritis, multifocal, with intralesional trematodes, moderate and Coelomitis, multifocal, granulomatous, with intralesional trematodes, moderate

S 37- NAF

S 38-
Coelomic fat – There is a single histiocyte-rich focus of inflammation. No organisms are present.
Morphological diagnosis
1. Mural enteritis, multifocal, with intralesional trematodes, numerous
2. Steatitis, focal, histiocytic

S 39-
Morphological diagnosis
1. Cestode, luminal intestinal, moderate

S 40-
Morphological diagnosis
1. Myocarditis, multifocal, necrotizing, mild

S 41-
Morphological diagnosis
1. Cestode, luminal intestinal, few
2. Branchitis, distal, focally extensive, hyperplastic, moderate with intralesional crustaceans

S 42-
Coelomic 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
4. Mural enteritis, multifocal, with intralesional trematodes, few
5. Cestode, luminal intestinal, few

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
2. Cestode, luminal intestinal, moderate

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

S 50-
Morphological diagnosis
1. Branchitis, distal, focally extensive, hyperplastic, mild

S 51-
Morphological diagnosis
1. Cestode, luminal intestinal, few

S 52-
Morphological diagnosis
1. Branchitis, distal, focally extensive, hyperplastic, mild with intralesional crustaceans

S 53-

Morphological diagnosis
1. Gill, intravascular foreign body, few
2. Cestode, luminal intestinal, few
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
4. Trematode, likely endomeningial, encysted, focal

S 55-
Morphological diagnosis
1. Cestode, luminal intestinal, moderate
2. Branchitis, distal, focally extensive, hyperplastic, mild
3. Gill, intravascular foreign body, few

S 56-
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-
Morphological diagnosis
1. Branchitis, distal, focally extensive, hyperplastic, mild with intralesional crustaceans
2. Cestode, luminal intestinal, few

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, ~10 mm in diameter protozoans.
Morphological diagnosis
1. Branchitis, distal, focally extensive, hyperplastic, moderate with intralesional crustaceans
2. Mural enteritis, multifocal, with intralesional trematodes, moderate
3. Cestode, luminal intestinal, moderate
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

S 60-
Morphological diagnosis
1. Myocarditis, multifocal, necrotizing, mild
2. Cestode, luminal intestinal, few

Spring

S 1-
Morphological diagnosis
1. Cestode, luminal intestinal, few
2. Epicarditis, multifocal, mild
3. Endocarditis, proliferative, focally extensive

S 2-
Morphological diagnosis
1. Cestode, luminal intestinal, few
2. Branchitis, focal, mild

S 3-
Morphological diagnosis
1. Cestode, luminal intestinal, few
2. Branchitis, focal, mild
3. Gill, intravascular foreign body, few

S 4-
Morphological diagnosis
1. Cestode, luminal intestinal, few
2. Branchitis, focal, mild
3. Gill, intravascular foreign body, few
4. Enteritis, submucosal, focal with intralesional apicomplexan

S 5-
Morphological diagnosis
1. Enteritis, submucosal, focal with intralesional apicomplexan

S 6- NAF

S 7-
Morphological diagnosis
1. Hepatitis, granulomatous, multifocal, mild with intralesional metazoan
2. Cholangiohepatits, multifocal, mild
3. Cestode, luminal intestinal, moderate
4. Epicarditis, myocarditis, multifocal, mild
5. Endocarditis, proliferative, focally extensive

S 8-
Morphological diagnosis
1. Endocarditis, proliferative, focally extensive, moderate
2. Cholangiohepatits, multifocal, mild

S 9-
Morphological diagnosis
1. Cholangiohepatits, multifocal, mild

S 10-
Morphological diagnosis
1. Mural, enteritis, multifocal, with intralesional trematodes, moderate
2.Branchitis, multifocal, mild

S 11- NAF

S 12-
Morphological diagnosis
1. Hepatitis, multifocal, mild

S 13-
Morphological diagnosis
1. Branchitis, multifocal, mild
2. Gill, intravascular foreign body, few
3. Hepatitis, necrotizing, multifocal, mild
4. Cestode, luminal intestinal, moderate

S 14-
Morphological diagnosis
1. Nephritis, interstitial, multifocal
2. Mural, enteritis, multifocal, with intralesional trematodes, moderate
3. Enteritis, submucosal, multifocal, mild

S 15-
Morphological diagnosis
1. Hepatitis, multifocal, mild

S 16-
Morphological diagnosis
1. Nephritis, interstitial, multifocal
2. Myxosporidiosis, renal, multifocal
3. Mural enteritis, multifocal, with intralesional trematodes, few
4. Trematodiasis, endomeningial, encysted, focal

S 17-

Morphological diagnosis
1. Nephritis, interstitial, multifocal
2. Myxosporidiosis, renal, multifocal
3. Mural enteritis, multifocal, with intralesional trematodes, few

S 18-
Morphological diagnosis
1. Branchitis, multifocal, mild
2. Endocarditis, proliferative, focally extensive, moderate
3. Cestode, luminal intestinal, few

S 19-
Morphological diagnosis
1. Nephritis, interstitial, multifocal
2. Mural enteritis, multifocal, with intralesional trematodes, moderate

S 20-
Morphological diagnosis
1. Nephritis, interstitial, multifocal
2. Mural enteritis, multifocal, with intralesional trematodes, moderate

S 21- NAF

S 22-
Morphological diagnosis
1. Hepatitis, multifocal, mild

S 23-
Morphological diagnosis
1. Nephritis, interstitial, multifocal
2. Mural enteritis, multifocal, with intralesional trematodes, moderate

S 24- NAF

S 25- NAF

S 26-
Morphological diagnosis
1. Endocarditis, proliferative, focally extensive, moderate
2. Mural enteritis, multifocal, with intralesional trematodes, moderate

S 27-
Morphological diagnosis
1. Branchitis, focally extensive, moderate to marked with intralesional trematode
2. Nephritis, interstitial, multifocal
3. Mural enteritis, multifocal, with intralesional trematodes, moderate

S 28- NAF

S 29-
Morphological diagnosis
1. Branchitis, multifocal, mild
2. Mural enteritis, multifocal, with intralesional trematodes, numerous
3. Nephritis, interstitial, multifocal
4. Myxosporidiosis, renal, multifocal

S 30-
Morphological diagnosis
1. Cestode, luminal intestinal, numerous
2. Branchitis, distal, focally extensive, hyperplastic, mild to moderate
3. Epicarditis, myocarditis, multifocal, mild
4. Endocarditis, proliferative, focally extensive

Table 8. Summary of notable lesions and organisms in the walleye (*Sander vitreus* –W) collected during the summer of 2006 (n=60) and the spring of 2007 (n=30) from Lake Winnipeg and surrounding waters.

Walleye (*Sander vitreus*) Tissue/agent	Summer 2006 – Sixty fish	Spring 2007 – Thirty fish
Gills	1. Trematode egg (27/60) 2. *Trichodina* sp., *Caprinia* sp., etc. (36/56) 3. Crustacean (3/60)	1. Trematode egg (5/30) 2. *Trichodina* sp., *Caprinia* sp., etc. (7/30)
Stomach/ Intestine/ peritoneum	1. Intestinal cestode (28/60) 2. Mural intestinal trematode (3/60) 3. Granuloma (3/60)	1. Intestinal cestode (8/30) 2. Mural intestinal trematode (3/30)
Skeletal muscle and skin	1. Dermal sarcoma (2/60) 2. Lymphocystis (1/60)	
Liver and bile ducts		1. Granuloma, metazoan (2/30)
Kidney		1. Myxosporean, tubular and glomerular (4/30) 2. Intravascular trematode (1/30)
Heart	1. Myocarditis (39/60) 2. Proliferative endocarditis (37/60) with luminal *Sanguinicola* sp. (9/60)	1. Proliferative endocarditis (6/30)

Summer sample

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.

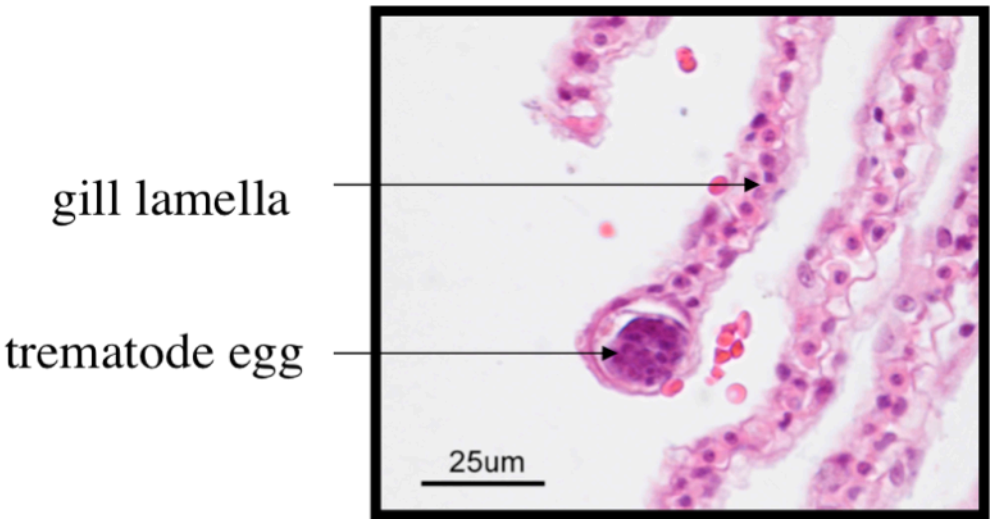
A common lesion that was not evaluated in a systematic fashion was a mild (occasionally moderate) lymphocytic cholangiohepatitis and perihepatopancreatitis. Occasionally these foci are

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.

Spring sample
Individual tissues were dissected in half the cases (W1- W15) with fifteen fish (W16 – W30) consisting of longitudinal sections of whole fish. Tissues routinely examined included skeletal muscle, skin, 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 spleen often contained excessive intracellular and extracellular hemosiderin (golden-brown pigment).

Lesions or organisms noted in both summer and spring.

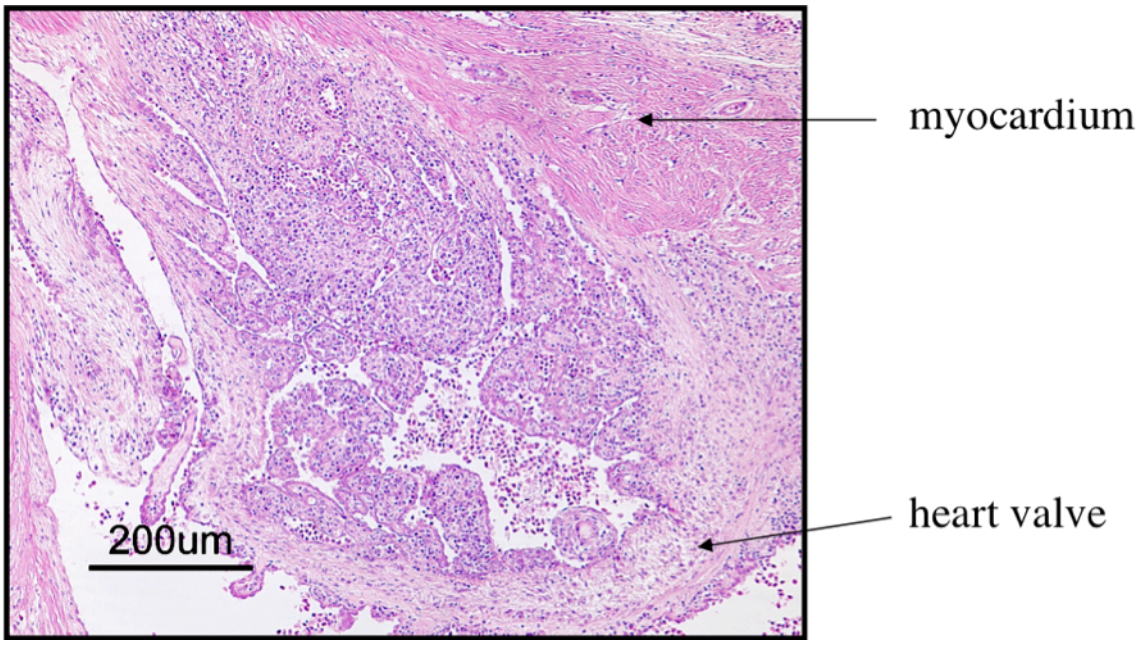
Intracardial trematode 'complex'
Intravascular metazoan 'eggs' – Gill, intravascular foreign body. Trematode egg, presumptive. There are few to numerous intravascular multicellular bodies that expand the lamellar pillar cell channel. The highly basophilic cells form a round ~10 to 15 μm egg that appears to have a very thin case. These same bodies are rarely (once S54) seen intimately associated with the multifocal myocardial lesions described below.
Summer ---- 27 of 60 affected. Spring ---- 5 of 30 affected.

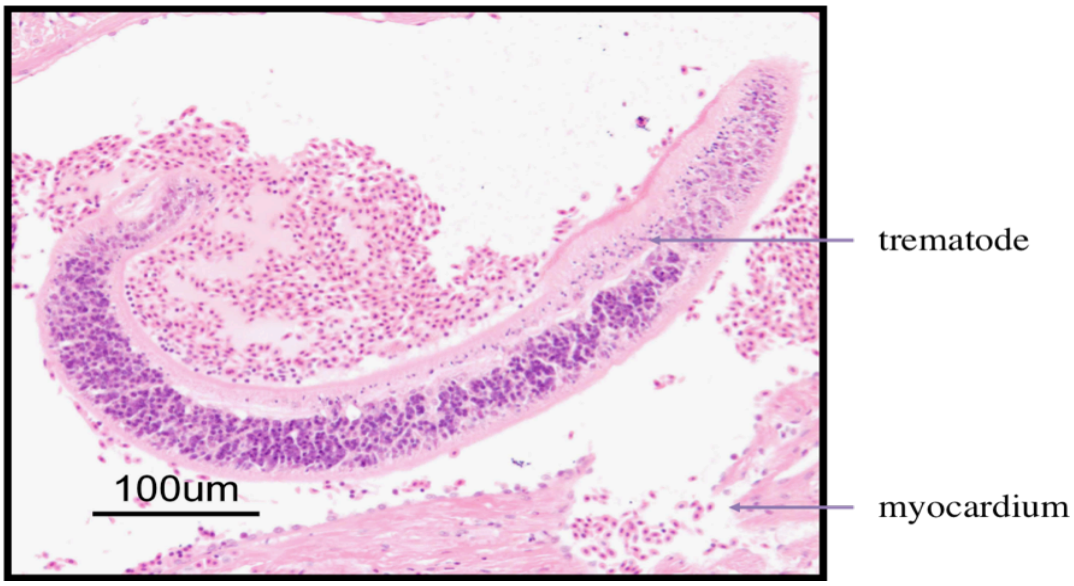


Multifocal necrotizing myocarditis – Myocarditis, multifocal, necrotizing.
There are multiple small hypercellular foci scattered in the myocardium of the atrium and ventricle. The bulbous is rarely affected. These have dense populations of small leukocytes

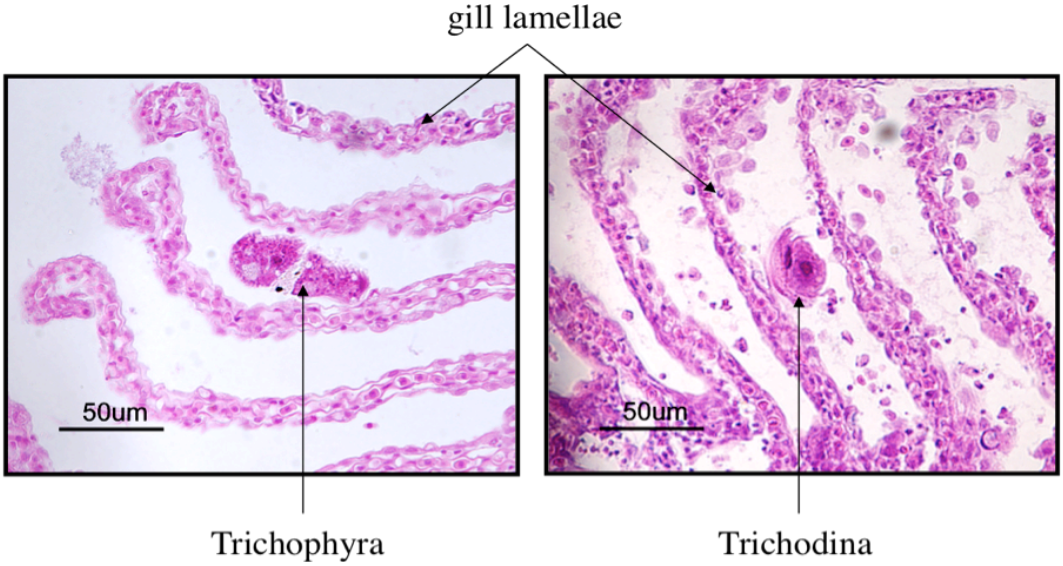
(neutrophils presumably) and endocardial/myocardial cells that are undergoing degeneration and necrosis. Affected myocardial trabeculae are hypereosinophilic, often with loss of striations. In one fish, these are associated with multicelluar bodies similar to those described within intravascular channels in gill lamellae. There are no organisms associated with these lesions otherwise. Summer ----- 39 of 60 affected. Spring – very few fish were affected, not enumerated. This is likely since the majority of walleye collected at this time were very small. See the sauger section for photo.

Proliferative endocarditis - Endocarditis, proliferative, focally extensive
There is a focally extensive hyperplastic plaque of hypertrophied endocardial cells subtended by fibrous hyperplasia. This is occasionally up to 400 μm 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 ~100 x 1200 μm metazoan with a tegument and parenchymatous matrix but no calcareous corpuscles or intestine or cuticle (Phylum Platyhelminthes, Class Trematoda). Summer ---- 37 of 60 affected, with an intralesional trematode(s) ---- 10 of 60 affected. Spring ---- 6 of 30 affected.

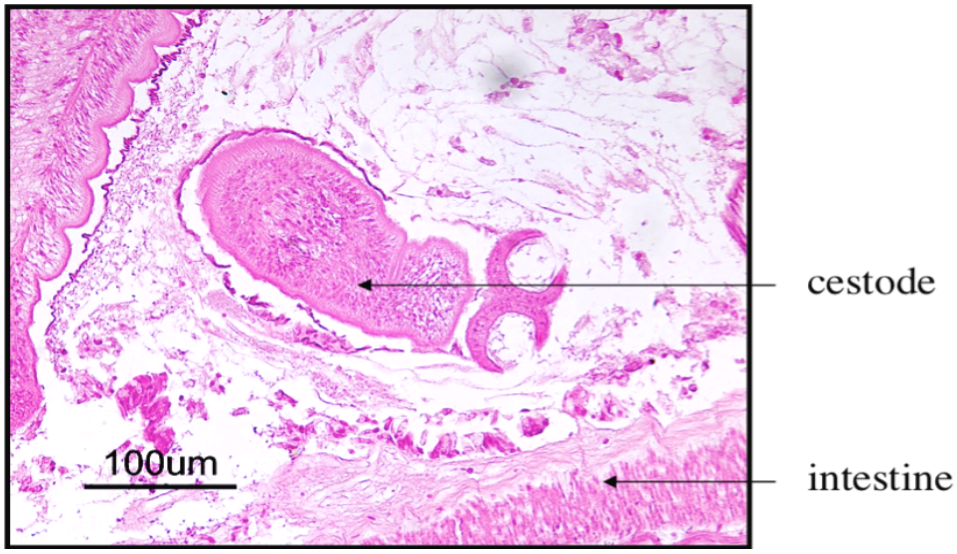




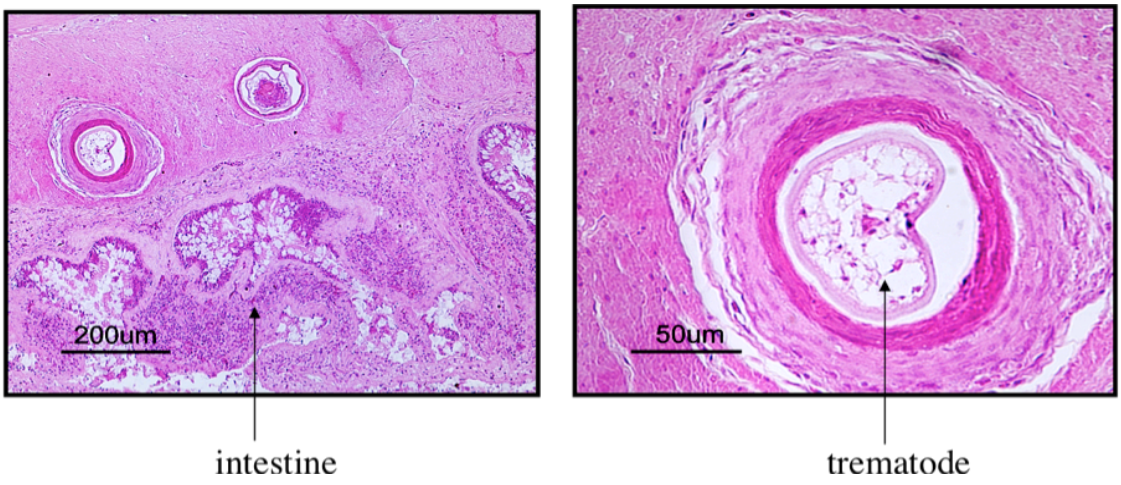
Branchial protozoans – Branchitis, diffuse, with lamellar protozoans
There are single to very numerous (100's) of ~20 x 50 μm slightly dorsoventrally-flattened protozoans with a basophilic nucleus and thin ~2 μm multiple tentacles (these are not uniformly arranged and are opposite from the surface in 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 ~25 μm 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. Summer ---- 36 of 56 affected. Spring ---- 7 of 30 affected.



Intestinal cestodes – Cestode, luminal intestinal
These are ~150 to 200 µm wide and have a parenchymatous matrix and tegument, often thrown into distinct folds and occasionally have calcareous corpuscles (Phylum Platyhelminthes, Class Cestoidea). Summer ---- 28 of 60 affected. Spring ---- 8 of 30 affected.

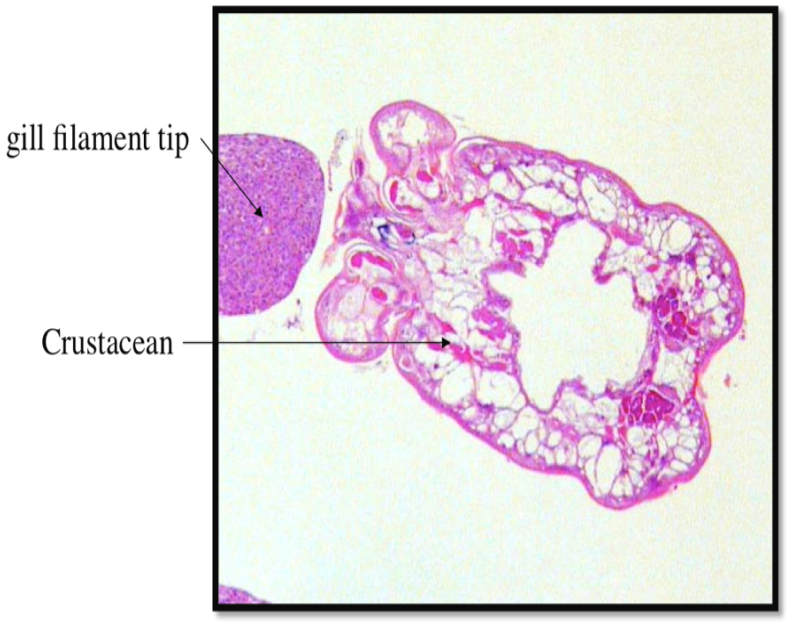


Mural intestinal/gastric trematodiasis – Mural enteritis, multifocal, with intralesional trematodes.
Single to multiple cross sections (80 to 200 µm) of a metazoan are encysted within various sections of the smooth muscle of the intestinal wall. The trematodes are characterized by having a thin eosinophilic 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. Occasionally only remnants of an encysted metazoan(s) are also present within these mural lesions (Phylum Platyhelminthes, Class Trematoda). Summer ----- 3 of 60 affected. Spring ----- 3 of 30 affected.

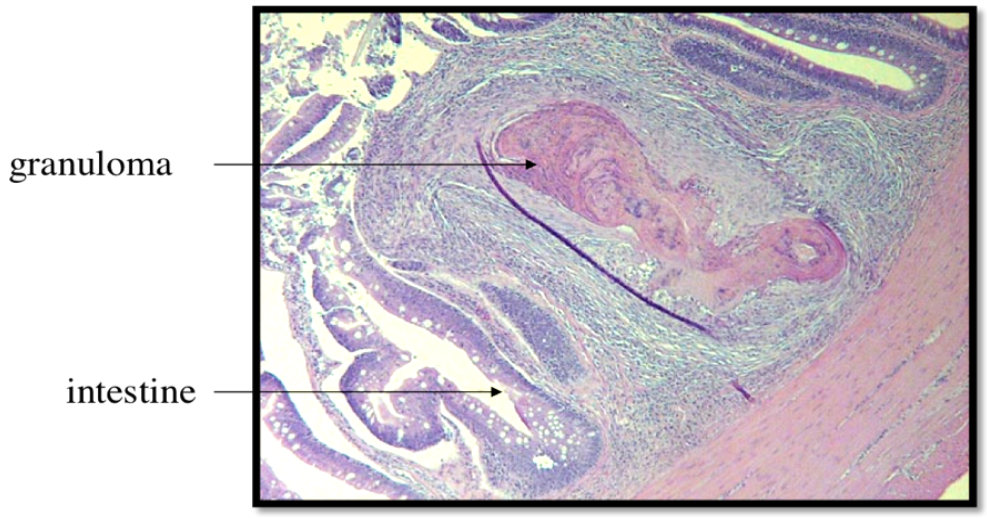


Lesions or organisms only noted in summer.

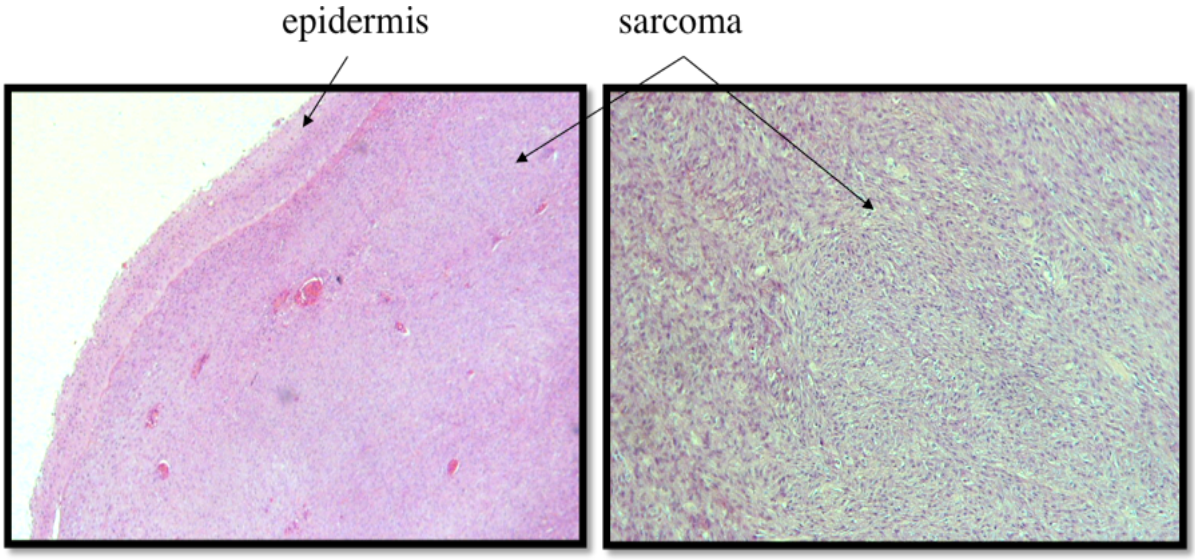
Branchial crustacean - Branchitis, distal, focally extensive, hyperplastic, with (or without) intralesional crustaceans
Few to several filaments have regionally extensive epithelial hyperplasia that is particularly severe at the distal portion. There is a moderate infiltrate of morphologically mixed inflammatory cells surrounding a ~400 x 700 μm 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). The crustacean is shaped somewhat like an Argulus. ---- 6 of 60 affected.



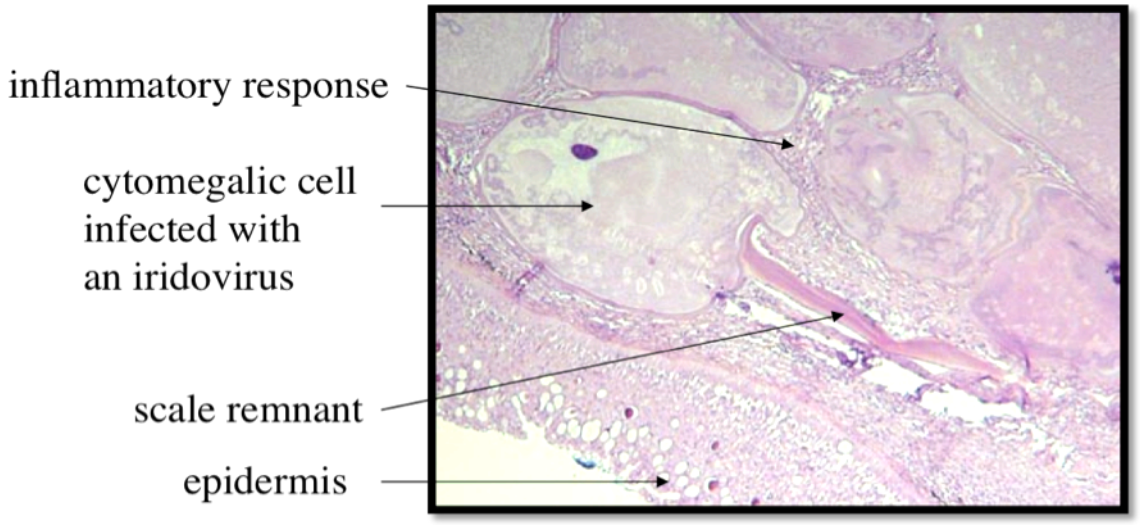
Granulomatous enteritis – Granulomas, intestinal or gastric, lamina proprial to submucosal, focal to multifocal
There single to several, large up to ~2-3 mm, masses composed of very large numbers of hypertrophied macrophages that surround eosinophilic amorphous content with necrotic cellular debris. 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.



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 strap-shaped to bulbous cytoplasmic features resembling muscle cells. ----- 2 of 60 affected.

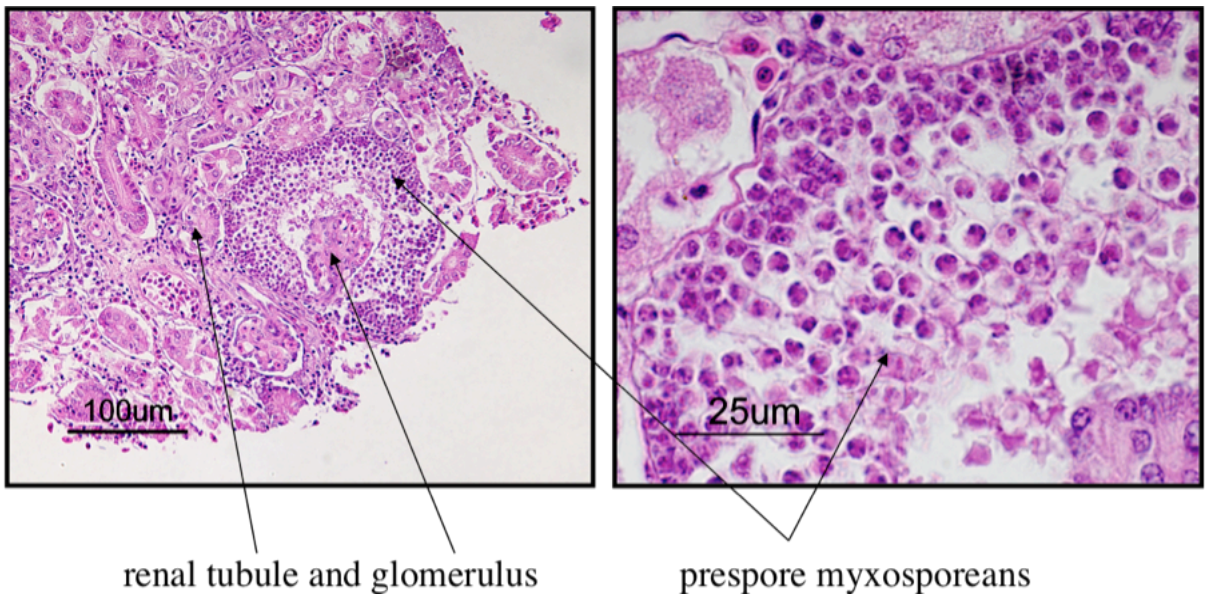


Lymphocystis – Cytomegaly, dermal fibroblasts with cytoplasmic iridoviral inclusions
There are numerous markedly cytomegalic fibroblasts (~1-2 mm in diameter) that expand the dermis and elevate the epidermis. The affected cells have a cytoplasm filled with basophilic granular material. There is a marked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



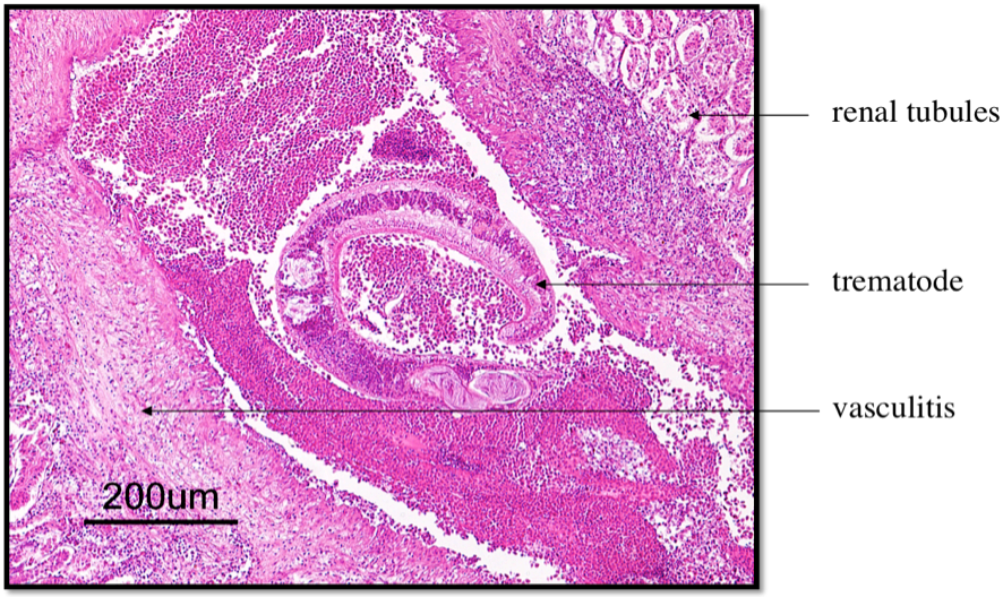
Lesions or organisms only noted in spring.

Renal myxosporidiosis – Myxosporidiosis, renal, multifocal.
Large numbers of 7 to 10 μm bi-nucleate sporoplasm, that appear to be within the glomerular parietal epithelium and greatly expands Bowman's capsule of scattered glomeruli. Similar multinucleate sporoplasms are loose within the urinary space. In some sections, there is mild to moderate numbers of leukocytes surrounding affected glomeruli. (Phylum Myxozoa, Class Myxosporea). ---- 4 of 30 affected.



Intravascular, renal trematodiasis – Trematodiasis, intravascular with regionally extensive, proliferative vasculitis. Within the lumen of a large renal vein of the caudal kidney, there is a tangential section of a trematode parasite that is approximately 100 μm wide and 1 mm in length. The trematode is characterized by a thin eosinophilic tegument surrounding a parenchymatous

matrix with the absence of calcareous corpuscles. Reproductive tissues are present, however no digestive tract was identified (Phylum Platyhelminthes, Class Trematoda). Along a regionally extensive section of the renal vein, there is there is a focally extensive hyperplastic plaque of hypertrophied endothelial cells underlined by fibrous hyperplasia and thrown into papillary fronds. Within some sections, there are small to moderate numbers of leukocytes expanding spaces between vascular tunics and extending into the renal interstitium. ----- 1 of 30 affected.



Hepatic parasitic granulomas – Hepatitis, granulomatous, multifocal, with intralesional metazoan. Embedded within the hepatic parenchyma there are multifocal accumulations of plump and attenuated amphiphilic macrophages surrounding remnants of a metazoan parasite. ---- 2 of 30 affected.

Summaries of lesions or organisms noted in individual fish.

Summer

W 1 –
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

W 2 –
Eye – There is a diffuse periscleral infiltration of lymphocytes. No organisms are present.
Morphologic diagnosis
1. Branchitis, diffuse, with lamellar protozoans, numerous.

W 3 –

Morphologic diagnosis
1. Gill, intravascular foreign body, few
2. Branchitis, diffuse, with lamellar protozoans, few
3. Cestode, luminal intestinal, numerous
4. Granulomas, intestinal, lamina proprial to submucosal, focal to multifocal
5. Mural enteritis, multifocal

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

W 5 – NAF

W 6 –
Morphologic diagnosis
1. Cestode, luminal intestinal, numerous

W 7 –
Morphologic diagnosis
1. Gill, intravascular foreign body, few
2. Branchitis, diffuse, with lamellar protozoans, few

W 8 –
Morphologic diagnosis
1. Myocarditis, multifocal, necrotizing, mild
2. Cestode, luminal intestinal, few

W 9 –
Morphologic diagnosis
1. Myocarditis, multifocal, necrotizing, mild
2. Branchitis, diffuse, with lamellar protozoans, moderate

W 10 –
Morphologic diagnosis
1. Endocarditis, proliferative, focally extensive, mild
2. Branchitis, diffuse, with lamellar protozoans, few
3. Cestode, luminal intestinal, moderate
4. Granulomas, gastric, lamina proprial to submucosal, focal to multifocal
5. Granulomas, hepatic, metazoan-type

W 11 –

Morphologic diagnosis
1. Gill, intravascular foreign body, few
2. Myocarditis, multifocal, necrotizing, mild
3. Endocarditis, proliferative, focally extensive
4. Branchitis, diffuse, with lamellar protozoans, few

W 12 –
Morphologic diagnosis
1. Myocarditis, multifocal, necrotizing, mild
2. Endocarditis, proliferative, focally extensive
3. Cestode, luminal intestinal, few
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

W 14 –
Morphologic diagnosis
1. Cestode, luminal intestinal, few
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

W 17 –
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

W 20 –
Morphologic diagnosis
1. Myocarditis, multifocal, necrotizing, very mild
2. Branchitis, diffuse, with lamellar protozoans, numerous
3. Intestine, granuloma, focal, metazoan contents

W 21 –
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

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

W 23 –
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

W 25 –
Morphologic diagnosis
1. Gill, intravascular foreign body, few
2. Endocarditis, proliferative, focally extensive, moderate with myocardial necrosis at the base of the atrio-ventricular valves
3. Branchitis, diffuse, with lamellar protozoans, few

W 26 –
Morphologic diagnosis
1. Gill, intravascular foreign body, moderate
2. Endocarditis, proliferative, focally extensive, mild
3. Branchitis, distal, focally extensive, hyperplastic, moderate with intralesional crustaceans

W 27 –
Morphologic diagnosis.
1. Myocarditis, multifocal, necrotizing, mild
2. Branchitis, diffuse, with lamellar protozoans, moderate
3. Cestode, luminal intestinal, few

W 28 –
There is a single focus of gliosis in the molecular layer of the optic lobe.
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

W 29 –
There are two wedge-shaped foci of necrosis in the compact myocardium and extending ~2 mm 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

W 30 –
Morphologic diagnosis
1. Myocarditis, multifocal, necrotizing, moderate
2. Cestode, luminal intestinal, few

W 31 –
Morphologic diagnosis
1. Myocarditis, multifocal, necrotizing, mild

2. Endocarditis, proliferative, focally extensive, moderate
3. Cestode, luminal intestinal, numerous

W 32 –
Morphologic diagnosis
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

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

W 34 –
Morphologic diagnosis
1. Gill, intravascular foreign body, few
2. Branchitis, distal, focally extensive, hyperplastic, moderate to severe with intralesional crustaceans
3. Cestode, luminal intestinal, numerous

W 35 –
Morphologic diagnosis
1. Gill, intravascular foreign body, numerous
2. Endocarditis, proliferative, focally extensive, moderate
3. Branchitis, diffuse, with lamellar protozoans, few

W 36 –
Morphologic diagnosis
1. Branchitis, diffuse, with lamellar protozoans, numerous

W 37 –
Morphologic diagnosis
1. Endocarditis, proliferative, focally extensive, mild with portion of a metazoan
2. Cestode, luminal intestinal, numerous
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

W 39 –
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

W 40 –
Morphologic diagnosis
1. Gill, intravascular foreign body, numerous
2. Branchitis, diffuse, with lamellar protozoans, few
3. Cestodes, luminal, intestinal, numerous

W 41 –
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)

W 42 –
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

W 45 –
Morphologic diagnosis
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 ~10 μm 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
6. Cestodes, luminal, intestinal, few

W 47 –
Kidney – There are two foci, one that appears to be in the interstitium and the other that surrounds a glomerulus, that are ~60-80 μm in diameter and are composed of structures similar to ~5-7 μm multinucleate plasmodia arranged in rows. Presumptive myxosporean cyst containing unsporulated plasmodia. No spores and no inflammation are present.
Morphologic diagnosis
1. Cestode, luminal intestinal, numerous

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

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

W 50 –
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.
Morphologic diagnosis
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

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

W 55 –
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

W 57 –
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 intralesional trematode
6. Cytomegaly, dermal fibroblasts with cytoplasmic iridoviral inclusions

W 58 –
Morphologic diagnosis
1. Gill, intravascular foreign body, few
2. Myocarditis, multifocal, necrotizing, mild
3. Endocarditis, proliferative, focally extensive, moderate
4. Cestode, luminal intestinal, few
5. Branchitis, distal, focally extensive, hyperplastic, moderate with intralesional crustaceans

W 59 –
Intestine – There are two sections of a nematode, ~150 μm 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 –
Morphologic diagnosis
1. Gill, intravascular foreign body, few (one with a clear egg case)
2. Cestode, luminal intestinal, few

Spring

W 1 -
Morphologic diagnosis
1. Mural enteritis, multifocal, with intralesional trematodes.

W 2 –
Morphologic diagnosis
1. Gill, intravascular foreign body, few
2. Endocarditis, proliferative, focally extensive, moderate
4. Branchitis, multifocal to regionally extensive, with lamellar protozoans, numerous
5. Hepatitis, granulomatous, multifocal, with intralesional metazoan
6. Mural enteritis, multifocal, with intralesional trematodes

W 3 –
Morphologic diagnosis
1. Gill, intravascular foreign body, few
2. Endocarditis, proliferative, focally extensive, moderate
3. Branchitis, multifocal to regionally extensive, with lamellar protozoans, numerous
4. Mural enteritis, multifocal, with intralesional trematode

W 4 –

Morphologic diagnosis
1. Gill, intravascular foreign body, few
2. Trematodiasis, intravascular with focally extensive, proliferative vasculitis.
3. Branchitis, diffuse, with lamellar protozoans, few
4. Cestode, luminal intestinal, numerous

W 5 –
Morphologic diagnosis
1. Nephritis, multifocal, mild to moderate

W 6 –
Morphologic diagnosis
1. Nephritis, multifocal, mild to moderate
2. Nephrocalcinosis, multifocal, mild
3. Granuloma, renal interstitium, focal

W 7 –
Morphologic diagnosis
1. Nephritis, multifocal, mild to moderate
2. Branchitis, diffuse, with lamellar protozoans, few

W 8 –
Morphologic diagnosis
1. Hepatitis, granulomatous, multifocal, with intralesional metazoan
2. Myxosporidiosis, renal, multifocal
3. Nephritis, multifocal, mild to moderate
4. Branchitis, multifocal, mild

W 9 –
Morphologic diagnosis
1. Gill, intravascular foreign body, few
2. Endocarditis, proliferative, focally extensive, moderate
3. Branchitis, multifocal to regionally extensive, with lamellar protozoans, numerous
4. Nephritis, multifocal, mild to moderate
5. Cestode, luminal intestinal

W 10 –
Morphologic diagnosis
1. Cestode, luminal intestinal

W 11 –
Morphologic diagnosis
1. Gill, intravascular foreign body, few
2. Endocarditis, proliferative, focally extensive, moderate
3. Branchitis, multifocal to regionally extensive, with lamellar protozoans, numerous
4. Vasculitis, splenic, focal

W 12 –
Morphologic diagnosis
1. Nephritis, multifocal, mild to moderate
2. Myxosporidiosis, renal, multifocal
3. Cestode, luminal intestinal, few
3. Granuloma, splenic, focal

W 13 –
Morphologic diagnosis
1. Nephritis, multifocal, mild to moderate

W 14 –
Morphologic diagnosis
1. Endocarditis, proliferative, focally extensive, moderate
2. Nephritis, multifocal, mild to moderate
3. Myxosporidiosis, renal, multifocal

W 15 –
Morphologic diagnosis
1. Endocarditis, proliferative, focally extensive, moderate
2. Branchitis, multifocal to regionally extensive, with lamellar protozoans, numerous
3. Nephritis, multifocal, mild to moderate
4. Myxosporidiosis, renal, multifocal

W 16 – NAF

W 17 –
Morphologic diagnosis
1. Cestode, luminal intestinal

W 18 –
Morphologic diagnosis
1. Cestode, luminal intestinal

W 19 –
Morphologic diagnosis
1. Cestode, luminal intestinal

W 20 – NAF

W 21 – NAF

W 22 – NAF

W 23 – NAF

W 24 – NAF

W 25 – NAF

W 26 –
Morphologic diagnosis
1. Cestode, luminal intestinal

W 27 – NAF

W 28 – NAF

W 29 – NAF

W 30 – NAF

Table 9. Summary of notable lesions and organisms in the white bass (*Morone chrysops* – WB) collected during the summer of 2006 (n=60) and the spring of 2007 (n=30) from Lake Winnipeg and surrounding waters.

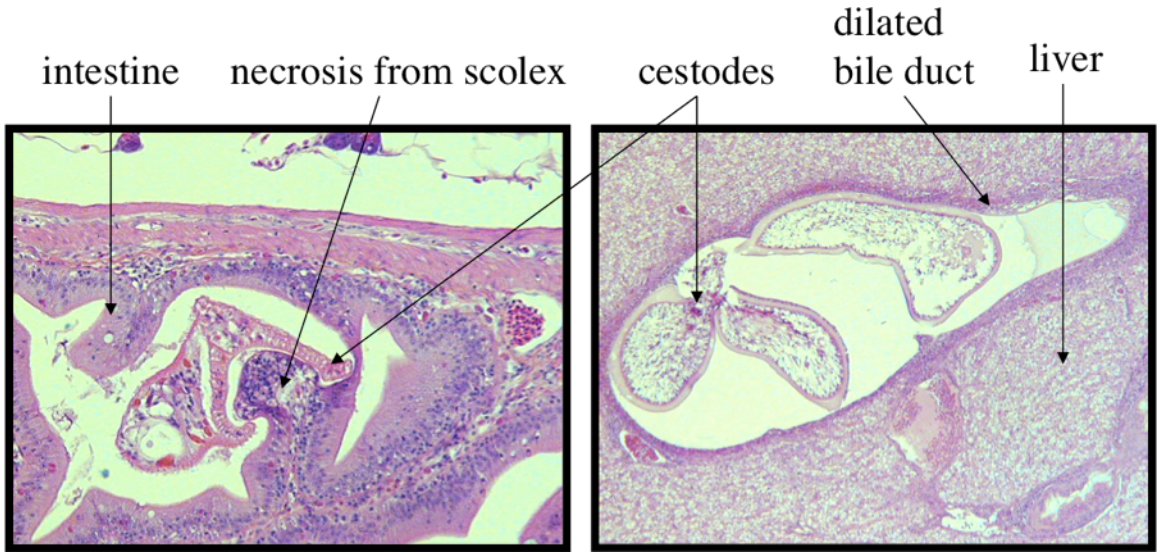
White Bass (*Morone chrysops*) Tissue/agent	Summer 2006 – Sixty fish	Spring 2007 – Thirty fish
Gills	1. Protozoan (numerous) (36/59) 2. Epitheliocystis (23/59) 3. Monogene - small (2/59) 4. Monogene - large (51/59) 5. Microsporean (2/59)	1. Protozoan (*Caprinia* sp.) (11/30) 2. Epitheliocystis (2/30) 3. Monogene X (1/30)
Stomach/ Intestine/ peritoneum	1. Intestinal cestode (35/59)	1. Intestinal cestode (5/30)
Skeletal muscle and skin	1. Trematode (5/59)	
Brain and meninges		1. Encephalitis (1/30)
Liver and bile ducts	1. Biliary cestode (2/59) 2. Cholangiohepatitis	1. Metazoan granuloma (1/30) 2. Cholangiohepatitis
Heart		1. Epi/myocarditis (7/30)

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.

Lesions or organisms noted in both summer and spring.

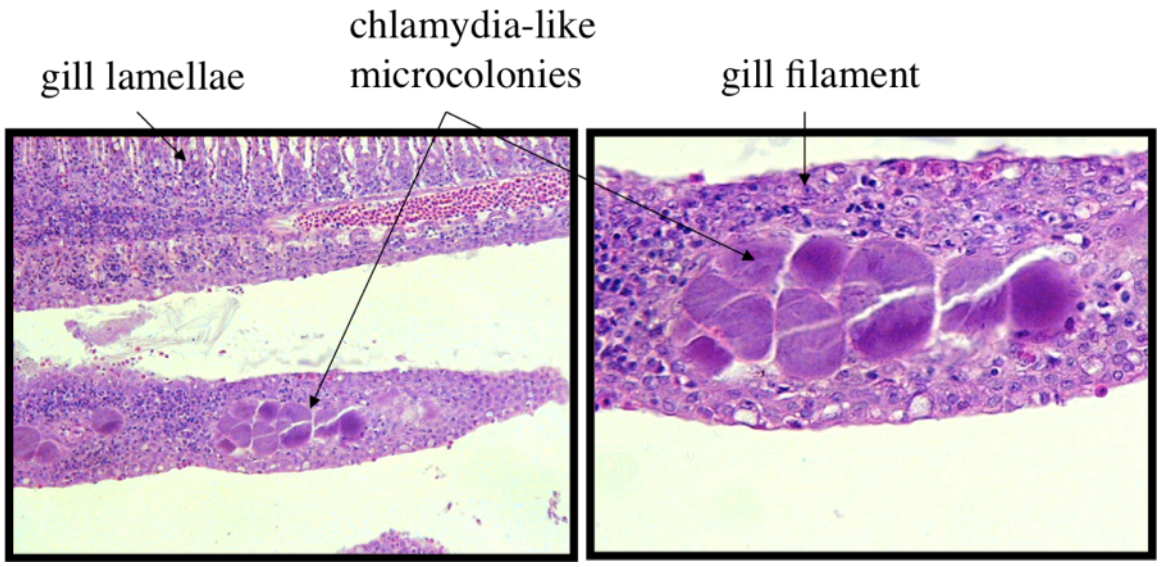
Intestinal cestodes – Cestodes, intraluminal, intestinal
There are single to very numerous large ~300 to 400 μm in width metazoans with a thick (~40 μm) eosinophilic tegument and a parenchymatous matrix with calcareous corpuscles and lacking in digestive tubes (Cestode). Occasionally, there is segmentation and numerous eggs contained within ovaries and less often free in the intestinal lumen.
Tissue reaction is minimal but occasionally there is a holdfast visible in the section with clamped mucosa undergoing necrosis (see Figure). Very heavily infested sections of intestine are dilated with minimally thinned mucosa (Phylum Platyhelminthes, Class Cestoidea). Summer ----35 of 59 affected. Spring ---- 5 of 30 affected.

Intrahepatic (biliary) cestodes (likely same as above) – 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 (Phylum Platyhelminthes, Class Cestoidea). Summer only ---- 2 of 59 affected.

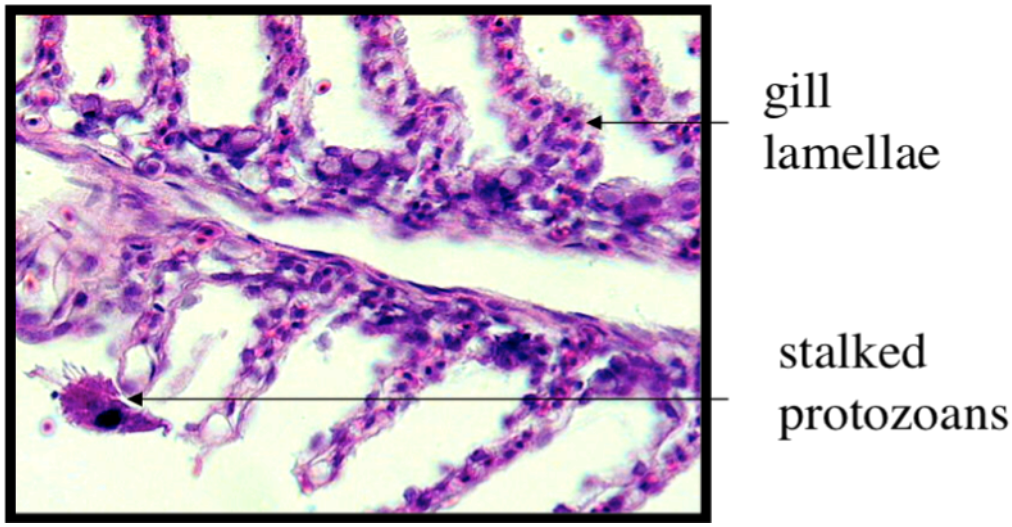


Branchial epitheliocystis – Branchitis, focal to multifocal or regionally extensive, with filamental interepithelial bacterial microcolonies
There are single to several closely spaced ~70 to 150 μm tightly-packed basophilic foci with a thin ~5 μm eosinophilic limiting membrane that delineates each microcolony. The microcolony is filled with ~1 μm (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?). Summer ---- 23 of 59 affected. Spring ---- 2 of 30 affected.



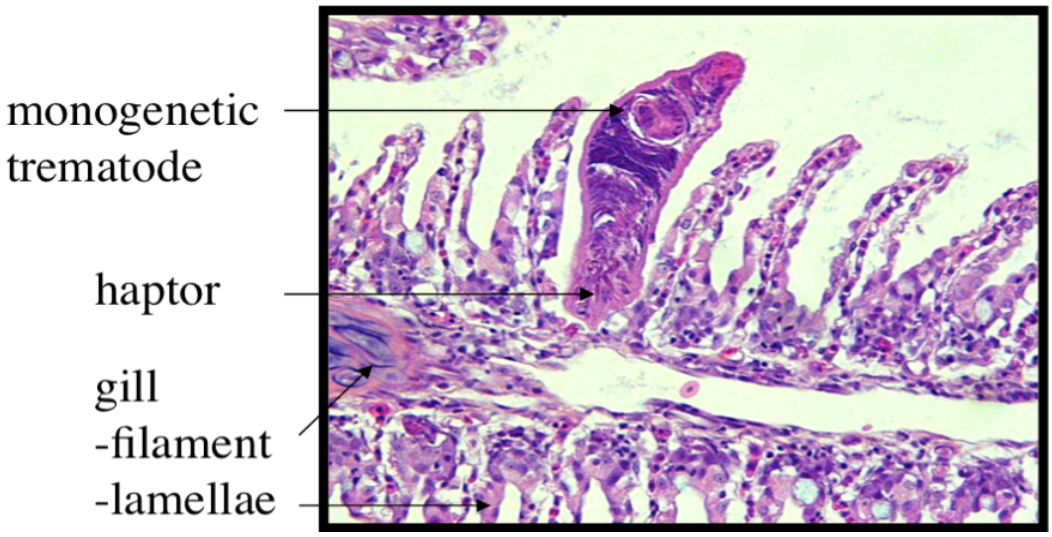
Branchial protozoans – Branchitis, multifocal, with lamellar protozoans
There are single to very numerous ~20 x 50 μm slightly dorsoventrally-flattened protozoans with a basophilic nucleus and thin ~2 μm multiple tentacles (these are not uniformly arranged and are opposite from the surface in contact with the epithelium) present on the lamellar epithelial surface. (Phylum Ciliophora, Subclass Suctoria, *Caprinia* sp. (formerly *Trichophyra*). There are also small to moderate numbers of ~25 μm 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. Summer ---- 36 of 59 affected. Spring ---- 11 of 30 affected.



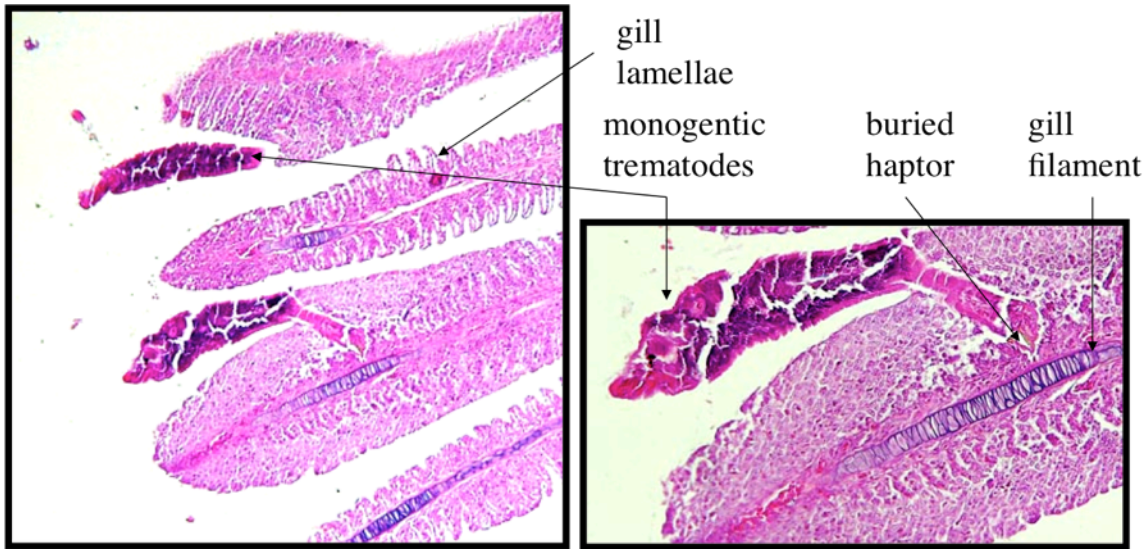
Multifocal cholangiohepatitis - This lesion was very common and was not enumerated in a systematic fashion. Most lesions were mild to moderate. No organisms were seen associated with these lesions.
Typically, these were collars of inflammatory cells consisting of lymphocytes with fewer macrophages, plasma 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 the pancreatic tissue appears to be effaced to some degree. This lesion was present in spring and summer.

Lesions or organisms only noted in summer.

Branchial monogenetic trematode (smaller) - Branchitis, with single intralesional trematode with haptor (distinct from species with holdfast)
There is a single cross-section of an ~50 x 300 μm metazoan organism characterized by a non-segmented thin tegument supported by a row of sub-tegumental cells and loose reticular parenchymal matrix. A terminal haptor is visible in contact with the interlamellar epithelium. Mild focal epithelial necrosis can be seen. ---- 2 or 3 of 59 affected.

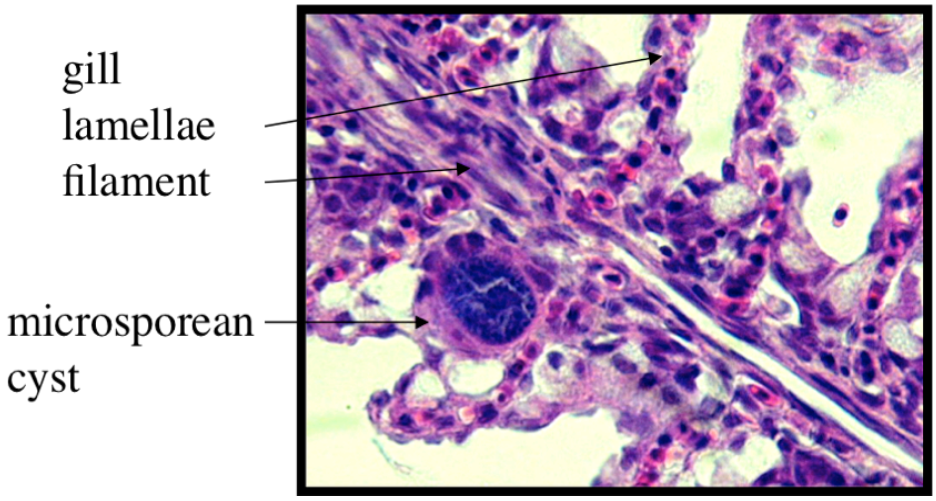


Branchial monogenetic trematode – Branchitis, distal, focally extensive, hyperplastic, with intralesional trematodes (distinct from smaller trematode above)
Gills – Numerous filaments have diffuse epithelial hyperplasia that is particularly severe at the distal portion. There is a moderate infiltrate of morphologically mixed inflammatory cells and mild necrosis surrounding a 50 by 400 to 500 μm metazoan with a chitinous holdfast (haptor) buried within the filament. This metazoan has a tegument and granular basophilic contents and is without a reproductive system or intestine (Phylum Platyhelminthes, Class Trematoda). ---- 51 of 59 affected.

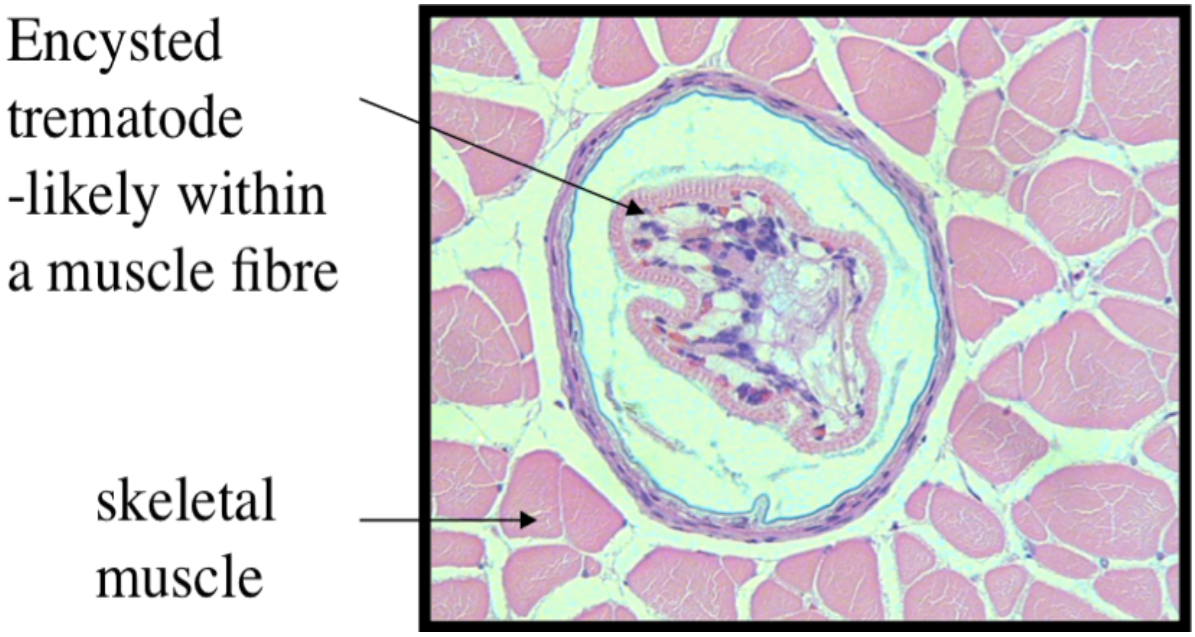


Branchial monogenetic trematodes – Branchitis, focally extensive, hyperplastic, with intralesional trematodes.
Multiple filaments have multifocal to focally extensive epithelial hyperplasia. There is a moderate infiltrate of morphologically mixed inflammatory cells and mild necrosis surrounding cross sections of a metazoan parasite (presumptive monogenean trematode). This metazoan has a tegument and granular basophilic contents and is without a reproductive system or intestine (Phylum Platyhelminthes, Class Trematoda). Spring ---- 1 of 30 affected. Due to the lack of detail present it was not possible to determine with a degree of certainty if this organism was similar to either of the two identified during summer. It more closely resembles the first described.

Branchial microsporidean – Branchitis, multifocal, with few microsporidian cysts
There are single to several round to oval ~40 to 50 μm structures buried within the interlamellar epithelium, that have a granular basophilic content made up of ~2 μm spores and a thin ~3 μm hyaline eosinophilic cyst wall (Phylum Microspora, Family Microsporea). There is no to limited tissue reaction but these cysts minimally distort the adjacent lamellae. ---- 2 of 59 affected.



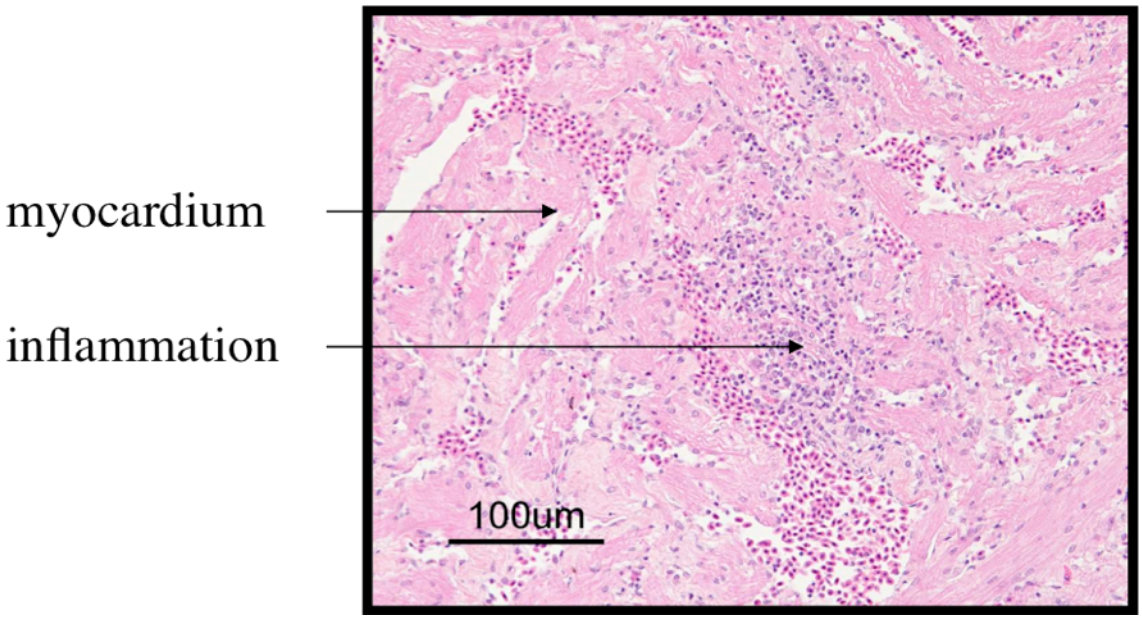
Intramuscular trematode – Trematode, encysted intramuscular, skeletal.
Muscle – single encysted metazoan – There is a ~200 μm metazoan within a fibrous encapsulated wall (likely within a myocyte with compressed parenchyma). There is no host reaction. The metazoan has a thin eosinophilic tegument and a parenchymatous matrix with no calcareous corpuscles (Phylum Platyhelminthes, Class Trematoda). ---- 5 of 59 affected.



Lesions or organisms only noted in spring.

Hepatic parasitic granulomas – Hepatitis, granulomatous, multifocal, with intralesional metazoan. Embedded within the hepatic parenchyma there are multifocal accumulations of plump and attenuated amphophilic macrophages surrounding remnants of a metazoan parasite. ---- 1 of 30 affected. (no photo)

Epicarditits / myocarditis - focal to multifocal, mild to moderate**.**
There are single and occasionally multiple small hypercellular foci scattered in the epicardium and myocardium of the ventricle. These populations are made of small to moderate numbers of small leukocytes (neutrophils and lesser numbers of lymphocytes presumably) and occasionally endocardial/myocardial cells that are undergoing degeneration and necrosis. Affected myocardial trabeculae are hypereosinophilic, often with loss of striations. There are no organisms associated with these lesions. ----- 7 of 30 affected.



Liver calcification – Hepatitis, multifocal, mild with calcification.
Scattered throughout the hepatic parenchyma, there are multiple small foci of cellular calcification surrounded by small numbers of hepatocytes undergoing degeneration and necrosis. Minimal numbers of leukocytes are associated with these lesions and no organisms were identified. ---- 1 of 30 affected. (no photo)

Encephalitis, focal, mild.
Within sections of the neuropil, there is a single accumulation of moderate numbers of glial cells surrounding vascular capillaries and viable neurons. No organisms were associated with this lesion. ---- 1 of 30 affected. (no photo)

Summaries of lesions or organisms noted in individual fish.

Summer

WB 1-
Morphological diagnosis
1. Branchitis, diffuse, with numerous lamellar protozoans
2. Branchitis, moderate, distal, focally extensive, hyperplastic, with moderate intralesional trematodes
3. Cestodes, moderate intraluminal, intestinal
4. Kidney – mild amounts of eosinophilic material in tubules, occasionally mineralized.

WB 2 -
Morphological diagnosis
1. Branchitis, diffuse, with moderate lamellar protozoans
2. Branchitis, focal to multifocal or regionally extensive, with filamental interepithelial bacterial microcolonies

3. Kidney – mild amounts of eosinophilic material in tubules

WB 3- No fish/slide

WB 4-
Morphological diagnosis
1. Branchitis, distal, focally extensive, hyperplastic, with moderate intralesional trematodes
2. Cestodes, fewintraluminal, intestinal

WB 5-
Morphological diagnosis
1. Branchitis, focal to multifocal or regionally extensive, with moderate filamental interepithelial bacterial microcolonies
2. Cestodes, few intraluminal, intestinal
3. Trematode, encysted intramuscular, skeletal

WB 6 -
Morphological diagnosis
1. Branchitis, mild, distal, focally extensive, hyperplastic, with intralesional trematodes
2. Cestodes, few intraluminal, intestinal
3. Stomach – There are small multifocal accumulations of lymphocytes with loose foci on macrophages in the submucosa. No organisms are visible.
4. Cestode with no discernible anatomic location

WB 7 -
Morphological diagnosis
1. Branchitis, moderate, distal, focally extensive, hyperplastic, with moderate intralesional trematodes
2. Cestodes, few intraluminal, intestinal

WB 8-
Morphological diagnosis
1. Branchitis, diffuse, with few lamellar protozoans
2. Branchitis, mild, distal, focally extensive, hyperplastic, with moderate intralesional trematodes

WB 9-
Morphological diagnosis
1. Branchitis, diffuse, with numerous lamellar protozoans
2. Branchitis, moderate, distal, focally extensive, hyperplastic, with few intralesional trematodes
3. Cestodes, few intraluminal, intestinal
4. Stomach - For many of the fish there are varying numbers of epithelial apoptotic bodies in the mucosa. There are also mild infliltrates of mixed inflammatory cells (lymphocytes and macrophages most commonly) in the submucosa and lamina propria.

WB 10-
Morphological diagnosis

1. Branchitis, focal to multifocal or regionally extensive, with single filamental interepithelial bacterial microcolonies
2. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes
3. Cestodes, numerous intraluminal, intestinal
4. Trematode, encysted intramuscular, skeletal, few

WB 11-
Morphological diagnosis
1. Branchitis, focal to multifocal or regionally extensive, with numerous filamental interepithelial bacterial microcolonies
2. Branchitis, moderate, distal, focally extensive, hyperplastic, with numerous intralesional trematodes

WB 12-
Morphological diagnosis
1. Branchitis, diffuse, with few lamellar protozoans
2. Branchitis, mild, distal, focally extensive, hyperplastic, with moderate intralesional trematodes
3. Cestodes, few intraluminal, intestinal

WB 13 -
Morphological diagnosis
1. Branchitis, diffuse, with moderate lamellar protozoans
2. Branchitis, moderate, distal, focally extensive, hyperplastic, with moderate intralesional trematodes
3. Cestodes, few intraluminal, intestinal

WB 14 -
Morphological diagnosis
1. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes
2. Cestodes, few intraluminal, intestinal

WB 15-
Morphological diagnosis
1. Branchitis, focal to multifocal or regionally extensive, with numerous filamental interepithelial bacterial microcolonies
2. Branchitis, moderate, distal, focally extensive, hyperplastic, with few intralesional trematodes
3. Trematode, encysted intramuscular, skeletal
4. Kidney – There are expanded melanomacrophage centers with debris and golden brown pigment (hemosiderin)
5. 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.

WB 19 -
Morphological diagnosis
1. Branchitis, diffuse, with moderate lamellar protozoans

2. Branchitis, moderate, distal, focally extensive, hyperplastic, with intralesional trematodes
3. Trematode, encysted intramuscular, skeletal

WB 20 -
Morphological diagnosis
1. Branchitis, diffuse, with moderate lamellar protozoans
2. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies
3. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes
4. Cestodes, few intraluminal, intestinal
5. Branchitis, multifocal, with few microsporidian cysts
6. 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

WB 21-
Morphological diagnosis
1. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies
2. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes
3. Small granuloma near thymus – no contents

WB 22-
Morphological diagnosis
1. Branchitis, diffuse, with numerous lamellar protozoans
2. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies
3. Branchitis, moderate, distal, focally extensive, hyperplastic.
4. Cestodes, few intraluminal, intestinal
5. Histiocytic focus, skeletal muscle, single
6. Branchitis, multifocal, with few microsporidian cysts

WB 23-
Morphological diagnosis
1. Branchitis, diffuse, with moderate lamellar protozoans
2. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies
3. Branchitis, moderate to severe, distal, focally extensive, hyperplastic, with few intralesional trematodes
4. Cestodes, few intraluminal, intestinal
5. Granuloma, skeletal muscle, single focal

WB 24-
Morphological diagnosis
1. Branchitis, diffuse, with moderate lamellar protozoans
2. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes
3. Cestodes, few intraluminal, intestinal

WB 25-
Morphological diagnosis
1. Branchitis, diffuse, with numerous lamellar protozoans
2. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes
3. Dermatitis (branchial cavity wall) focal, hyperplasitic with intralesional trematode (same as for gills above)
4, Cestodes, few intraluminal, intestinal
5. Granuloma, skeletal muscle, single focal

WB 26-
Morphological diagnosis
1. Branchitis, diffuse, with few lamellar protozoans
2. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies
3. Branchitis, mild to moderate, distal, focally extensive, hyperplastic.
4. Cestodes, few intraluminal, intestinal
5. Stomach – An ingested fish contains a trematode

WB 27-
Morphological diagnosis
1. Branchitis, mild to moderate, distal, focally extensive, hyperplastic, with few intralesional trematodes

WB 28-
Morphological diagnosis
1. Branchitis, diffuse, with numerous lamellar protozoans
2. Branchitis, moderate, distal, focally extensive, hyperplastic
3. Cestodes, few intraluminal, intestinal
4. Stomach – An ingested fish contains a cestode in the intestine
5. Skeletal muscle and skin. 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. This has the appearance of a previous penetrating wound.

WB 29-
Morphological diagnosis
1. Branchitis, diffuse, with few lamellar protozoans
2. Branchitis, moderate, distal, focally extensive, hyperplastic, with numerous intralesional trematodes
3. Cestodes, few intraluminal, intestinal
4. Stomach – There is an ingested fish in which there are numerous sporulating and sporulated myxosporean cysts that distort the lamellae.

WB 30-
Morphological diagnosis

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

WB 31-
Morphological diagnosis
1. Branchitis, diffuse, with few lamellar protozoans
2. Branchitis, mild, distal, focally extensive, hyperplastic

WB 32-
Morphological diagnosis
1. Branchitis, diffuse, with few lamellar protozoans
2. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies
3. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes

WB 33 -
Morphological diagnosis
1. Branchitis, focal to multifocal or regionally extensive, with moderate filamental interepithelial bacterial microcolonies
2. Branchitis, mild to moderate, distal, focally extensive, hyperplastic.
3. Trematode, encysted, cranial connective tissue
4. Intestine – There is a submucosal zone of edema and with inflammatory cells exiting from blood vessel – no organisms are present.

WB 34-
Morphological diagnosis
1. Branchitis, diffuse, with moderate lamellar protozoans
2. Branchitis, focal to multifocal or regionally extensive, with moderate filamental interepithelial bacterial microcolonies
3. Branchitis, mild to moderate, distal, focally extensive, hyperplastic, with few intralesional trematodes

WB 35-
Morphological diagnosis
1. Branchitis, diffuse, with numerous lamellar protozoans
2. Cestodes, few intraluminal, intestinal
3. Liver – There is a single focus of nodular hyperplasia

WB 36-
Morphological diagnosis
1. Branchitis, diffuse, with very numerous lamellar protozoans
2. Branchitis, moderate, distal, focally extensive, hyperplastic, with moderate intralesional trematodes
3. Cestodes, moderate intraluminal, intestinal

4. 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.

WB 37-
Morphological diagnosis
1. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes

WB 38-
Morphological diagnosis
1. Branchitis, mild, distal, focally extensive, hyperplastic.
2. Cestodes, numerous intraluminal, intestinal
3. Mesentery – encysted metazoan – indistinct likely trematode.
4. Cholangiohepatitis, focally extensive, with intraluminal cestodes and with hepatic atrophy
5. Stomach – There is mild submucosal lymphocytic gastritis and esophagitis. There are no organisms present.

WB 39-
Morphological diagnosis
1. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies
2. Lymphohistiocytic focus, skeletal muscle, single

WB 40-
Morphological diagnosis
1. Branchitis, diffuse, with few lamellar protozoans
2. Branchitis, moderate, distal, focally extensive, hyperplastic.
3. Cestodes, few intraluminal, intestinal

WB 41-
Morphological diagnosis
1. Branchitis, diffuse, with few lamellar protozoans
2. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies
3. Branchitis, moderate, distal, focally extensive, hyperplastic, with few intralesional trematodes
4. Cestodes, few intraluminal, intestinal
5. Large metazoan with no discernible anatomic location

WB 42-
Morphological diagnosis
1. Branchitis, diffuse, with few lamellar protozoans
2. Branchitis, moderate, distal, focally extensive, hyperplastic, with moderate intralesional trematodes
3. Histiocytic focus, skeletal muscle, few

4. Mesentery – There is a single ~100 μm granuloma with golden-brown pigment and that contains no visible organisms.

WB 43 -
Morphological diagnosis
1. Branchitis, diffuse, with few lamellar protozoans
2. Cestodes, few intraluminal, intestinal
3. Mild submucosal edema and inflammation as for previous fish
4. Free trematode with no discenible anatomic location
5. Skeletal muscle. There is a large encapsulated metazoan (remnants) with 30-40 cell thick collar of inflammation with macrophages, lymphocytes and few melanomacrophages.

WB 44-
Morphological diagnosis
1. Branchitis, diffuse, with few lamellar protozoans
2. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies
3. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes
4. Cestodes, few intraluminal, intestinal

WB 45-
Morphological diagnosis
1. Cestodes, few intraluminal, intestinal

WB 46-
Morphological diagnosis
1. Branchitis, diffuse, with few lamellar protozoans
2. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies
3. Branchitis, moderate, distal, focally extensive, hyperplastic, with few intralesional trematodes

WB 47-
Morphological diagnosis
1. Branchitis, diffuse, with numerous lamellar protozoans
2. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes
3. Cestodes, few intraluminal, intestinal
4. Branchitis, with single intralesional trematode with haptor (distinct from species with holdfast)
5. 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.

WB 48-
Morphological diagnosis
1. Branchitis, focal to multifocal or regionally extensive, with moderate filamental interepithelial bacterial microcolonies
2. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes
3. Cestodes, few intraluminal, intestinal

WB 49-
Morphological diagnosis
1. Branchitis, moderate to severe, distal, focally extensive, hyperplastic, with numerous intralesional trematodes
2. Trematode, encysted intramuscular, skeletal

WB 50-
Morphological diagnosis
1. Branchitis, diffuse, with few lamellar protozoans
2. Branchitis, moderate, distal, focally extensive, hyperplastic, with numerous intralesional trematodes

WB 51-
Morphological diagnosis
1. Branchitis, diffuse, with few lamellar protozoans
2. Branchitis, focal to multifocal or regionally extensive, with few filamental interepithelial bacterial microcolonies
3. Branchitis, moderate, distal, focally extensive, hyperplastic.

WB 52-
Morphological diagnosis
1. Branchitis, focal to multifocal or regionally extensive, with numerous filamental interepithelial bacterial microcolonies
2. Dermatitis, focal to multifocal or regionally extensive, with numerous filamental interepithelial bacterial microcolonies
3. Branchitis, moderate, distal, focally extensive, hyperplastic.
4. Cestodes, few intraluminal, intestinal
5. Two cestodes with eggs that have no discernible anatomic location

WB 53 -
Morphological diagnosis
1. Branchitis, focal to multifocal or regionally extensive, with few numerous filamental interepithelial bacterial microcolonies
2. Branchitis, moderate to severe, distal, focally extensive, hyperplastic, with moderate intralesional trematodes
3. Cholangiohepatitis, focally extensive, with intraluminal cestodes and with hepatic atrophy
4. Mesenteric metazoan granuloma – no content

WB 54-
Morphological diagnosis
1. Cestodes, few intraluminal, intestinal
2. Gills – Mild multifocal epithelial hyperplasia. Single section of monogenetic trematode
3. Intestine – One section of intestine is notably edematous.

WB 55-

Morphological diagnosis
1. Branchitis, diffuse, with numerous lamellar protozoans
2. Branchitis, focal to multifocal or regionally extensive, with few numerous filamental interepithelial bacterial microcolonies
3. Branchitis, moderate, distal, focally extensive, hyperplastic, with few intralesional trematodes
4. Skin – There is a focal lymphocytic dermatitis. No organisms are present.

WB 56-
Morphological diagnosis
1. Branchitis, moderate, distal, focally extensive, hyperplastic, with numerous intralesional trematodes
2. Cestodes, numerous intraluminal, intestinal

WB 57-
Morphological diagnosis
1. Branchitis, diffuse, with numerous lamellar protozoans
2. Branchitis, focal to multifocal or regionally extensive, with moderate numerous filamental interepithelial bacterial microcolonies
3. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes
4. Branchitis, with single intralesional trematode with haptor (distinct from species with holdfast)
5. Cestodes, few intraluminal, intestinal

WB 58-
Morphological diagnosis
1. Branchitis, diffuse, with numerous lamellar protozoans
2. Branchitis, mild, distal, focally extensive, hyperplastic, with few intralesional trematodes

WB 59-
Morphological diagnosis
1. Branchitis, diffuse, with few lamellar protozoans
2. Branchitis, moderate, distal, focally extensive, hyperplastic, with numerous intralesional trematodes

Spring

WB 1-
Morphological diagnosis
1. Branchitis, multifocal, with numerous lamellar protozoans

WB 2-
Morphological diagnosis
1. Branchitis, multifocal, with mild lamellar protozoans

WB 3- NAF

WB 4- NAF

WB 5- NAF

WB 6-
Morphological diagnosis
1. Branchitis, multifocal, with mild lamellar protozoans

WB 7-
Morphological diagnosis
1. Epicarditis, focal, mild
2. Cestodes, few intraluminal, intestinal with diffuse mild to moderate submucosal enteritis

WB 8-
Morphological diagnosis
1.Dermatitis, focal, mild

WB 9-
Morphological diagnosis
1. Branchitis, multifocal, mild
2. Myocarditis, focal, mild to moderate

WB 10-
Morphological diagnosis
1. Cestode, intraluminal, intestine

WB 11-
Morphological diagnosis
1. Branchitis, multifocal, with few lamellar protozoans

WB 12-
Morphological diagnosis
1. Branchitis, multifocal, with few lamellar protozoans
2. Branchitis, focal to multifocal or regionally extensive, with filamental inter-epithelial bacterial microcolonies

WB 13– No fish / no slide

WB 14- NAF

WB 15-
Morphological diagnosis
1.Myocarditis, focal, mild

WB 16-
Morphological diagnosis

1. Nematodiasis, intraluminal, multiple

WB 17-
Morphological diagnosis
1. Branchitis, focally extensive, hyperplastic, with intralesional trematodes

WB 18- NAF

WB 19 -
Morphological diagnosis
1. Hepatitis, multifocal, mild with calcification

WB 20 -
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate
2. Nephrocalcinosis, multifocal, mild

WB 21-
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate
2. Epicarditis, multifocal, mild
3. Myocarditis, multifocal, mild
4. Cestodes, intraluminal, intestinal

WB 22-
Morphological diagnosis
1. Branchitis, multifocal, with moderate numbers of lamellar protozoans
2. Enteritis, granulomatous, submucosal, focal

WB 23-
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate
2. Myocarditis, multifocal, mild
3. Encephalitis, focal, mild

WB 24-
Morphological diagnosis
1. Branchitis, multifocal, mild to moderate

WB 25-
Morphological diagnosis
1. Branchitis, multifocal, moderate

WB 26-
Morphological diagnosis
1. Branchitis, multifocal to focally extensive, with few lamellar protozoans

2. Epicarditis, multifocal, mild
3. Cestodes, few intraluminal, intestinal

WB 27-
Morphological diagnosis
1. Branchitis, multifocal to focally extensive, with few lamellar protozoans
2. Hepatitis, granulomatous, multifocal, with intralesional metazoan
3. Cestodes, few intraluminal, intestinal

WB 28-
Morphological diagnosis
1. Branchitis, multifocal to focally extensive, with few lamellar protozoans
2. Epicarditis, multifocal, mild

WB 29-
Morphological diagnosis
1. Branchitis, multifocal to focally extensive, with few lamellar protozoans

WB 30-
Morphological diagnosis
1. Branchitis, multifocal to focally extensive, with few lamellar protozoans
2. Branchitis, focal to multifocal or regionally extensive, with filamental interepithelial bacterial microcolonies
3. Cestodes, few intraluminal, intestinal

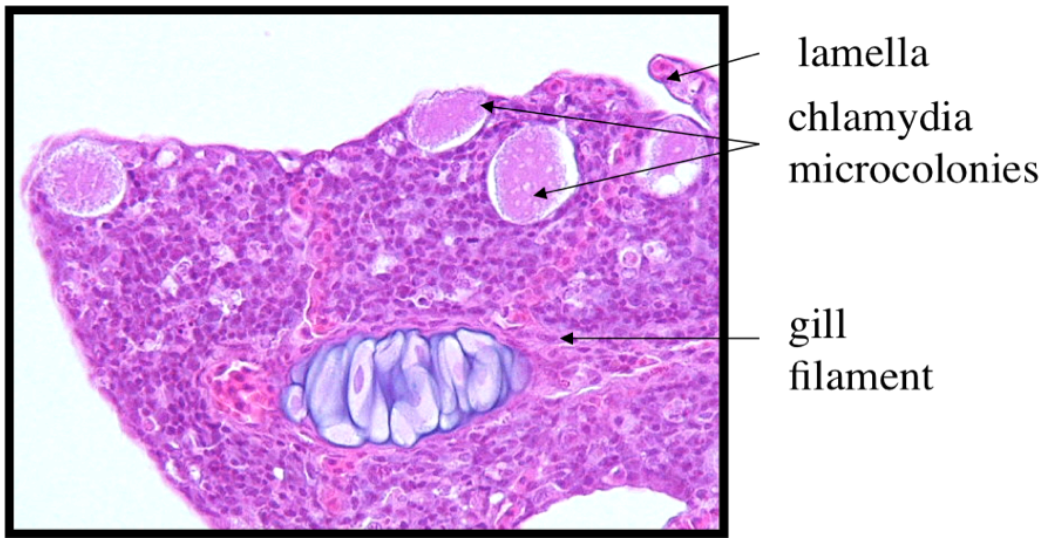
Table 10. Summary of notable lesions and organisms in the yellow perch (*Perca flavescens* – YP) collected during the summer of 2006 (n=60) and the spring of 2007 (n=30) from Lake Winnipeg and surrounding waters.

Yellow Perch (*Perca flavescens*) Tissue/agent	Summer 2006 – Sixty fish	Spring 2007 – Thirty fish
Gills	1. Epitheliocystis (4/60) 2. *Caprinia* sp. (12/60)	1. Epitheliocystis (3/30) 2. *Caprinia* sp., *Trichodina* sp. (15/30)
Stomach/ Intestine/ peritoneum	1. Intestinal mural nematodes (2/60) 2. Peritoneal nematodes (4/60) 3. Intestinal cestode (17/60) 4. Intestinal protozoan (apicomplexan) (1/60)	1. Intestinal mural trematode (1/30) 2. Intestinal nematode (luminal) (1/30) 3. Peritoneal (and sb) trematode (2/30) 4. Intestinal cestode (5/30)
Skeletal muscle and skin	1. Dermal *Trichodina* sp. (5/60) 2. Muscular trematode (3/60) 3. Muscular (also peritoneal) trematode (3/60) 4. Dermal larval metazoan (embryo?) (1/60)	
Brain and meninges	1. Peri-chondritis (1/60) 2. Spinal cord myxosporean (1/60)	1. Spinal cord myxosporean (2/30)
Liver and bile ducts	1. Myxosporean, likely *Myxobolus* sp. (3/60) 2. Trematode (1/60)	
Kidney	1. Myxosporean, ureteral (6/60)	

The tissues 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. Not all tissues are present on each slide examined.

Lesions or organisms noted in both summer and spring.

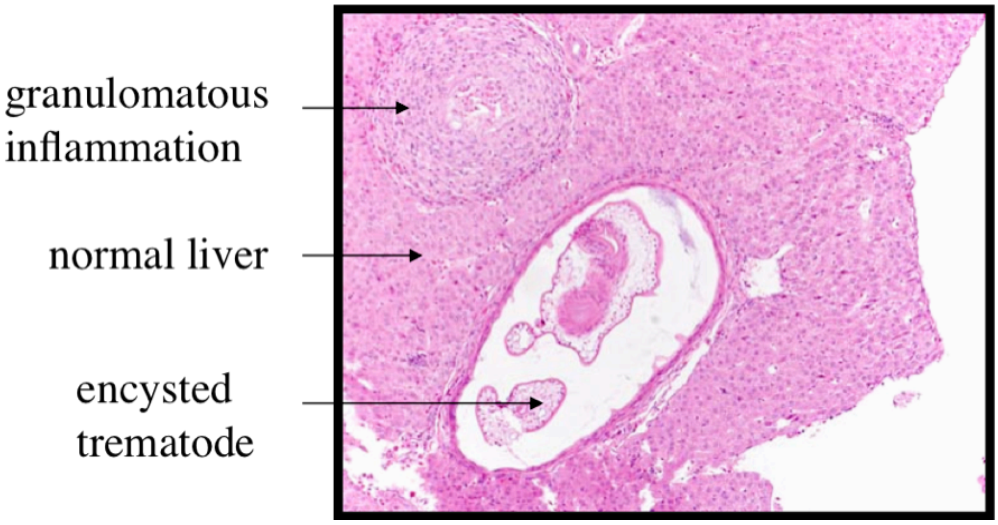
Branchial Epitheliocystis - Branchitis, focal to multifocal, with filamental interepithelial bacterial microcolonies (chlamydia-like bacteria).
Along scattered filaments, There are single to several closely spaced ~25 - 50 μm tightly-packed basophilic foci with a thin ~2-3 μm eosinophilic limiting membrane that delineates each microcolony. The microcolony is filled with ~1 μm (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. Summer ---- 4 of 60 affected. Spring ---- 3 of 30 affected.



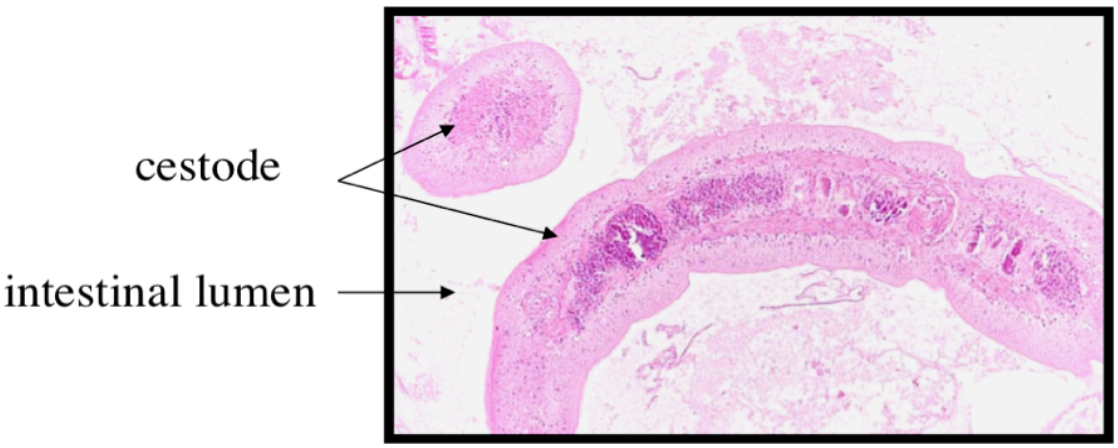
Intramuscular and peritoneal encysted trematodes – Trematodiasis, intramuscular and peritoneal, encysted.
Encysted by few to several layers of thin attenuated macrophages there are solitary to multifocal sections of a trematode parasite within skeletal myofibers of the body wall and within connective 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). Summer ---- 3 of 60 affected. Spring ---- 1 of 30 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.

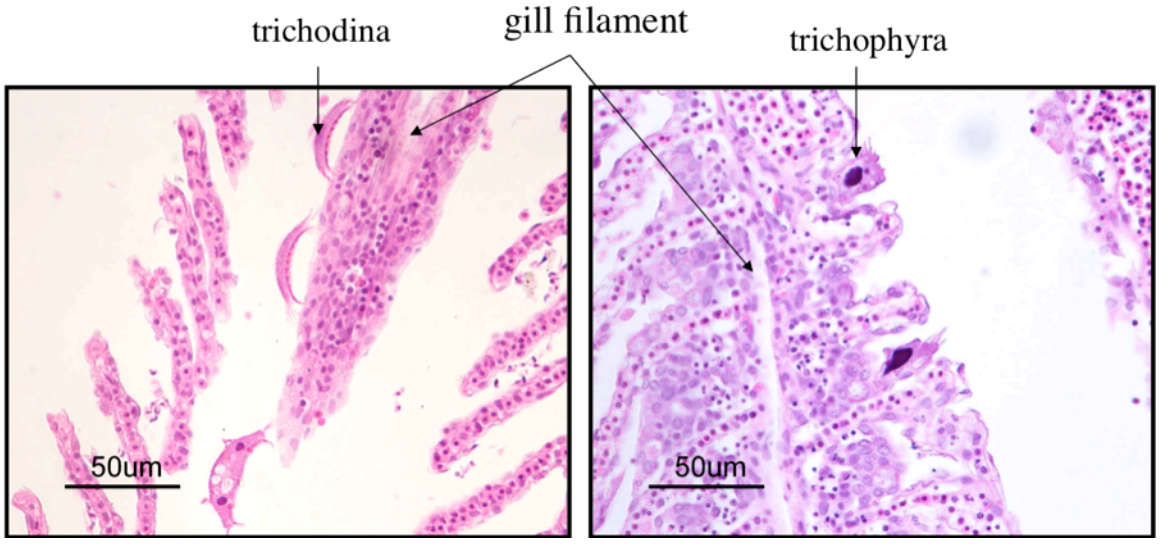


Intestinal cestodes – Cestodes, intraluminal, intestinal
There are multiple sections of a metazoan organism filling the lumen of the gastrointestinal tract of numerous fish. The metazoan is characterized by an eosinophilic tegument thrown into regularly spaced folds (proglottids), a lack of digestive 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. Summer ---- 17 of 60 affected. Spring ---- 5 of 30 affected.

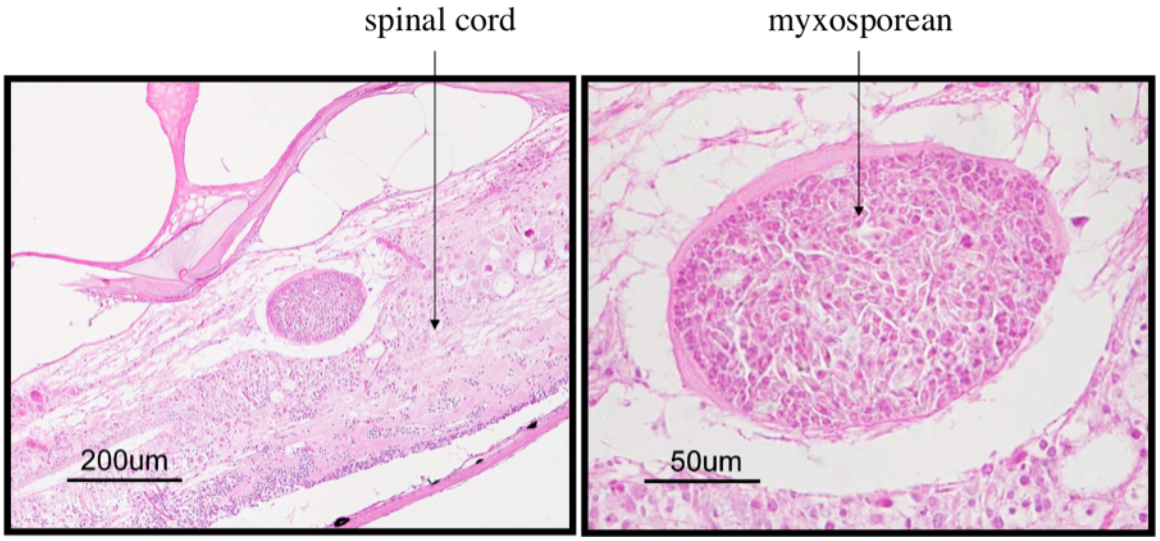


Branchial protozoan - Protozoa (*Caprinia* species), branchial, multifocal.
There are small numbers of protozoan organisms attached to lamellae of scattered filaments. The shape of the protozoan is variable but more it is often elongate (45 x 90 µm) or sac-like with a centrally located nucleus and numerous 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*). There are

also small to very numerous ~25 - 50 µm dorsoventrally flattened protozoans with a chitinous endoskeleton (denticular ring) and a 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. Summer ---- 12 of 60 affected. Spring ---- 15 of 30 affected.



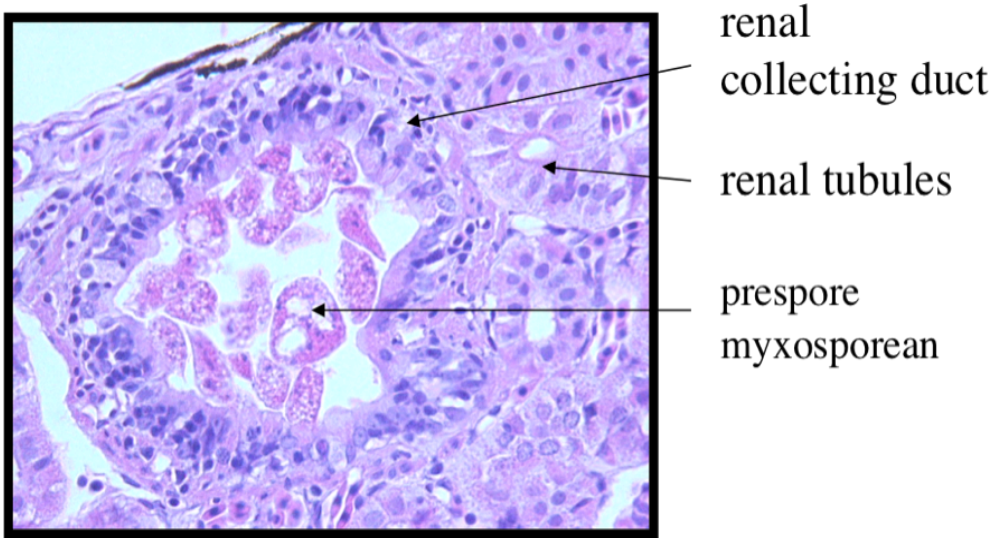
Spinal column myxosporean cyst - Myxosporidiosis, intra-spinal, encysted.
Single and multiple, approximately 100 to 150 µm in diameter, myxosporean cysts containing both immature multinucleate plasmodium and mature spores are encysted within the neuropil of the spinal cord. (Phylum Myxozoa, Class Myxosporea). Summer ---- 1 of 60 affected. Spring ---- 2 of 30 affected.



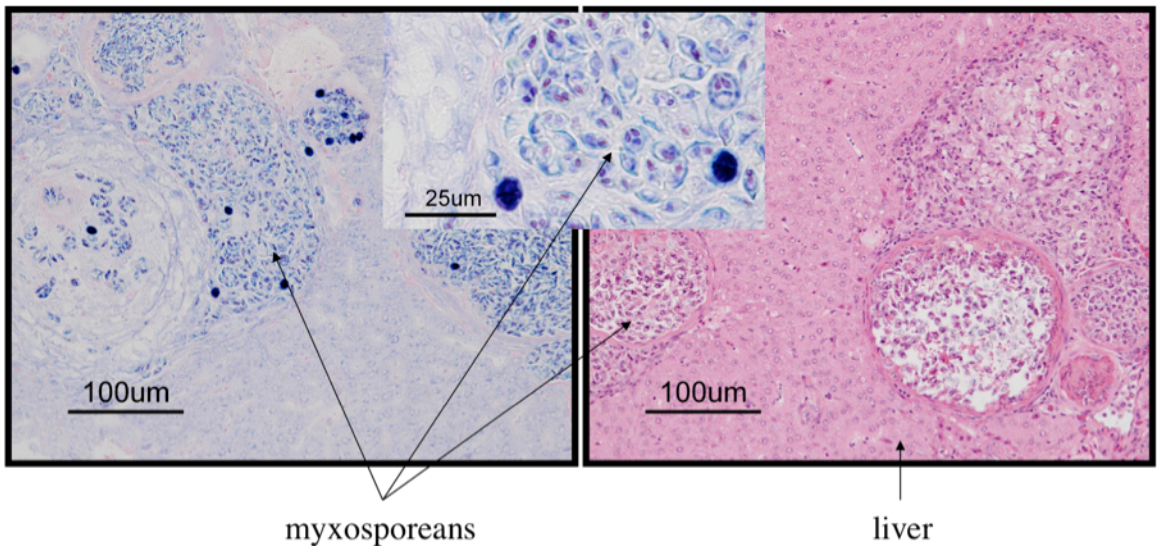
Lesions or organisms only noted in summer.

Intestinal Apicomplexans - Enteritis, multifocal, mild with intralesional 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)

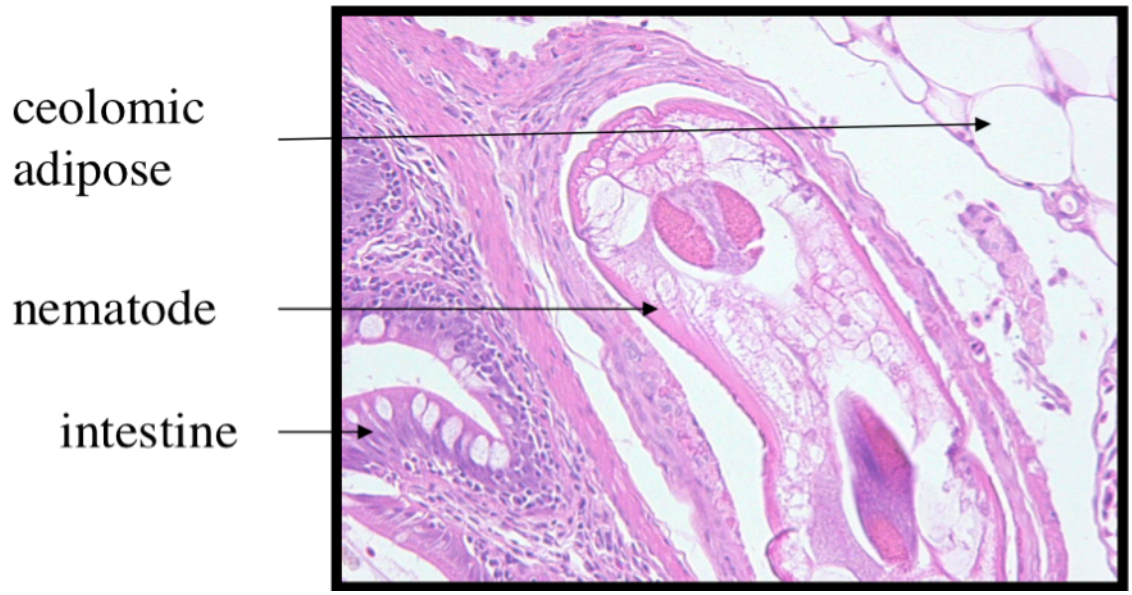
Renal myxosporidiosis – Myxosporidiosis, renal.
Within the lumen of both ureters of the caudal kidney, there are low to moderate numbers of multinucleated plasmodium-like protozoan (~ 15 to 25 µm in diameter) organisms. There are increased numbers of mononuclear cells and lesser numbers of eosinophilic 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.



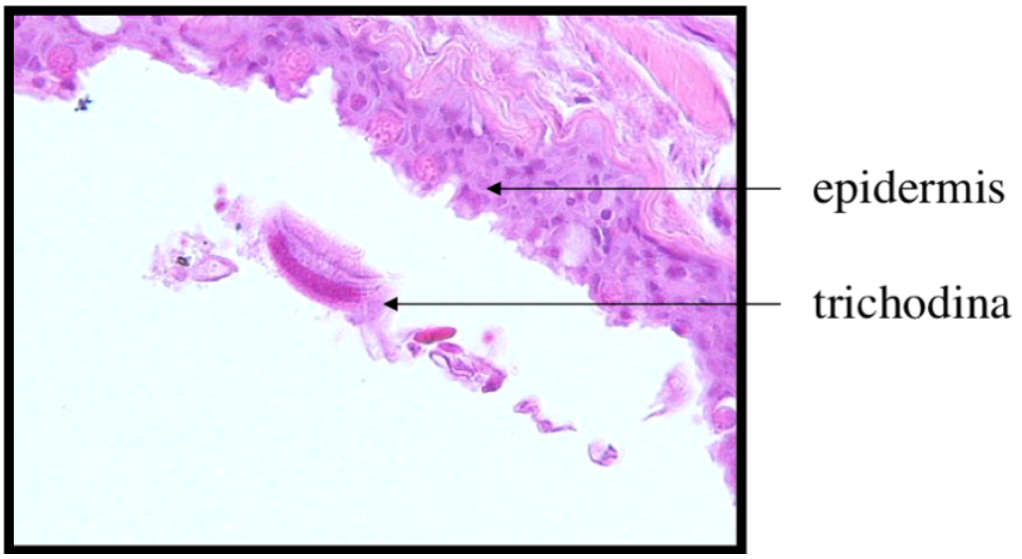
Hepatic myxosporidiosis - Myxosporidiosis, hepatic, multifocal.
There are multiple small and large accumulations of pyriform myxosporean spores (~ 10 µm in length) embedded within hepatic parenchyma and around portal triads. The spores are surrounded by several layers of thin macrophages admixed with 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, Family Myxobolidae, presumptive *Myxobolus* sp.) ---- 3 of 60 affected. Giemsa stain at left and middle, below.



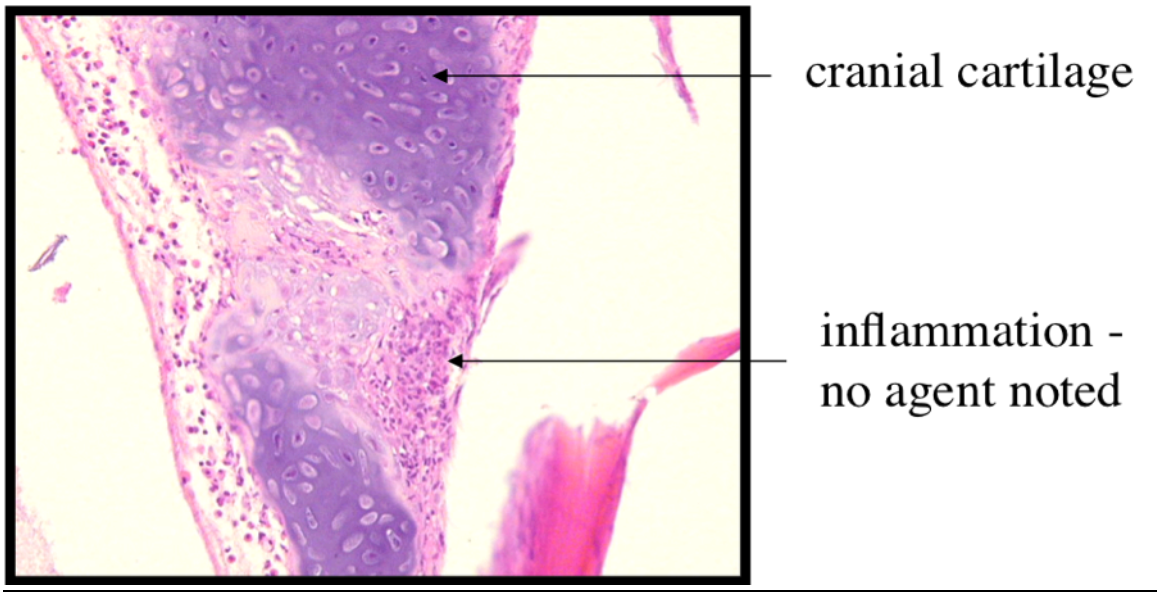
Intestinal encysted nematodes – Nematodiasis, intestinal wall, encysted.
Encysted within longitudinal muscle of the gut, there are focal and multifocal cross-sections of a metazoan parasite characterized by the presence of a digestive tract, psuedocoelom and cuticle (Phylum Nemathelminthes, Class Nematoda).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



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.

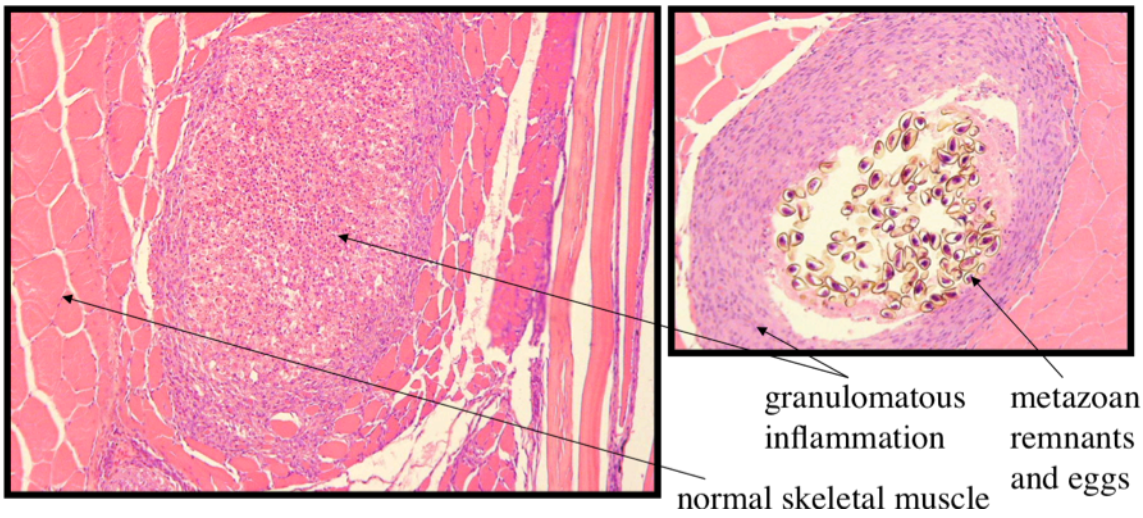


Cranial Chondritis – Perichondritis, multifocal, mild.
Within sections of the capitus, there is multifocal chondrocyte degeneration associated with small numbers of macrophages and lesser numbers of lymphocytes and granulocytic cells. Increased numbers of macrophages, lymphocytes and lesser numbers of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



Intramuscular granuloma / encysted trematodes – Myositis, granulomatous, multifocal, mild to moderate with occasional sections of an intralesional metazoan, likely trematode.
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 trematode characterized by an

eosinophilic tegument, a lack of digestive tract embedded within a loose parenchymatous matrix, is present within the center of the granuloma. ---- 3 of 60 affected.



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)

Lesions or organisms only noted in spring.

Intestinal nematodiasis – Nematodes, intraluminal, intestine.
There is a single cross-section of a nematode organism between villi of the distal intestine. The nematode is characterized by a thick eosinophilic and ridged cuticle and muscular pharynx. (Phylum Nemathelminthes, Class Nematoda). There is mild to moderate enteritis within sections of intestine surrounding the nematode. ---- 1 of 30 affected. (no photo)

Peritoneal/swim bladder metazoan, suspect trematodes – Trematodiasis, peritoneal, encysted.
Embedded within the connective tissue underlying the swim bladder, there are cross sections of 100 to 200 μm, encysted trematodes. The trematodes are characterized by a thin tegument surrounding a layer of sub-tegumental cells, a loose parenchyma and muscular oral sucker. Several layers of plump amphophilic macrophages and low numbers of eosinophilic granular cells surround both metazoans. (Phylum Nemathelminthes, Class Trematoda) ---- 2 of 30 affected. (no photo)

Summaries of lesions or organisms noted in individual fish.

Summer

<u>YP 1</u> –

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, intraluminal, intestinal
2. Myositis, granulomatous, focal
3. Trichodinosis, dermal, small numbers

YP 2 –
Morphological diagnosis
1. Epitheliocystis, multifocal, mild

YP 3 – NAF

YP 4 - NAF

YP 5 –
Morphological diagnosis
1. Cestodes, intraluminal, intestinal

YP 6 –
Morphological diagnosis
1. Cestodes, intraluminal, intestinal
2. Protozoa (*Caprinia* species, formerly *Trichophyra* species), branchial, multifocal, small numbers

YP 7 -
Morpological diagnosis
1. Myositis, granulomatous, multifocal, mild with intralesional cestode
2. Branchitis, mild, multifocal

YP 8 –
Morphological diagnosis
1. Protozoa (*Caprinia* species), branchial, multifocal, small numbers
2. Branchitis, moderate, multifocal with epithelial hyperplasia and multifocal single cell necrosis.
3. Trichodinosis, dermal, low numbers

YP 9 -
Morphological diagnosis
1. Perichondritis, mild, multifocal

YP 10 - NAF

YP 11 –
Gill – There is regionally extensive filling of interlamellar 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
1. Cestodes, intraluminal, intestinal
2. Granulomas, multifocal, peritoneal, parasitic
3. Branchitis, multifocal, mild to moderate

YP 14 –
Morphological diagnosis

2. Branchitis, regionally extensive, mild to moderate, histiocytic and eosinophilic with multifocal epithelial hyperplasia and single cell necrosis
3. Myxosporidiosis, hepatic, multifocal, large numbers

YP 15 –
Morphological diagnosis

1. Branchitis, multifocal, mononuclear and granulocytic, moderate numbers.
2. Protozoa (*Caprinia* species), branchial, multifocal, small numbers
3. Cestodes, intraluminal, intestinal
4. Nematodiasis, intestinal wall, encysted
5. Myxosporidiosis, renal, small numbers
6. Enteritis, multifocal, mild with intralesional accumulations of an intracellular protozoan organism
7. Epicarditis, lymphocytic, focal, mild

YP 16 -
Morphological diagnosis

1. Epitheliocystis, focal
2. Protozoa (*Caprinia* species), branchial, multifocal, small numbers
3. Cestodes, intraluminal, intestinal

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 intralesional enterocyte degeneration.

2. Splenitis, multifocal, mild
3. Trichodinosis, branchial, solitary

YP 18 -
Morphological diagnosis

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

YP 19
Morphological diagnosis

1. Cestodes, intraluminal, intestinal
2. Protozoa (*Caprinia* species), branchial, multifocal, small numbers

YP 20 -
Morphological diagnosis

1. Myxosporidiosis, multifocal, hepatic, large numbers

YP 21 –
Morphological diagnosis

1. Myxosporidiosis, renal, small numbers
2. Cestodes, intraluminal, intestinal

YP 22 -
Morphological diagnosis

1. Branchitis, multifocal, mild
2. Cestodes, intraluminal, intestinal

YP 23 -
Morphological diagnosis

1. Protozoa (*Caprinia* species), branchial, multifocal, small numbers

YP 24 -
Morphological diagnosis

1. Branchitis, multifocal, interlamellar, mild with scattered lamellar synechae
2. Cestodes, intraluminal, intestinal

YP 25 -
Morphological diagnosis

1. Protozoa (*Caprinia* species), branchial, multifocal, small numbers

YP 26 –
Morphological diagnosis

1. Branchitis, multifocal, mild.
2. Protozoa (*Caprinia* species), branchial, multifocal, small numbers

YP 27 -

Morphological diagnosis

1. Cestodes, intraluminal, intestinal

YP 28 -

Morphological diagnosis

1. Branchitis, multifocal, mild to moderate
2. Protozoa (*Caprinia* species), branchial, multifocal, small numbers
3. Myxosporidiosis, renal, small numbers

YP 29 –

Morphological diagnosis

1. Branchitis, multifocal, mild.

YP 30 –

Morphological diagnosis

1. Cestodes, intraluminal, intestinal
2. Nematodiasis, intestinal wall, encysted

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
2. Myositis, multifocal, mild with occasional myocyte degeneration

YP 32

Morphological diagnosis

1. Cestodes, intraluminal, intestinal
2. Protozoa (*Caprinia* species), branchial, multifocal, small numbers
3. Nematodiasis, peritoneal, encysted

YP 33

Morphological diagnosis

1. Trematodiasis, hepatic, multifocal
2. Myxosporidiosis, renal, moderate numbers
3. Branchitis, multifocal mild
4. Protozoa (*Caprinia* species), branchial, multifocal, small numbers

YP 34

Morphological diagnosis

1. Protozoa (*Caprinia* species), branchial, multifocal, small numbers

YP 35

Morphological diagnosis

1. Trematodiasis, intramuscular, solitary
2. Protozoa (*Caprinia* species), branchial, multifocal, small numbers

YP 36
Morphological diagnosis
1. Protozoa (*Caprinia* species), branchial, multifocal, small numbers
2. Myxosporidiosis, renal, moderate numbers

YP 37
Morphological diagnosis
1. Protozoa (*Caprinia* species), branchial, multifocal, small numbers

YP 38
Morphological diagnosis
1. Myxosporidiosis, intra-spinal, solitary
2. Branchitis, multifocal, mild

YP 39
Morphological diagnosis
1. Cestodes, intraluminal, intestinal
2. Nematodiasis, intestinal wall, encysted
3. Myxosporidiosis, renal, moderate numbers
4. Branchitis, multifocal, mild

YP 40
Morphological diagnosis
1. Branchitis, multifocal mild with occasional intralesional foci of necrosis
2. Trichodinosis, dermal, small numbers

YP 41
Morphological diagnosis
1. Protozoa (*Caprinia* species), branchial, multifocal, moderate numbers

YP 42
Morphological diagnosis
1. Nematodiasis, peritoneal, encysted, multifocal

YP 43
Morphological diagnosis
1. Protozoa (*Caprinia* species), branchial, multifocal, moderate numbers
2. Branchitis, multifocal mild with occasional intralesional foci of necrosis
3. Epicarditis, lymphocytic, focal, mild

Slide 44
Morphological diagnosis
1. Nematodiasis, peritoneal, encysted, multifocal
2. Splenitis, histiocytic, multifocal, mild.

YP 45
Morphological diagnosis
1. Trichodina, solitary, oral cavity
2. Protozoa (*Caprinia* species), branchial, multifocal, small numbers
3. Cestodes, intraluminal, intestinal

YP 46
Morphological diagnosis
1. Cestodes, intraluminal, intestinal
2. Protozoa (*Caprinia* species), branchial, multifocal, small numbers

YP 47
Morphological diagnosis
1. Branchitis, multifocal with moderate to marked interlamellar 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 (presumptive)

YP 49
Morphological diagnosis
1. Cestodes, intraluminal, intestinal

YP 50 - NAF

YP 51
Morphological diagnosis
1. Protozoa (*Caprinia* species), branchial, multifocal, small numbers
2. Branchitis, multifocal, mild.
3. Nematodiasis, peritoneal, encysted, multifocal

YP 52
Morphological diagnosis
1. Myxosporidiosis, renal, moderate numbers
2. Protozoa (*Caprinia* species), branchial, multifocal, small numbers
3. Trichodinosis, small numbers
4. Branchitis, multifocal, mild
5. Cestodes, intraluminal, intestinal

YP 53
Morphological diagnosis
1. Protozoa (*Caprinia* species), branchial, multifocal, small numbers
2. Branchitis, multifocal, mild

YP 54
Morphological diagnosis
1. Branchitis, multifocal, mild
2. Nematodiasis, peritoneal, encysted, multifocal

YP 55
Morphological diagnosis
1. Nematodiasis, peritoneal, encysted, solitary
2. Epitheliocystis, multifocal
3. Branchitis, multifocal, mild
4. Myxosporidiosis, hepatic, multifocal
5. Myxosporidiosis, renal, multifocal

YP 56
Morphological diagnosis
1. Branchitis, regionally extensive, mild
2. Myxosporidiosis, renal, multifocal
3. Myositis, histiocytic, focal with intralesional myocyte degeneration
4. Granuloma, parasitic, enteric, encysted
5. Trematodiasis, *intramuscular*, encysted

YP 57
Morphological diagnosis
1. Myxosporidiosis, renal, multifocal
2. Trematodiasis, intramuscular, encysted
3. Trichodinosis, branchial, low numbers

YP 58
Morphological diagnosis
1. Branchitis, multifocal, mild
2. Trematodiasis, peritoneal, encysted
3. Cestodiasis, enteric
4. Myositis, histiocytic, multifocal, mild with intralesional myocyte degeneration
5. Splenitis, histiocytic, multifocal, mild with intralesional foci of cellular degeneration and necrosis

YP 59
Morphological diagnosis.
1. Cestodes, intraluminal, intestinal

YP 60

Morphological diagnosis.

1. Cestodes, intraluminal, intestinal

Spring.

YP 1 -
Morphological diagnosis
1. Branchitis, multifocal, mild

YP 2 –
Morphological diagnosis
1. Branchitis, multifocal, mild with numerous lamellar protozoans

YP 3 –
Morphological diagnosis
1. Branchitis, multifocal, mild

YP 4 -
Morphological diagnosis
1. Branchitis, multifocal, mild with numerous lamellar protozoans

YP 5 –
Morphological diagnosis
1. Branchitis, multifocal, mild with numerous lamellar protozoans

YP 6 –
Morphological diagnosis
1. Branchitis, multifocal, mild with numerous lamellar protozoans

YP 7 -
Morphological diagnosis
1. Branchitis, multifocal, mild with numerous lamellar protozoans
2. Stomatitis, regionally extensive, mild to moderate

YP 8 –
Morphological diagnosis
1. Branchitis, multifocal, mild with low numbers of lamellar protozoans
2. Enteritis, granulomatous, submucosal, focal
3. Cellulitis and myositis, cranial, focal, moderate

YP 9 -
Morphological diagnosis
1. Branchitis, multifocal, mild

YP 10 -

Morphological diagnosis
1. Branchitis, multifocal, mild with numerous lamellar protozoans

YP 11 –
Morphological diagnosis
1. Branchitis, multifocal, mild with numerous lamellar protozoans
2. Cestodes, intraluminal, intestinal

YP 12 -
Morphological diagnosis
1. Peritonitis, regionally extensive, moderate to marked

YP 13 –
Morphological diagnosis
1. Dermatitis, focal, mild to moderate

YP 14 – NAF

YP 15 –
Morphological diagnosis
1. Granuloma, parasitic, peritoneal

YP 16 -
Morphological diagnosis
1. Cestodes, intraluminal, intestinal

YP 17 –
Morphological diagnosis
1. Branchitis, multifocal, mild with numerous lamellar protozoans
2. Cestodes, intraluminal, intestinal

YP 18 -
Morphological diagnosis
1. Branchitis, multifocal, mild with low numbers of lamellar protozoans
2. Cestodes, intraluminal, intestinal
3. Trematodiasis, peritoneal, encysted

YP 19
Morphological diagnosis
1. Branchitis, multifocal, mild

YP 20 - NAF

YP 21 – NAF

YP 22 -
Morphological diagnosis
1. Branchitis, multifocal, mild with low numbers of lamellar protozoans
2. Myxosporidiosis, intra-spinal, encysted, focal

YP 23 -
Morphological diagnosis
1. Branchitis, multifocal, mild with low numbers of lamellar protozoans
2. Trematodiasis, peritoneal, encysted

YP 24 -
Morphological diagnosis
1. Branchitis, multifocal, mild with low to moderate numbers of lamellar protozoans

YP 25 –
Morphological diagnosis
1. Nematodes, intraluminal, intestine

YP 26 –
Morphological diagnosis
1. Branchitis, focal to multifocal, with filamental interepithelial bacterial microcolonies
2. Cestodes, intraluminal, intestinal

YP 27 -
Morphological diagnosis
1. Branchitis, multifocal, with filamental interepithelial bacterial microcolonies
2. Myxosporidiosis, intra-spinal, encysted, multifocal
3. Trematodiasis, intestinal wall, encysted

YP 28 -
Morphological diagnosis
1. Branchitis, multifocal, mild with low to moderate numbers of lamellar protozoans

YP 29 –
Morphological diagnosis
1. Branchitis, multifocal, with filamental interepithelial bacterial microcolonies
2. Branchitis, multifocal, mild with low to moderate numbers of lamellar protozoans

YP 30 –
Morphological diagnosis
1. Branchitis, multifocal, mild with low to moderate numbers of lamellar protozoans

Lake Winnipeg Disease Surveillance Program 2006/2007
University of Guelph, Department of Pathobiology
& Department of Fisheries and Oceans, Freshwater Institute, Winnipeg.

STANDARD OPERATING PROCEDURES

Histology, Bacteriology and Virology Sampling and Sample Submission Protocol.

Procedure:

1. Make sure that you are wearing all the protective clothing, including: lab coat, nitrile gloves, closed-toed shoes & protective eyewear when working with formalin.
2. Label one BHIA or TSA plate, one container of appropriate size for each fish filled with prepared neutral 10% buffered formalin (9:1, formalin to fish ratio), printed microscope slides (2 wells per fish) and one 2oz Whirlpack bag for a 5 fish pool (1 species per bag). Fish species and number must be labelled using the following code: Goldeye, G1-60; Fathead minnow, FM1-60; Channel catfish, CC1-60; Walleye, W1-60; Sauger, S1-60; White Bass, WB1-60; Yellow Perch, YP1-60; Emerald Shinner, ES1-60; Brook Stickleback, BS1-60. All labels must include the species, fish number and the date.
3. Lay out 2-3 sheets of paper towel in a tray, spray with 70% isopropyl alcohol and place the fish on top of the paper towel along the length of the tray. Spray the fish with 70% isopropyl alcohol and wipe the excess alcohol.
4. Dip scissors in a 50mL beaker half filled with 70% isopropyl alcohol and flame over the Bunsen burner.
5. Pinch the fish's abdomen just anterior to the vent and make a transverse cut with the scissors in front of the vent. Place the blunt end of the scissors in the incision and continue to cut along the midline toward the head stopping once you've reached the heart chamber.
6. Sterilize forceps as previously done with scissors. Holding the top skin flap with the forceps, bring the scissors back to the original incision point and cut toward the dorsal side of the fish, outlining the peritoneal cavity to remove the skin flap.
7. Cut the lower intestine at the vent and remove the viscera from the peritoneal cavity. Flame a scalpel blade and sear a small section of the kidney. With a sterile scalpel make a small incision in the kidney. Flame the inoculation loop, let cool and move from side to

side inside the incision without contaminating by touching anything other than the inside of the kidney.

8. Streak a BHIA or TSA agar plate and perform two dilutions by flaming the loop, letting cool and running over the previous dilution once or twice.
9. Using a synthetic cotton swab, vigorously rub over a small section of the mid-kidney. Transfer a fine film of cells from the kidney over two adjacent wells of printed glass microscope slides. Allow the slides to air dry then place inside a plastic slide box.
10. Remove a maximum of 0.5g of kidney and spleen from each fish and pool 5 fish samples in a single Whirlpack bag. Using a scalpel, make two transverse incisions in the kidney, delineating the sample area and scoop the sample out with the scalpel blade. Hold the spleen with forceps and use the scissors to extract the appropriate sized sample.
11. The procedures for histology will vary depending on the size of the fish. Use the following guidelines: For fish < 22cm, make an incision along the midline to expose the viscera and place the fish whole in a labelled clear plastic container with formalin. For fish >22cm, first remove the operculum and collect a gill arch, also collect samples of anterior kidney, posterior kidney, liver, heart, skin & muscle, intestine, pyloric cecae, stomach, spleen, eye, brain, swim bladder and gonads and place in a labelled clear plastic container with formalin. Leave tissues to fix for a minimum of 24 hours.
12. Virology and bacteriology samples must be shipped within 24 hours of sampling, histology can be shipped as the tissues are fixed (after 24 hours minimum) and kidney smears can be shipped in a single slide box at the end of the sampling period. See the packing and shipping protocol for further details on these procedures.

Packaging and Shipping of Histology, Bacteriology and Virology Samples.

Procedure:

1. Make sure that you are wearing all the protective clothing, including: lab coat, nitrile gloves, closed-toed shoes & protective eyewear when working with formalin.
2. All of the work with formalin fixed tissues must be conducted under a high ventilation chemical fume hood.
3. Label 3 Ziplock bags per fish using the following code: Goldeye, G1-60; Fathead minnow, FM1-60; Channel catfish, CC1-60; Walleye, W1-60; Sauger, S1-60; White Bass, WB1-60; Yellow Perch, YP1-60; Emerald Shinner, ES1-60; Brook Stickleback, BS1-60. All labels must include the fish species, number and date.
4. Separate fixed tissues from formalin by using a tissue-screening funnel over the formalin waste container and pouring the contents of your tissue container.
5. Place paper towel on a tray and soak with 95% isopropyl alcohol or ethanol.
6. Place fixed tissues at the center of the paper towels and gently wrap the tissues, covering all exposed surface area.
7. Place individual fish in appropriately labelled bags (all fish must be triple bagged). All bags with fish must be combined into a regular garbage bag sealed with a tie wrap.
8. Place the bag of fish inside a hard-sided cooler with a list of all fish being shipped in that particular cooler. Seal the cooler lid with duct tape and place the appropriate hazard stickers and waybill on the cooler.
9. Seal the slide box containing kidney smears with tape.
10. Seal individual bacteriology plates with parafilm then place 20 plates in a sleeve. Wrap each sleeve in two layers of bubble wrap.
11. Pack both the slide box and bacteriology plates in the same cooler but separate these with a divider. Fill the excess space inside the cooler with foam or paper filler.
12. Seal the cooler lid with duct tape and place the appropriate hazard stickers and waybill on the cooler.
13. Place all Whirlpack bags with virology samples in a single large Ziplock bag. Place the virology samples in a small cooler with a divider; have the samples on one side and ice packs on the other in order to avoid direct sample contact with ice.

14. Seal the cooler lid with duct tape and place the appropriate hazard stickers and waybill on the cooler.
15. For treatment of 10% formalin waste: Collect formalin waste in the designated 50L formalin reaction container. Add 1L of Formalex to each 4L of formalin waste collected (i.e. fill the 50L formalin waste carboy to the 40L mark and top up with Formalex to the 50L mark). Secure lid on reaction container and agitate container to mix solutions. Mixed solution will become cloudy and/or turbid in 30 minutes to 3 hours. When cloudy solution settles to the bottom of the reaction container, the treatment is complete. Agitate or invert reaction container to mix treated solution with non-liquid headspace area in the reaction container. Carefully pour waste solution in to sanitary sewer, with cold running tap water. Rinse container thoroughly with cold tap water preparing it for reuse.

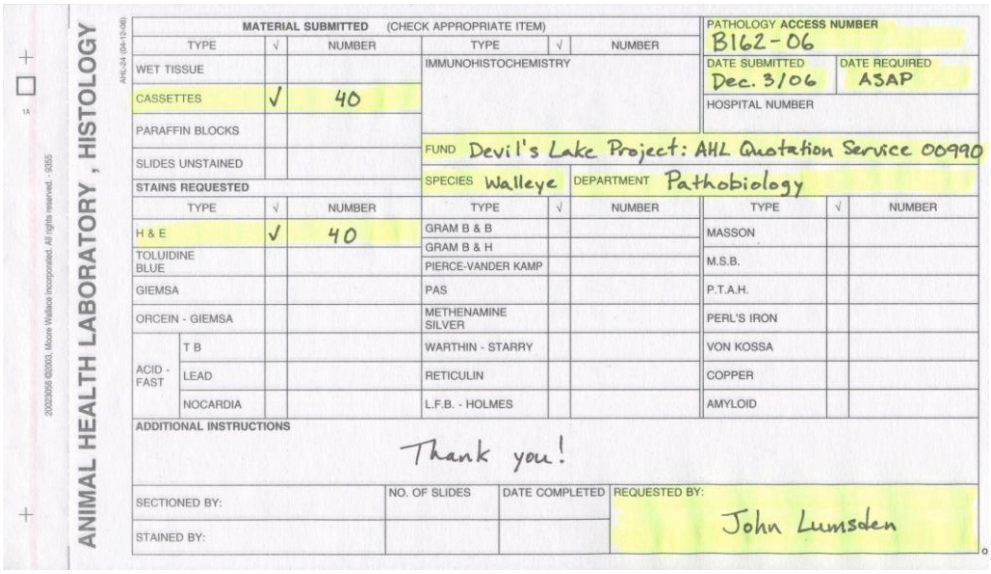
Tissue Trimming Protocol.

Procedure:

1. Prepare the fume hood for trimming:
    a. Put a pair of gloves on and keep on at all times when handling anything that has come into contact with formalin.
    b. Put a handful of blue-green cassettes on a paper plate and set inside the fume hood.
    c. Place a jar of clean formalin and a fresh jar of decal inside the fume hood.
    d. Place specimen bags inside the fume hood.
    e. Place a cutting board, a few razor blades and scalpel blades, forceps, a scalpel handle and pointed scissors inside the fume hood.
2. Bring the glass panel down so that there is sufficient ventilation (check the negative pressure with a piece of paper) and room to work comfortably with your hands inside the fume hood.
3. Open the bags with the fixed fish tissues and remove tissues from the paper towel wrapping.
4. Separate tissues into like groups (bits of liver together, kidney together, etc).
5. The following tissues must be submitted for histology: gills, eye, heart, kidney, spleen, intestine, pyloric cecae, stomach, brain, liver, gonads, skin/muscle and swim bladder. Using a sharp scalpel, cut a cross-section of each tissue type that is approximately 3mm thick. The gill arches can be sectioned to a reasonable size and laid flat at the bottom of cassettes. Keep the cut as clean as possible, using a single cutting motion rather than a sawing action.
6. Place tissue cross-section carefully in cassette, with the best cut edge face down. Small fish can be placed whole or with the head and body separated in a cassette; make a sagittal section through the entire length of the fish and place each half inside the cassette with the midline down. If a fish or section is too thin (< 2mm), it can be elevated by placing a piece of sponge at the bottom of a cassette.
7. Place as many tissues in each cassette as you can without crushing any of them. There should be some room around each cross-section so the adjacent tissues are not touching each other. In addition, try to organize tissue by toughness, i.e., you can place gill arch,

eye and skin/muscle sections together while liver, kidney, heart and intestine, which are softer and easier to cut, can be placed together.

8. When the cassette is full, break off its lid, flip it over, and snap it in place. Ensure that each cassette is firmly closed.
9. Label the front of all cassettes with the same case number (e.g.: B162-06). The side of the cassette should be labelled with the designated fish species and number code and use letter coding for multiple cassettes for a single fish (e.g. for the first cassette of the third walleye: W3-A).
10. Place any bony, cartilaginous or highly fibrous tissues in decal (eye, skin, gills) for one to two hours. Place all other tissues in the fresh jar of formalin. Once you take the other tissues out of decal, also place these in formalin.
11. Place all unused bits of tissue back in their original wrapping and bags.
12. Fill out the form for each species. Follow the example below (you must fill out the highlighted fields):



ANIMAL HEALTH LABORATORY , HISTOLOGY

MATERIAL SUBMITTED (CHECK APPROPRIATE ITEM)

TYPE	√	NUMBER
WET TISSUE		
CASSETTES	√	40
PARAFFIN BLOCKS		
SLIDES UNSTAINED		

TYPE	√	NUMBER
IMMUNOHISTOCHEMISTRY		

PATHOLOGY ACCESS NUMBER: B162-06
DATE SUBMITTED: Dec. 3/06
DATE REQUIRED: ASAP
HOSPITAL NUMBER:
FUND: Devil's Lake Project: AHL Quotation Service 00990
SPECIES: Walleye
DEPARTMENT: Pathobiology

STAINS REQUESTED

TYPE	√	NUMBER
H & E	√	40
TOLUIDINE BLUE		
GIEMSA		
ORCEIN - GIEMSA		
ACID - FAST: T B		
ACID - FAST: LEAD		
ACID - FAST: NOCARDIA		

TYPE	√	NUMBER
GRAM B & B		
GRAM B & H		
PIERCE-VANDER KAMP		
PAS		
METHENAMINE SILVER		
WARTHIN - STARRY		
RETICULIN		
L.F.B. - HOLMES		

TYPE	√	NUMBER
MASSON		
M.S.B.		
P.T.A.H.		
PERL'S IRON		
VON KOSSA		
COPPER		
AMYLOID		

ADDITIONAL INSTRUCTIONS: Thank you!

SECTIONED BY:	NO. OF SLIDES	DATE COMPLETED	REQUESTED BY:
STAINED BY:			John Lumsden

Submit the form and formalin jar with cassettes to the histology laboratory.