

Health Assessment of Slimy Sculpin in Three Yukon Streams

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EXECUTIVE SUMMARY

The Clinton Creek mine is an abandoned asbestos mine near Dawson City, Yukon. Site management activities are currently funded by the Federal Contaminated Sites Action Plan Program. Because Clinton Creek fish are periodically exposed to elevated asbestos concentrations, stakeholders expressed concerns about fish health in Clinton Creek. The primary objective of this work was to identify short and long term effects of asbestos exposure on fish health in this system to inform the closure planning process for the site. A secondary objective was to identify seasonal and stream-specific differences in cellular changes as assessed by histopathology.

We studied microscopic features of slimy sculpin (*Cottus cognatus*) in Clinton Creek and in two reference creeks near Whitehorse, Yukon (Croucher Creek and Wolf Creek). Slimy sculpin were captured in the spring, summer, and fall in each creek. Data recorded at necropsy included length, weight, and external lesions; organs sampled for histopathology included liver, heart, spleen, gill, gonad, kidney, skin/skeletal muscle, head, and gastrointestinal tract. Tissue sections were then analyzed for several microscopic indicators of fish health. The multi-organ analysis approach provides information on physiologic condition (glycogen or lipid stores in the liver, gastrointestinal food, mesenteric adipose tissue, gonad development), parasites (multiple species distributed throughout numerous organs are common in wild fish), and potential exposure to toxins (of particular importance are the liver, kidney, and gill). Water quality variables (nutrients, metals, asbestos, and flow) were measured at approximately the same time as when fish were sampled.

Slimy sculpin were abundant in all of our Clinton Creek trap sets, and the fish had little medical evidence that they were less healthy than fish from the other two creeks (one fish had liver lesion that might have been pre-cancerous). The histopathologic profile of each creek was unique, but there was no clear evidence that one population was healthier than the other. One of the most interesting findings in this study was the high degree of variability in microscopic findings among the three creeks. Findings in the Clinton Creek slimy sculpin were different from the two Whitehorse-area creeks, as might be expected from their geographic separation, but even the Whitehorse-area creeks had major differences.

Water quality results exceeded CCME guidelines for several variables:

1. total aluminum in Croucher Creek in spring and in Wolf Creek in spring and fall;
2. total chromium and total iron in Clinton Creek on all sampling occasions;
3. total copper in Clinton Creek in summer; and,
4. total iron in Croucher Creek in spring.

The concentrations of sulphate, sulfur, and magnesium were greater in Clinton Creek than in Croucher and Wolf creeks on all 3 sampling occasions, but no CCME guideline exists for these analytes. Asbestos occurred only in Clinton Creek in fall and in Croucher Creek in spring; in both cases, concentrations were less than 10^6 fibres·L⁻¹.

An important "big picture" lesson from this work was that environmental assessment involving comparison of potentially impacted streams with reference streams must be interpreted with caution to ensure that observed differences are not simply a result of differences in season or stream ecology that is independent of the suspect perturbation. Ideally, health profiles for a target fish population would be developed prior to any development and would be monitored during the construction, operation, and closure periods.

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1.0 INTRODUCTION

The Clinton Creek mine is an abandoned asbestos mine that is approximately 100 km northwest of Dawson City in the Yukon Territory. Between 1967 and 1978, the mineral serpentine (chrysotile asbestos) was mined at this site. The site is currently in the remediation/risk management phase under the Federal Contaminated Sites Action Plan (FCSAP) program.

While the mine was in production, geotechnical instability of waste rock and tailings resulted in slope failures. These slope failures led to partial impoundment of both Clinton Creek (creating Hudgeon Lake) and Wolverine Creek (ponding of water behind/between tailings slumps) (Figure 1).

Clinton Creek passes through the mine site, from its headwaters to the west in Alaska, through Hudgeon Lake; 11 km downstream of the lake outlet it joins the Forty Mile River, which is a tributary to the Yukon River. Clinton Creek is used by juvenile Chinook salmon (*Oncorhynchus tshawytscha*) as a non-natal rearing and over-wintering stream. Other common species in Clinton Creek include arctic grayling (*Thymallus arcticus*), burbot (*Lota lota*), longnose sucker (*Catostomus catostomus*), and slimy sculpin (*Cottus cognatus*) (Dawson District Renewable Resource Council 2009; von Finster 2005). Wolverine Creek currently does not support fish (a hanging culvert at its confluence with Clinton Creek is a barrier to upstream movement) and the extent of pre-mine fish use of the creek is unknown. Wolverine Creek discharges to Clinton Creek approximately 1.5 km downstream of the Hudgeon Lake outlet (Figure 1).

The effects of asbestos on fish are not well studied and little information on potential effects of asbestos on fish health are available. An environmental assessment of the effects of the mine on Clinton Creek, including a bioassay of fish histopathology and toxicology, was completed in the mid-1970s by Environment Canada (Landucci 1978). To determine the health status of the fish in Clinton Creek in 2010, we sampled slimy sculpin from Clinton Creek for histopathologic assessment, and compared our findings with similar assessment of slimy sculpin from two streams near Whitehorse, Yukon Territory. The primary objective of the work was to identify short and long term effects of asbestos exposure on fish health in Clinton Creek to inform the closure planning process for the site. A secondary objective was to identify seasonal and stream-specific differences in histopathology.

Slimy sculpin is a bottom-dwelling fish species without a swim bladder that is relatively common in the creeks sampled and is amenable to capture using minnow traps. Because slimy sculpin are constantly exposed to sediments, and thought to be relatively sedentary (Cunjak et al 2005), exposure to asbestos-rich rock and tailings was expected to be higher in slimy sculpin than in other fish species that use the creeks only seasonally (e.g., Arctic grayling) or during some stage of their life cycle (e.g., juvenile Chinook rearing and/or over-wintering).

Juvenile Chinook salmon use all three of the creeks as rearing and over-wintering habitat. Chinook salmon are a highly valued species throughout the Yukon River basin and, in Clinton Creek, recent DNA analyses suggested that juveniles from a variety of upstream populations (e.g., Yukon main [between mouth of Tatchun Creek and mouth of Pelly River], Teslin River,

Big Salmon River, Little Kalzas River, Nordenskiöld River, Mayo River, Chandindu River) rear in the creek in their first year (Mackenzie-Grieve, 2010).

PREVIOUS RELATED WORK ON ASBESTOS ON FISH

Chrysotile is the type of asbestos that occurs at the abandoned Clinton Creek mine; it is considered to be less harmful to humans than amphibole asbestos (ATSDR 2001). Chrysotile and amphibole asbestos caused maximum mortality in *Artemia salina*, a planktonic filter-feeding crustacean, between 10^7 - 10^8 fibers·L⁻¹, and toxicity was independent of fiber chemistry (Stewart and Schurr 1980 in Belanger et al 1986b). Acute mortality is insignificant for adult mollusks and a variety of fish species (Halsband 1974; Woodhead et al 1983; Belanger et al 1986a) although increased mortality in clam larvae and a decrease in the release of larvae by adult clams was linked to asbestos concentrations of 10^4 to 10^8 fibres·L⁻¹ (Belanger et al 1986a). Other work, however, suggests that exposure of aquatic organisms to asbestos results in chronic or sublethal effects which manifest as pathological and behavioral effects (e.g., Belanger et al 1985). Asbestos can occur naturally in most water supplies, and concentrations are usually less than 1.0×10^6 fibres·L⁻¹ (Faust 1995).

Lesions associated with asbestos exposure are diverse and reflect subtle stress effects (Belanger et al 1986b). Asbestos concentrations below 10^6 fibres·L⁻¹ do not appear to have either chronic or acute effects on fish. For example, larval coho salmon (*Oncorhynchus kisutch*) exposed to asbestos at 10^6 fibres·L⁻¹, were lethargic; microscopic changes included epidermal hypertrophy, hyperplasia and selective vacuolation near the branchial region, and degradation of the lateral line system (Belanger et al. 1985). Japanese medaka (*Oryzias latipes*) exposed to asbestos concentrations of 10^6 fibres·L⁻¹ developed slight but significant increases in epidermal thickness (Belanger et al 1990). Juvenile mollies (*Poecilia formosa*) exposed to chrysotile (concentrations not reported in fibres·L⁻¹) developed epithelial hypertrophy, selective necrosis in kidney cells, and vacuolation in heart cells (Woodhead et al. 1983). In addition, asbestos fibres sometimes accumulate in the tissues of aquatic organisms. For example, in lake trout (3 to 4 years old) exposed to amphibole asbestos (estimated at between <1 to 100×10^6 fibres·L⁻¹) as a result of exposure to tailings discharged into Lake Superior, higher concentrations of fibres accumulated in the kidney compared to the muscle and liver (Batterman and Cook 1981). In Asiatic clams (*Cobocula sp.*) fibres accumulated in gill and visceral tissues (Belanger et al 1986a).

2.0 METHODS

STUDY AREA

Study sites included Clinton Creek (64.448211°N 140.701868°W), Wolf Creek (60.594139°N 134.955355°W), and Croucher Creek (60.780820°N 135.035538°W) (Figure 2). Wolf and Croucher Creek are direct tributaries to the Yukon River and Clinton Creek discharges to the Forty Mile River which in turn discharges to the Yukon River. Fish health in Clinton Creek was the focus of this study; Wolf and Croucher Creeks were also studied to provide a basis of comparison for Clinton Creek observations.

The section of Clinton Creek sampled was shallow (between 0.1 and 0.2 m), warm (warmer than 13°C mid-summer), and lotic habitats predominated; there were few lentic habitats (pools and eddies) within the stream reach that could be sampled using minnow traps. Elevated asbestos ($>1 \times 10^7$ fibres·L⁻¹) had been previously noted throughout Clinton Creek (Appendix 1).

Wolf Creek is lake-headed (Coal Lake) but is colder in the summer than the other two study creeks (Fisheries and Oceans Canada, unpublished data). Elevated asbestos ($>1 \times 10^7$ fibres·L⁻¹) had been previously noted in Wolf Creek (Roach and Mitchell, 1998). Development in the immediate vicinity of the creek is currently limited to roads and road crossings (one of which was used by a now-reclaimed copper mine in the late 1970s and early 1980s), limited residential development, a campground, and an un-used railway crossing. The section of Wolf Creek sampled was easily accessible and was diverse in its habitats: it contained several pools and lentic habitats with woody debris, shallow riffle habitats, and less turbulent run habitats.

Croucher Creek summer temperatures are generally warmer than Wolf Creek, but colder than Clinton Creek (Fisheries and Oceans Canada, unpublished data). Current development in the immediate vicinity of Croucher creek is restricted to road crossings and one or two seasonal dwellings. In Croucher Creek, the stream section sampled was selected primarily based on ease of access, suitability of the habitat to sampling with minnow traps (i.e., lentic habitats were available), and presence of adequate numbers of slimy sculpin. Minnow traps were set in a section of the creek that was between 0.3 and 0.5 m deep in the middle, included several pools (between 0.5 and 1m deep) along one of the banks, and included some shallower lentic habitats. Also, a series of beaver dams downstream of the sampling area produced a backwater effect on the sampled section of the creek.

FISH PATHOLOGY

Slimy sculpin were sampled in 2010 from each of three creeks in spring (May - early June), summer (late July), and fall (late September - early October). In spring, GDM trained JMG to conduct necropsies and JMG completed the remaining summer and fall necropsies.

Gee-type minnow traps were used to capture fish at each of the study sites. Most traps were deployed 14 – 26.5 hours before fish were harvested except for the spring sampling event at Clinton Creek, where traps were deployed for approximately 100 hours. Traps were baited with Yukon River Chinook salmon roe.

For processing, slimy sculpin were separated from other species captured. The ten largest slimy sculpin were then selected for necropsy and placed in a separate bucket. All captured fish, including other species and smaller slimy sculpin, were counted and most were also weighed.

One at a time, slimy sculpin for necropsy were anaesthetized with tricaine methane sulfonate (250 mg·dl⁻¹) that was buffered with an equal mass of sodium bicarbonate. Total length and body mass (± 0.1 g) were recorded. Fish were examined for gross lesions and semi-quantitative scores were assigned as 0 (none), 1 (mild), 2 (moderate), or 3 (severe) for five external lesions: caudal fin fraying, caudal fin reddening, other fin fraying, fin base reddening, and focal skin reddening.

Following external examination, each sculpin was then bled by making a transverse cut through the caudal peduncle. For smaller fish (usually <3 g), the left operculum and left body wall were removed, and the remainder of the fish was preserved whole in 10% neutral buffered formalin. For larger fish, individual organs were harvested, including gill, liver and kidney (head and trunk), gonad, skin, skeletal muscle, brain, heart, spleen and intestine. The multi-organ analysis used here provided information on physiologic condition (glycogen or lipid stores in the liver, gastrointestinal food, mesenteric adipose tissue, gonad development), parasites (multiple species in numerous organs are common in wild fish), and potential exposure to toxins (of particular importance are the liver, kidney, and gill). For larger fish from which viscera were removed, two additional gross findings were semi-quantitatively scored: relative kidney volume and visceral white foci. Scoring for visceral white foci was based on the number of foci enumerated: 0 (none), 1 (1-5 foci), 2 (6 to 10 foci), 3 (>10 foci).

All tissues (from both large and small fish) were placed in coded histocassettes so that all further processing was done without knowledge of the source creek (blinded study). The coded histocassettes were then placed in 10 % neutral buffered formalin. After approximately 24 hours in 10% formalin, tissues were transferred to tap water and sent to the Animal Health Centre in Abbotsford, British Columbia for processing.

At the Animal Health Center, samples containing bone were decalcified in 10% Ethylenediaminetetraacetic Acid (EDTA) solution for 24 hours (for small samples like gill) to 6 days (for larger samples like whole bodies). For fish small enough to fit into a standard histocassette, the body was transected midsagittally, processed into paraffin, and embedded so that the resultant slide contained both cut faces; from most of these paraffin blocks, one or two additional step sections were cut at approximately 250 μ m depth intervals. Organs from larger fish were processed, embedded, and sectioned individually. Sections were cut at 3 μ m and slides were stained routinely with H&E. Periodic Acid-Schiff stain with and without amylase digestion was used to confirm the presence of glycogen in hepatocytes on a subset of the samples. Schmorl's lipofuscin and Perl's iron stain were used on a subset of samples that contained foci of yellow-brown pigment. Published methods were used for systematic histopathology (Marty & Heintz 2010; Marty, Heintz & Hinton 1997) and optimization of photomicrograph illumination and colour balance (Marty 2007). Tissue sections from each season's samples were analyzed by one pathologist (GDM).

For histopathology, common diagnoses were identified for each organ and scored on a 4 point scale as 0 (none), 1 (mild/small amounts), 2 (moderate), or 3 (severe/abundant). Uncommon microscopic findings were described in a comments column for the appropriate organ in the scoring spreadsheet (Appendix 2). After scores for each season's samples were assigned, the source creek of the samples was revealed, the data were sorted, and summary statistics were calculated for each creek. Trends in the data were identified by comparing prevalence and mean score ($\pm$ SEM). Site differences were identified when the standard error of the means for each of the characteristics scored from each creek did not overlap.

On each of the three sampling occasions, statistical significance in length and mass differences of fish from each of the three sites was assessed with a one way analysis of variance (ANOVA).

WATER QUALITY

At Clinton Creek, water samples were collected at the same time that fish sampling was completed (May 26-spring, July 20-summer, and September 21-fall). Spring water sampling was on May 25 at Croucher Creek and on June 10 at Wolf Creek. Water samples were collected from both creeks on August 9 (summer) and September 30 (fall). Samples were collected from the same location in the stream on each sampling occasion. On these occasions, water samples were collected (nutrients, total metals, dissolved metals, asbestos), field variables were measured, and flow was estimated.

Water samples for metals and nutrients were analyzed by Exova Labs in Surrey, B.C., and were collected as follows:

- Nutrients - 1 L plastic container;
- Total metals - 125 mL plastic container preserved with 1mL nitric acid; and,
- Dissolved metals - 125 mL plastic container, field-filtered with a 0.45 µm syringe filter and preserved with 1 mL nitric acid.

Water samples for asbestos analyses were analyzed by McMaster University's Occupational and Environmental Health Laboratory in Hamilton, ON. Samples were processed according to McMaster's standard laboratory procedures for asbestos samples.

In the field, all samples were stored in a cooler and kept at 4°C. Lab-specific protocols to ensure appropriate QA/QC procedures were followed in all cases.

Water quality results were compared with Canadian Council of Ministers of the Environment (CCME) guidelines and Yukon Contaminated Sites Regulations (Yukon CSR) aquatic life standards where they existed. In cases where CCME guidelines did not exist (e.g., asbestos), results were compared with published values in the scientific literature.

Flow estimates

Flow estimates for each of the three study creeks were completed at the same time as water samples were taken. Approximately the same transect was used on each sampling occasion. Flow measurements were made according to the midsection method (velocity-area method) outlined in Mosley and McKerchar (1993) using a Marsh-McBirney flow meter.

3.0 RESULTS AND DISCUSSION

FISH SAMPLING

Fish capture

Slimy sculpin catch per unit effort ($CPUE = \# \cdot \text{trap}^{-1} \cdot \text{hour}^{-1}$) was high in Croucher Creek in spring ($CPUE = 0.38$), but decreased in summer ($CPUE = 0.089$) and in fall ($CPUE = 0.055$).

Previous sampling completed by Fisheries & Oceans suggested that sculpin were the dominant species within this section of the creek (unpublished observations).

Slimy sculpin CPUE was generally low in Wolf Creek; it was 0.045 in spring, 0.050 in summer, and 0.028 in the fall. In the fall it was necessary to sample the creek for two consecutive days to obtain the desired sample size. Past sampling by Fisheries and Oceans using electrofishing techniques, however, captured many sculpin within the riffle/run features of the creek, but these areas were not amenable to the minnow trap techniques employed in our study.

Slimy sculpin CPUE at Clinton Creek was higher in the fall (CPUE = 0.12) than it was in the spring (CPUE = 0.055) and summer (CPUE = 0.045); however, the soak time for the spring samples (about 100 hours) was much longer than any other soak time (all <30 hours). Previous sampling completed by Fisheries & Oceans suggested that sculpin were common captures in minnow traps within the section of the creek sampled.

Fish Size

Although no significant differences in length or mass occurred in the spring, the long soak time for Clinton Creek fish in spring likely influenced observed mass in particular (Figure 4).

In summer, significant differences in length ($F_{2,29} = 15.31, p = 0.000029$) and mass ($F_{2,29} = 10.43, p = 0.00039$) of samples from the three creeks occurred; slimy sculpin from Clinton Creek were larger (length and mass) than those in either Croucher or Wolf Creeks (Tukey-Kramer, $p < 0.05$).

In the fall, significant differences in length ($F_{2,35} = 5.31, p = 0.0096$) and mass ($F_{2,35} = 3.98, p = 0.028$) occurred; slimy sculpin from Clinton creek were larger (length and mass) than those captured in Wolf and Croucher Creeks.

Sculpin included in the above analyses were the largest fish captured in each of the creeks by minnow trapping. The samples, therefore, are biased towards the largest fish in each of the three creeks, but the bias should be consistent between each of the creeks.

Fish pathology

Gross lesions and macroscopic findings

External lesions were uncommon. None of the fish sampled had caudal fin fraying, caudal fin reddening, or focal skin reddening. Other fin fraying occurred only in one fish captured from Wolf Creek in the spring. Mild fin base reddening occurred in about ¼ of summer Croucher Creek samples and in one sample from Wolf Creek in spring. It occurred in all summer and in more than half of the fall samples from Clinton Creek (Appendix 2). Visceral white foci were most common in fish from Croucher Creek.

Microscopic findings

Energy stores

Hepatocellular (liver cell) glycogen is a measure of the readily available energy stores in the fish. Mesenteric adipose tissue (fat located within the abdominal cavity) also provides some indication of the nutritional status of the fish.

Hepatocellular glycogen and mesenteric adipose tissue scores in spring were both less in Clinton Creek than in either Croucher Creek or Wolf Creek (Table 1). Because slimy sculpin captured in Clinton Creek in spring remained in traps for about 100 hours, feeding opportunities may have been reduced and energetic requirements may have been elevated (energy was expended to maintain position in trap). Throughout the remainder of the study, sampling effort was standardized across sites and sampling events to ensure results obtained were not an artifact of sampling methodology.

In summer, hepatocellular glycogen and mesenteric adipose tissue scores were greater in Wolf Creek than in either Croucher Creek or Clinton Creek (Table 1).

In fall, hepatocellular glycogen and mesenteric adipose scores in Croucher Creek were less than in either Wolf Creek or Clinton Creek (Table 1). In the fall, the water level at the Croucher Creek sampling location was high compared to previous years (unpublished observations); the high water may have resulted from a backwatering effect from downstream beaver dams. This may have affected productivity of the system (i.e., food supply), access to the food for sculpin, or energetic costs to sculpin inhabiting the area.

In Wolf Creek, hepatocellular glycogen and mesenteric adipose tissue scores were less in the fall than in the spring and summer (Table 1).

In Croucher Creek, mean scores for hepatocellular glycogen and mesenteric fat were greatest in the spring and tended to decrease through the summer and fall (Table 1). Less mesenteric adipose tissue in the fall might decrease chances of overwinter survival.

In Clinton Creek, mean scores for hepatocellular glycogen and mesenteric fat increased over the study period although low spring mean scores were likely an artifact of an extended period of restriction within minnow traps (Table 1).

Parasites

Parasite diversity and prevalence were highly variable among seasons and creeks, even for the two creeks in close proximity (i.e., Wolf and Croucher Creeks). Also, seasonal differences were sometimes absolute: in some cases a parasite was identified in one sampling event but was not identified in any other sampling event.

Coccidians are protists that occur in a number of tissues including the liver and intestine; they can prevent normal organ function by destroying tissue (necrosis) and/or making infected fish more prone to infection by other, more harmful, microbes (www.environment-agency.gov.uk/static/documents/Research/Coccidian_parasites.pdf). Coccidians in the liver (Figure 5)

occurred in Croucher Creek in all three seasons; they did not occur in Wolf Creek and occurred in Clinton Creek samples only in the spring (Table 1). Two types of coccidians occurred in the intestine. One type occurred on the apical surface of epithelial cells (Figures 6A - 6E); the other type occurred within the lamina propria and the base of the epithelium (Figures 6F and 6G). Neither form was associated with significant inflammation, even when the parasites were abundant (Figure 6). The superficial epithelial coccidians did not occur in any of the spring samples; they were common in Wolf Creek samples in the summer and fall, but they occurred in Clinton Creek only in the summer and in Croucher Creek (one fish) only in the fall (Table 1). The intraepithelial coccidians occurred in all creeks and all seasons (except Clinton Creek in the fall), and during all seasons prevalence was greatest at Wolf Creek (Table 1).

The intestinal tract sometimes contained one of three different nematode parasites, but none were associated with inflammation (Appendix 3 Plate A) - evidence that they were well adapted to their host. One of two types of small nematodes occurred in Wolf Creek during all three seasons, but occurred at Clinton Creek only in the summer and Croucher Creek only in the fall (Table 1). An adult nematode with embryonated eggs (i.e., an egg containing developing embryos) also occurred in Wolf Creek during all three seasons; it occurred in spring and fall Croucher Creek samples but never occurred in Clinton Creek samples (Table 1).

Epistylis is a colonial ciliated protozoan which attaches to the skin and gills of fish. *Epistylis* is not a true parasite, but its attachment may allow for invasion of secondary pathogens (Meyers et al. 2007). In our samples, *Epistylis* infected 80 – 100% of the sampled fish from all three sites and seasons (Table 1). Even when they were abundant, they were not associated with inflammation (Appendix 3 Plate B, image D) - evidence that they were well adapted to their host. *Epistylis* was more common in sections of the gill than on the skin of the body or head (Table 1).

Trichodina sp. is a ciliated protozoan often found on skin, gills, and head. When parasite numbers are elevated, fish may lose condition and become weak thus becoming more susceptible to infection by bacterial pathogens in the water (Klinger and Floyd 1998). In our samples, *Trichodina sp.* was most common on the gills (Appendix 3 Plate B, image C and Appendix 3 Plate B, image D); it occurred in all seasons and all creeks except for Wolf Creek in the fall (Table 1).

Monogenean parasites (flukes or flatworms) are common on the gills, skin, or fins of fish (Reed et al 2009), and they occurred on the gills (Appendix 3 Plate B, image E) and head of our samples (Table 1). Tissues damaged by monogenean infection may cause fish to be more prone to secondary infection by bacteria and fungus (Reed et al 2009), but our samples had no evidence of these effects. Monogenean parasites in the gill occurred at all three creeks in the spring, when they were most common in Croucher Creek. Monogeneans occurred only sporadically in the summer (one fish from Croucher Creek) and fall (one fish from Wolf Creek).

Myxosporeans are multicellular spore-forming parasites that occur in a variety of organs; many myxosporeans are well adapted to their hosts, but a few can act as pathogens. In the kidneys of our fish, pansporoblasts and spores of the myxosporean *Myxobilatus yukonensis* (Arthur and Margolis 1975) expanded tubules and glomeruli (Figure 7). They occurred only in Croucher Creek and Clinton Creek in the spring (Table 1). Severity scores for the renal myxosporeans

were not clearly correlated with the gross examination scores for relative kidney volume; for example, among the two fish with relative kidney volume scored as severe/abundant, one fish had no renal myxosporeans and one fish had abundant renal myxosporeans (Appendix 2).

In the eyes, trematodes (flukes) within the lens/retina (Appendix 3 Plate C) were most common in Croucher Creek: the parasite occurred in 60 % of spring samples (mild-moderate score), 58 % of summer samples (mild-moderate score), and 67 % of fall samples (generally mild score) (Appendix 2). Although the parasite never occurred in Wolf Creek samples, it occurred in one Clinton Creek slimy sculpin in summer (scored as mild) and in 4 slimy sculpin in the fall (mild-moderate score).

Larval cestodes (tapeworms) occurred sporadically in the loose connective tissues of the head (Figures 8 A&B), intestinal lumen (Figures 8 C&D), coelomic cavity (Figures 8 E&F), and skeletal muscle (Figure 9). Cestodes outside of the intestinal lumen were usually associated with chronic inflammation that varied from lymphocytic to granulomatous. In contrast, cestodes within the intestinal lumen were not associated with inflammation. The cestodes were most common in fish from Croucher Creek, with prevalence varying from 10% in spring samples to 58 % in summer and fall samples (Appendix 2). In Wolf Creek, they occurred in 0% to 10% of seasonal samples. Larval cestodes did not occur in any of the Clinton Creek samples (Appendix 2).

Organ-specific lesions

Liver

Hepatocyte cytoplasm sometimes contained eosinophilic droplets that varied from 1 - 5 μm in diameter (Figure 10). The pigmented droplets contained iron (Figure 10E) and lipofuscin (Schmorl's lipofuscin stain, not shown). In all three creeks, mean scores and prevalence of these cytoplasmic pigments were greatest in the spring and least in the summer (Table 1). Among the three creeks, Croucher Creek consistently had the least mean scores and prevalence.

The majority of the livers in all samples had at least a few hepatocytes with enlarged nuclei, diagnosed as hepatocellular megalocytosis/karyomegaly (Figure 11). The lesion occurred in at least 50% of all samples, but lesion severity tended to be less in fish from Croucher Creek than the other two creeks (Table 1). One liver from Clinton Creek, sampled in July, had an eosinophilic focus of cellular alteration (Figure 11A) and a basophilic focus of cellular alteration (Figure 11D). The same liver also had scattered pigmented macrophage aggregates (Figure 11E), and a few hepatocytes had foci of membrane-bound cytoplasmic protein that were sometimes greater than 10 μm in diameter (Figure 11F).

Hepatocellular single cell necrosis or apoptosis occurred in 30% of the spring Clinton Creek samples, probably as a result of hepatic remodeling associated with the lack of feeding during the long time from trap placement to trap collection for this group of fish. The only other samples with this hepatocellular lesion occurred in spring and fall in Wolf Creek (one fish in each sample) (Table 1).

Stomach, intestine, and mesenteries

Fibrous capsule granulomas surrounding degenerating metacercariae (Appendix 3 Plate D) were common in the mesenteric connective tissues of Croucher Creek samples, where they occurred at greater than 50% prevalence during all three seasons. Some fish had similar granulomas in the head or surrounding the heart. Fibrous capsule granulomas did not occur in Wolf Creek, and in Clinton Creek occurred in only one fish in summer and another fish in fall (Table 1).

Peritonitis was scored when inflammation of the layer lining the viscera was not clearly associated with any other scored variable. In some cases, peritonitis was probably scored when the inciting cause (e.g., a fibrous capsule granuloma or larval cestode) was out of the plane of section examined). Peritonitis was most common in Croucher Creek where it occurred in 60 to 90 % of all samples and mean scores were always greater than for the two other creeks (Table 1). In Croucher Creek, mean scores were greatest in the summer. In one fish, sampled in the spring from Croucher Creek, mesenteric granulomatous inflammation between the spleen and the stomach surrounded myxosporean spores (Figure 12).

The distal intestine of one fish, sampled in the spring from Wolf Creek, was distended by homogeneous protein consistent with yolk material (Figure 13), perhaps from an ingested egg. The protein included foci of bacterial rods that might have adversely affected fish health.

The mesenteries of one fish, sampled from Wolf Creek in early October, had a multilobular expansile tumour composed of a reticular pattern of collagenous stroma separating tumour cells into packets about 20 μm in diameter (Appendix 3 Plate E). Tumour location and morphology are characteristic of an endocrine tumour of the pancreatic islets.

The mesenteries of one fish, sampled from Croucher Creek in May, contained an irregularly angular focus of granulomatous inflammation between the intestine and gallbladder. The inflammation contained scattered sporulating coccidian oocysts (Appendix 3 Plate F). The coccidian might be the same species as in the liver (Figure 5) or intestine (Figure 6G), but in an aberrant location.

Heart

Endocardial phagocytosis was diagnosed when cells lining the atrium of the heart were enlarged and contained cytoplasmic granules. Among the two moderate cases that were subjected to special stains, both contained lipofuscin (Schmorl's stain) and neither contained iron (Perl's stain). Endocardial phagocytosis was common in all three creeks during all three seasons (Table 1). Epicarditis and endocarditis were uncommon, never exceeding 10% prevalence in any sample (Appendix 2).

Spleen

Scores for pigmented macrophage aggregates in the spleen were consistently less in fish from Wolf Creek, and no fish from Wolf Creek had moderate or abundant pigmented macrophage aggregates that occurred among fish at both of the other creeks. The trend towards greater scores

in fish from Croucher Creek might be related to their greater load of visceral parasites and peritonitis. The trend towards greater scores in fish from Clinton Creek might be related to their larger size (suggestive of older age) and toxin exposure.

Thickening (hypertrophy) of the splenic ellipsoids (Appendix 3 Plate G) in spring samples occurred only among females (Table 1), and this might have been related to postspawning absorption of ovarian fluids. Among summer and fall samples, only a single fish had prominent splenic ellipsoids: a male from Croucher Creek sampled during the summer (scored as mild).

Gill

Refractile material in the gills, roughly 10 - 50 μm in diameter, occurred in 80 - 90% of samples from all three creeks in spring, and mean scores were greater in spring than in summer and fall. For all seasons and creeks, except for two fish gills had no more than moderate amounts of refractile material (Table 1). In the summer, refractile material was least common in Croucher Creek samples (25 % of samples) (Table 1). Mean scores for refractile material in the gills are not indicative of asbestos concentrations in the water; asbestos was only detected in Clinton Creek in fall and in Croucher Creek in spring (Table 2). Instead, refractile material in gills is likely associated with mineral particles in the water and/or sediment.

Lamellar epithelial hyperplasia/hypertrophy was more common in spring samples than in summer and fall samples. In the spring it occurred in 90 % of Croucher Creek samples, 60 % of Wolf Creek samples and about 30 % of the Clinton Creek samples. The lesion did not occur in any of the July samples, and occurred in only one Clinton Creek fish in the fall (Table 1).

Gonad

Slimy sculpin spawn during the spring, and many microscopic features of the gonads were related to the reproductive cycle. Features of postspawning ovaries included postovulatory follicles, unspawned eggs, and collapsed mature follicles (Appendix 3 Plate H). Gonadal foamy macrophages were more common in spring samples than in samples collected later in the year, and ovaries were more commonly affected than testes (Table 1). In summer and fall samples, no more than one fish per sample group had gonadal foamy macrophages, except for two affected fish in fall samples from Croucher Creek. One testes from a fall sample from Croucher Creek contained abundant macrophages, and the lesion had no organisms on a Twort's Gram stain. Ruptured follicles occurred only in spring samples, occurring in 75 % of Clinton Creek samples (3 of 4 slimy sculpin), 60 % of Wolf Creek samples, and 25 % of the Croucher Creek samples (Table 1).

Kidney

Tubular epithelial intracytoplasmic protein droplets (Appendix 3 Plate I) were most common in males from Croucher Creek sampled in the spring, where 4 of 5 had abundant droplets; in contrast, none of the 4 females sampled from Croucher Creek in the spring had these droplets (Appendix 2). The droplets were less common in spring samples from the other creeks, but from all sites only one female (from Wolf Creek) was affected, and this case has only small numbers

of droplets. These droplets occurred in none of the summer samples and only one fish sampled in the fall (a female from Wolf Creek; Appendix 2). Based on these findings, the condition seems to be associated with immediate post-spawning in males.

Golden epithelial pigment in tubules probably included lipofuscin and, therefore, is evidence of cell membrane turnover. All cases were scored as mild. Mean scores and prevalence tended to be greater among Clinton Creek samples and were always the lowest among Wolf Creek samples (Table 1).

Skin/skeletal muscle/spinal cord

Myositis was most common in Croucher Creek in the summer and the fall, where it was often associated with larval cestodes. In spring samples, myositis did not occur in Croucher Creek, but it did occur in one fish from each of the other creeks (Appendix 2).

Inflammation of the spinal cord, meningomyelitis, included scattered foci of lymphocytes and macrophages that were often associated with intralesional myxosporean spores in various stages of development (Figure 14). Meningomyelitis occurred in 75 - 100% of all seasonal Wolf Creek samples but never in more than 10% of seasonal samples from other creeks (Table 1); of the 32 fish sampled from Wolf Creek during the study, 29 (91%) had meningomyelitis, and 76% of these (22 of 29) contained myxosporean spores. No cases of meningomyelitis from the other creeks contained myxosporean spores in the spinal cord.

Spinal cords from three fish (two from Wolf Creek and one from Clinton Creek; all sampled in July) contained cylindrical multicellular parasites, possibly myxosporean trophozoites (Appendix 3 Plate J). The parasites, about 35 µm long and 15 µm in diameter, are associated with no more than minimal inflammation.

Descriptions of lesions and features examined as part of this work are included in Appendix 4.

WATER QUALITY AND FLOW

CCME guidelines for water quality were exceeded for: 1) total aluminum in Croucher Creek in spring and in Wolf Creek in spring and fall; 2) total iron in Clinton Creek on all occasions; 3) total copper in Clinton Creek in the summer; and 4), total iron in Croucher Creek in the spring (Table 2).

The concentrations of sulphate, sulfur, and magnesium were greater in Clinton Creek than in Croucher and Wolf creeks on all 3 sampling occasions (Table 2), although none of these analytes have a CCME Guideline.

Water sampling- asbestos

Asbestos fibres were not detected in Clinton Creek in either spring or summer, but they were detected in the fall sample where fibres were mostly <5µm (Table 2). A factor in determining the pathogenic potential of asbestos is the fiber-length distribution; it is generally believed that asbestos fibers shorter than 5 µm are not pathogenic (ACGIH, 1991). Repeated water sampling

for another study in Clinton Creek in 2010 detected asbestos occasionally at several, but not all, sites sampled (LES 2011). When asbestos was detected, concentrations ranged from 0.203 to $11.5 \times 10^6 \text{ fibres} \cdot \text{L}^{-1}$ (LES 2011). Locations sampled as part of that work did not overlap with those sampled in this study. Other work has shown asbestos concentrations as high as $7.3 \times 10^{11} \text{ fibres} \cdot \text{L}^{-1}$ (Delaney et al 1981), but repeated sampling was not completed as part of this work. These data suggest that there is considerable variation in asbestos concentration, both temporally and spatially, within the Clinton Creek system, but concentrations in 2010 were several orders of magnitude less than in 1981. Work by Schreier et al (1987) in the Sumas River suggested that asbestos concentrations were positively correlated with flow, but this was not the case in our work or that of LES (2011).

In Croucher Creek, asbestos fibres less than 5 μm size range were detected in the spring, but not in subsequent sampling events. In both cases asbestos fibre concentrations did not exceed $10^6 \text{ fibres} \cdot \text{L}^{-1}$ (Table 2). Asbestos was not found in any of the samples collected from Wolf Creek (Table 2).

Flow estimates

Clinton Creek flow varied little during this study, and it was similar to Croucher Creek. Wolf Creek flow was three to four times greater than Clinton Creek and Croucher Creek (Table 2). In fall, Clinton Creek flow decreased, but flow increased by about $1 \text{ m}^3 \cdot \text{s}^{-1}$ in the other two creeks (Table 2).

Although sampling at Clinton Creek completed as part of this study suggested little change in flow, there was a significant high-water event which was not captured in our dataset. Eastern Alaska, in the vicinity of Eagle, for example, experienced record-breaking summer rainfall; summer rainfall was much higher in 2010 than it was in either 2009 or 2011 (Figure 15). Effects of the elevated rainfall on infrastructure in the area were severe and resulted in washout of the Taylor Highway (highway between Tok & Eagle) for much of the late summer and fall. Analyses of regional rainfall data showed that between 8 and 10 cm of rain fell over the Forty Mile River Basin July 10-11, 2010. The rainfall caused a rapid rise in river level and resulted in a record-level stage of 94.3 feet (www.nws.noaa.gov/hic/flood_stats/summaries/WY2010.pdf).

The Clinton Creek watershed experienced at least some of this precipitation although at the time there were no on-site weather stations in place capable of recording precipitation. The elevated precipitation is thought to be the cause of a large number of landslides within the watershed that occurred in early August. Many of the larger landslides were in the headwater areas (upstream of Hudgeon Lake); these resulted in large log jams and considerable sediment transport downslope/downstream and into Hudgeon Lake. Several landslides downstream of Hudgeon Lake temporarily blocked the mine property access road and resulted in sediment inputs to the creek. High water levels resulted in changes to creek channel morphology throughout Clinton Creek. Damage to the gabion structures at the outlet of Hudgeon Lake were also documented (Hartshorne 2010a, b). The landslides are likely the result of a type of mass movement often called active layer detachment or active layer slope failures. These types of landslides are related to the characteristics of seasonally thawed ground. Movement generally occurs along the surface of the permafrost table. The underlying cause of these movements appears to be an excess of

pore water pressures at the base of the active layer. Many movements of this type appear to be triggered by rain (Gerrard, 1990).

Erosion of material resulting from the high water event(s) may explain asbestos detection in our August water sample. The August high-water event(s) likely resulted in erosion of waste rock and tailings material and re-suspension of previously deposited asbestos material in the Clinton Creek channel. We did not detect any effects on the slimy sculpin attributable to this event, however. Compared with the two reference sites, fish from Clinton Creek had similar amount of refractile material in the gills and no gill lesions that could be attributed to asbestos exposure.

SYNTHESIS

Consistent with abundant slimy sculpin in all of our Clinton Creek trap sets, there is no medical evidence that Clinton Creek sculpin are less healthy than those from the other two creeks. The histopathologic profile of each creek is unique, but there is no clear evidence that one population is healthier than the other.

Indeed, one of the most significant findings in this study is the high degree of variability in microscopic findings among the three creeks. Because of geographic separation, microscopic findings in Clinton Creek might be expected to be different from the two Whitehorse area creeks, but even these two creeks have major differences. For example, trematodes were common in the lens of fish from Croucher Creek, but none of the samples from Wolf Creek had these parasites. In contrast, myxosporean parasites were common in the spinal cord and brainstem of sculpin from Wolf Creek, but none of the samples from Croucher Creek had these parasites. Further, seasonal differences are sometimes absolute. For example, renal myxosporean parasites (probably *Myxobolus yukonensis*) were common in spring samples from Croucher and Clinton Creeks, but they did not occur in any summer or fall samples.

An important "big picture" lesson from this work is that environmental assessment involving comparison of potentially impacted streams with reference streams must be interpreted with caution to ensure that observed differences are not simply a result of differences in season or stream ecology independent of the suspect perturbation. Ideally, histopathologic profiles for a target fish would be developed prior to any industrial development. This "pre-assessment" could include several reference sites as well. The histopathologic profiles from target and reference systems could be revisited during the construction period, periodically over the life of the project, and in the remediation (long-term monitoring) phase of the project as well. Such an approach could assist in the identification of any changes to fish health as a result of development.

5.0 ACKNOWLEDGEMENTS

We would like to acknowledge Al von Finster (AVF Research and Development) for his assistance in trap placement in Clinton Creek and for his historical insight into fish habitat use of Clinton Creek and adjacent areas. Patrick Roach (Aboriginal Affairs and Northern Development Canada) provided support, field assistance, and historical insight. Paraffin sections for histopathology were prepared by S. Etheridge and J. Taylor (Animal Health Center, Abbotsford,

B.C.). We would also like to acknowledge the Yukon Government, Energy, Mines, and Resources, Assessment and Abandoned Mines, for covering costs of laboratory analyses of water samples.

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Table 1. Mean scores and standard error for necropsy findings in spring, summer and fall samples from three creeks in the Yukon Territory, 2010. Scores ranged from 0 (none) to 3 (abundant/severe). Refer to Appendix 2 for complete sampling, necropsy, and histopathology data.

	Spring						Summer						Fall					
	Croucher Creek		Wolf Creek		Clinton Creek		Croucher Creek		Wolf Creek		Clinton Creek		Croucher Creek		Wolf Creek		Clinton Creek	
	n=10		n=10		n=10		n=12		n=8		n=12		n=12		n=14		n=12	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
External/Gross Lesion Scores																		
fin base reddening	0.0	0.0	0.1	0.1	0.2	0.1	0.3	0.13	0.0	0.00	1.0	0.00	0.0	0.00	0.1	0.08	0.6	0.15
visceral white foci							1.3	0.25	0.0		0.0	0.00	1.0	0.00	0.0	0.00	0.1	0.14
Energy Stores																		
hepatocellular glycogen	2.9	0.1	2.7	0.2	0.3	0.2	0.8	0.25	2.6	0.26	1.1	0.29	0.8	0.27	1.9	0.30	1.8	0.24
mesenteric adipose tissue	1.7	0.3	2.3	0.2	0.4	0.2	1.4	0.23	2.3	0.16	1.2	0.21	0.5	0.19	1.6	0.17	1.4	0.15
Microscopic Lesion Scores																		
Liver																		
lipidosis	0.0	0.0	0.0	0.0	0.2	0.1	0.9	0.31	0.0	0.00	0.1	0.08	0.2	0.11	0.1	0.07	0.1	0.08
coccidian parasites	1.2	0.4	0.0	0.0	0.1	0.1	1.9	0.36	0.0	0.00	0.0	0.00	1.3	0.31	0.0	0.00	0.0	0.00
pigmented macrophage aggregates	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	0.00	0.2	0.11	0.0	0.00	0.1	0.07	0.0	0.00
pigment, hepatocellular, intracytoplasmic	0.5	0.2	1.0	0.3	0.7	0.2	0.0	0.00	0.1	0.13	0.3	0.13	0.3	0.13	0.6	0.14	0.7	0.22
focal/multifocal parenchymal leukocytes	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.08	0.1	0.13	0.2	0.17	0.0	0.00	0.1	0.07	0.2	0.11
perivascular lymphocytes/leukocytes	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.1	0.10	0.0	0.00
hepatocellular megalocytosis/karyomegaly	0.6	0.2	0.8	0.1	1.2	0.1	0.5	0.15	0.9	0.13	0.7	0.19	0.9	0.15	0.9	0.13	0.8	0.11
single cell necrosis (apoptosis)	0.0	0.0	0.1	0.1	0.5	0.3	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.1	0.07	0.0	0.00
Heart																		
endocardial phagocytosis (atrium)	0.8	0.3	0.8	0.3	1.5	0.2	0.7	0.14	0.6	0.26	0.8	0.17	1.0	0.21	0.9	0.13	0.8	0.17
Spleen																		
pigmented macrophage aggregates	0.6	0.2	0.1	0.1	0.9	0.3	0.5	0.15	0.1	0.13	1.5	0.28	1.1	0.31	0.4	0.13	0.9	0.19
congestion	0.8	0.1	0.6	0.2	0.9	0.2	0.5	0.15	1.4	0.38	0.9	0.16	1.5	0.19	1.9	0.23	1.7	0.26
ellipsoid hypertrophy	0.2	0.1	0.4	0.2	0.0	0.0	0.1	0.08	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00
granulomatous inflammation	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00
Stomach/intestine/intestinal ceca																		
fibrous capsule granuloma	0.7	0.3	0.0	0.0	0.0	0.0	0.8	0.24	0.0	0.00	0.1	0.08	0.6	0.19	0.0	0.00	0.1	0.08
nematode with embryonated eggs (intestine)	0.3	0.2	0.5	0.2	0.0	0.0	0.0	0.00	0.1	0.13	0.0	0.00	0.3	0.13	0.2	0.11	0.0	0.00
nematode (small) without eggs (intestine)	0.0	0.0	0.2	0.1	0.0	0.0	0.0	0.00	0.1	0.13	0.1	0.08	0.4	0.19	0.6	0.17	0.0	0.00
intraepithelial coccidian (<i>Eimeria</i> sp?)	0.1	0.1	0.9	0.3	0.2	0.2	0.1	0.08	0.6	0.18	0.3	0.14	0.2	0.11	0.3	0.13	0.0	0.00
superficial epithelial coccidian (<i>Goussia</i> ?)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	1.4	0.18	0.5	0.23	0.1	0.08	0.7	0.27	0.0	0.00
peritonitis (includes margin of spleen & kidney)	0.8	0.2	0.1	0.1	0.3	0.2	1.5	0.19	0.0	0.00	0.1	0.08	0.7	0.19	0.1	0.10	0.3	0.13
Gonad																		
gonadal foamy macrophages	0.3	0.2	0.2	0.1	0.6	0.2	0.1	0.08	0.0	0.00	0.1	0.08	0.3	0.26	0.1	0.07	0.2	0.17
oocyte atresia, mature follicles	0.0	0.0	0.2	0.2	0.0	0.0	0.0	0.00	0.0	0.00			0.5	0.50	0.0	0.00	2.0	0.58
ovarian ruptured follicles	0.3	0.3	0.6	0.2	1.3	0.5	0.0	0.00	0.0	0.00			0.0	0.00	0.0	0.00	0.0	0.00
oophoritis, nongranulomatous	0.0	0.0	0.0	0.0	0.8	0.5	0.0	0.00	0.0	0.00			0.0	0.00	0.0	0.00	0.0	0.00
number of females	4		5		5		3		3		0		2		4		3	
number of males	5		5		5		9		5		12		8		10		9	
Kidney																		
tubular epithelial protein (intracytoplasmic)	1.2	0.5	0.6	0.3	0.2	0.1	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.1	0.07	0.0	0.00
intratubular (luminal) protein casts	0.2	0.1	0.1	0.1	0.1	0.1	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00
golden epithelial pigment in tubules	0.3	0.2	0.1	0.1	0.4	0.2	0.1	0.08	0.0	0.00	0.3	0.14	0.5	0.15	0.1	0.10	0.6	0.15
tubular or glomerular myxosporean (<i>Myxobolus yukonensis</i>)	1.3	0.3	0.0	0.0	1.7	0.4	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00
Skin/skeletal muscle																		
<i>Epistylis</i> sp	0.9	0.2	0.9	0.1	0.5	0.2	0.0	0.00	0.6	0.18	0.2	0.11	0.3	0.13	0.4	0.14	0.1	0.08
fibrous capsule granuloma (probable metacercaria)	0.2	0.1	0.0	0.0	0.0	0.0	0.2	0.11	0.0	0.00	0.0	0.00	0.2	0.11	0.0	0.00	0.0	0.00
myositis	0.0	0.0	0.1	0.1	0.2	0.2	0.8	0.22	0.0	0.00	0.0	0.00	0.8	0.24	0.3	0.16	0.1	0.08
meningomyelitis	0.0	0.0	1.0	0.0	0.0	0.0	0.1	0.08	1.3	0.37	0.1	0.08	0.0	0.00	1.6	0.23	0.1	0.08

Table 1(con't)

	Spring						Summer						Fall					
	Croucher Creek		Wolf Creek		Clinton Creek		Croucher Creek		Wolf Creek		Clinton Creek		Croucher Creek		Wolf Creek		Clinton Creek	
	n=10		n=10		n=10		n=12		n=8		n=12		n=12		n=14		n=12	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Gill																		
<i>Epistylis</i> sp.	2.4	0.3	1.3	0.3	1.7	0.3	0.8	0.17	0.9	0.13	1.2	0.21	0.6	0.15	0.9	0.10	0.8	0.11
refractile material	1.1	0.2	1.2	0.2	1.2	0.3	0.3	0.13	0.9	0.13	0.5	0.15	0.7	0.14	0.5	0.14	0.7	0.14
<i>Trichodina</i> sp.	0.4	0.3	0.3	0.2	1.2	0.3	0.4	0.15	0.1	0.13	0.3	0.25	0.2	0.17	0.0	0.00	0.3	0.13
monogenean parasite	0.7	0.2	0.3	0.2	0.1	0.1	0.1	0.08	0.0	0.00	0.0	0.00	0.0	0.00	0.1	0.07	0.0	0.00
lamellar epithelial hyperplasia/hypertrophy	0.9	0.1	0.7	0.2	0.3	0.2	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.1	0.08
Head																		
<i>Epistylis</i> sp.	1.2	0.1	1.2	0.2	0.9	0.2	0.3	0.14	0.8	0.16	0.3	0.13	0.6	0.15	0.7	0.13	0.3	0.13
fibrous capsule granuloma (probable metacercaria)	0.3	0.2	0.0	0.0	0.0	0.0	0.3	0.18	0.0	0.00	0.0	0.00	0.6	0.19	0.0	0.00	0.1	0.08
lens trematode metacercariae (<i>Diplostomum</i> sp.?)	0.9	0.3	0.0	0.0	0.0	0.0	0.8	0.24	0.0	0.00	0.1	0.08	0.8	0.18	0.0	0.00	0.5	0.23
myodegeneration and necrosis	0.0	0.0	0.1	0.1	0.3	0.2	0.0	0.00	0.0	0.00	0.0	0.00	0.1	0.08	0.1	0.07	0.0	0.00
site differences within a season																		
Wolf Creek is the only site with myxosporeans in MML																		
blanks indicate not observed or not recorded																		

Table 2. Water quality summaries for Yukon creeks sampled in 2010. Refer to Appendix 5 for complete summary of water quality data.

Parameter	Units	CCME	Yukon CSR	Clinton Creek (CC-02)			Croucher Creek			Wolf Creek		
		Aquatic Life	Aquatic Life	26-May	20-Jul	21-Sep	25-May	9-Aug	30-Sep	10-Jun	9-Aug	30-Sep
flow	m ³ s ⁻¹			0.62	0.86	0.39	0.56	0.63	1.39	2.12	2.07	3.03
field conductivity	uS cm ⁻¹			287	311		123	160	105	100	85	75
field DO	mg L ⁻¹			9.34	9.27		8.99	8.17	10.75	10.23	10.68	12.14
field oxidation reduction potential (ORP)	mV			111.7	16.8	101.7	104.1	72.5	97.7	111.9	78.1	135.5
field pH	pH Units			7.96	7.93	7.80	7.74	7.73	7.79	8.16	8.15	8.35
field specific conductivity	µS cm ⁻¹			368	396	679	172	203	184	137	126	127
field TDS	g L ⁻¹			0.239	0.258	0.422	0.112	0.132	0.119	0.089	0.082	0.083
field temperature	°C			13.47	13.71	6.80	9.86	13.87	2.69	11.09	8.08	3.42
total suspended solids (TSS)	mg L ⁻¹			< 2	4	8	18	< 2	< 2	8	4	3
field turbidity	NTU			8.9	9.7		14.0	6.7	10.2	10.3	7.1	7.2
Asbestos - length < 5 µm	fb L ⁻¹			ND	ND	0.81 x 10 ⁵	0.24 x 10 ⁵	ND	ND	ND	ND	ND
Asbestos length > 5 µm	fb L ⁻¹			ND	ND	0.16 x 10 ⁵	ND	ND	ND	ND	ND	ND
Total Alkalinity	mg L ⁻¹			95	104	154	99	152	136	69	83	83
Hardness as CaCO ₃	mg L ⁻¹			227	382		142	128	71	82	84	
pH				7.82	7.95	7.87	7.82	7.96	7.68	7.88	8.12	7.80
Specific Conductivity	µS cm ⁻¹ at 25°C			397	423	693	181	272	244	137	171	176
Sulphate (SO ₄)	mg L ⁻¹		1000	98.60	108.00	208.00	1.76	1.80	3.23	7.09	9.52	9.70
Total Aluminum (Al)	mg L ⁻¹	0.1 (pH> 6.5)		0.126	0.131	0.089	0.233	0.009	0.039	0.224	0.033	0.207
Total Arsenic (As)	mg L ⁻¹	0.005		0.0011	0.0013	0.0015	0.0006	0.0008	0.0004	0.0006	0.0006	0.0006
Total Barium (Ba)	mg L ⁻¹			0.041	0.041	0.064	0.033	0.039	0.029	0.076	0.065	0.069
Total Boron (B)	mg L ⁻¹	¹ 1.5		0.047	0.043	0.062	0.007	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Total Cadmium (Cd)	mg L ⁻¹	² hardness dependent		0.00006	0.00004	0.00004	< 0.00001	< 0.00001	< 0.00001	< 0.00001	0.00001	< 0.00001
Total Calcium (Ca)	mg L ⁻¹			45.2	43.6	73.7	35.0	47.9	44.2	21.6	30.0	24.4
Total Chromium (Cr)	mg L ⁻¹	³ 0.001		0.0023	0.0021	0.0068	0.0009	0.0005	0.0005	0.0006	< 0.0004	0.0006
Total Cobalt (Co)	mg L ⁻¹			0.00052	0.00048	0.00083	0.00016	0.00005	0.00005	0.00015	0.00006	0.00014
Total Copper (Cu)	mg L ⁻¹	⁴ 0.002 - 0.004		0.003	0.005	0.003	0.001	< 0.001	< 0.001	0.001	< 0.001	0.002
Total Iron (Fe)	mg L ⁻¹	0.3		0.38	0.43	0.48	0.42	0.28	0.14	0.28	0.13	0.30
Total Kjeldahl Nitrogen	mg L ⁻¹			0.86	0.30	0.48	0.31	0.22	0.24	0.16	0.13	0.15
Total Lead (Pb)	mg L ⁻¹	⁵ hardness dependent		0.0002	0.0002	0.0002	0.0002	< 0.0001	0.0001	0.0002	0.0001	0.0003
Total Lithium (Li)	mg L ⁻¹			0.005	0.005	0.01	0.001	0.002	0.001	< 0.001	< 0.001	< 0.001
Total Magnesium (Mg)	mg L ⁻¹			30.9	30.0	50.7	4.7	6.3	6.1	5.1	7.3	5.9
Total Manganese (Mn)	mg L ⁻¹			0.0778	0.0642	0.246	0.0219	0.0076	0.01	0.0293	0.0136	0.023
Total Molybdenum (Mo)	mg L ⁻¹	0.073		0.0009	0.0011	0.0021	0.0024	0.0022	0.002	0.0006	0.0009	0.0009
Total Nickel (Ni)	mg L ⁻¹	⁶ hardness dependent		0.014	0.012	0.016	0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Total Phosphorus (P)	mg L ⁻¹			0.05	< 0.05	< 0.05	0.06	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Total Potassium (K)	mg L ⁻¹			0.7	0.1	0.9	0.6	0.5	0.8	0.7	0.9	0.8
Total Silicon (Si)	mg L ⁻¹			3.72	4.25	3.91	4.31	5.36	5.59	5	6.02	3.56
Total Sodium (Na)	mg L ⁻¹			2.38	2.49	4.58	2.50	3.53	3.69	2.92	4.22	3.62
Total Strontium (Sr)	mg L ⁻¹			0.255	0.253	0.423	0.158	0.235	0.184	0.193	0.211	0.202
Total Sulfur (S)	mg L ⁻¹			38.4	38.4	75.7	1.1	1	1.3	3.6	4.5	3.7
Total Titanium (Ti)	mg L ⁻¹			0.002	0.002	< 0.001	0.008	< 0.001	< 0.001	0.008	0.002	0.004
Total Uranium (U)	mg L ⁻¹	⁷ 0.015		0.0016	0.0015	0.0019	0.0024	0.0029	0.0031	0.0005	0.0007	0.0007
Total Vanadium (V)	mg L ⁻¹			0.0006	0.0006	0.0006	0.0011	0.0004	0.0003	0.0008	0.0004	0.0006
Total Zinc (Zn)	mg L ⁻¹	0.03		0.008	0.006	0.013	0.004	0.01	0.013	0.002	0.014	0.013
Total Zirconium (Zr)	mg L ⁻¹			0.0009	0.0011	0.0008	< 0.0001	0.0002	< 0.0001	< 0.0001	0.0002	< 0.0001
ND = non detect												
exceedances of CCME Guidelines for the Projection of Aquatic Life indicated depend on # significant digits of the CCME criteria												
exceedances of CCME Guidelines for the Protection of Aquatic Life are highlighted in yellow												
¹ boron: used "long-term" CCME guideline value												
² cadmium: guideline value calculated according to 10 ^{5.280log 10(hardness)+3.2} and converted to mg L ⁻¹ ; separate guideline calculated for each sample												
³ chromium: assumed chromium was in a hexavalent state; trivalent state guideline is 0.0089 mg L ⁻¹												
⁴ copper: guideline value calculated according to e ^{0.8846log(hardness)+1.486} and converted to mg L ⁻¹												
⁵ lead: guideline value calculated according to e ^{1.2732log(hardness)+4.708}												
⁶ nickel: guideline value calculated according to e ^{0.762log(hardness)+1.06} and converted to mg L ⁻¹												
⁷ uranium: used "long-term" exposure guideline												

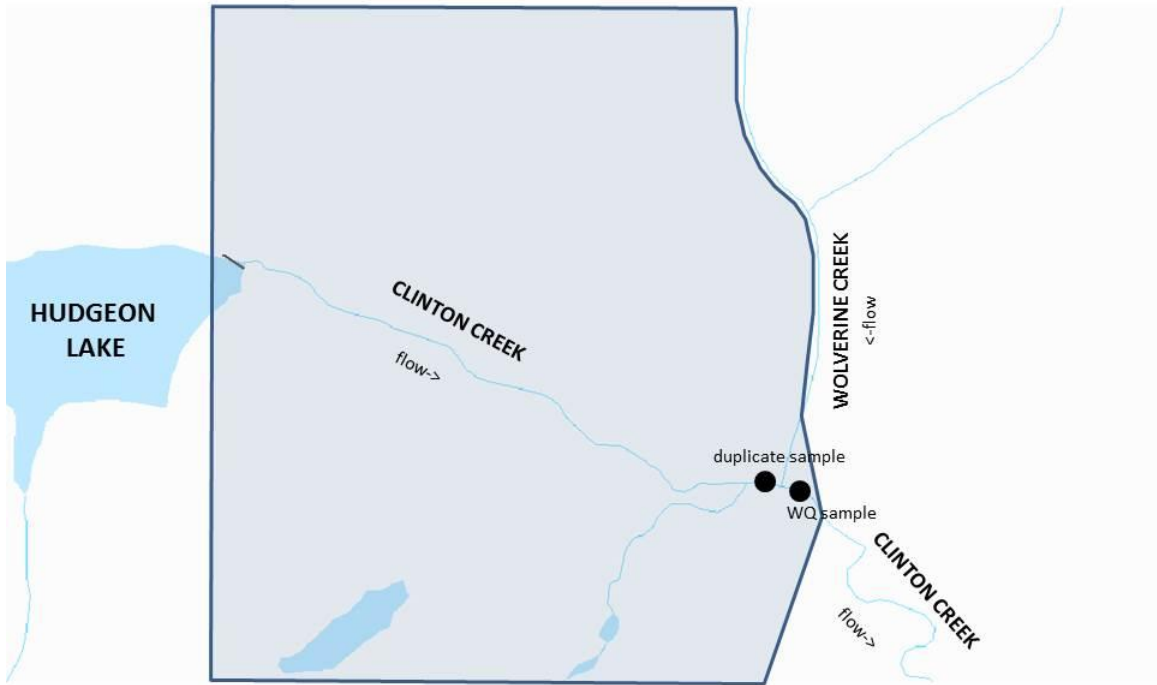


Figure 1. Location of water quality sampling within Clinton Creek mine site. Shading indicates generalized outline of mine site.

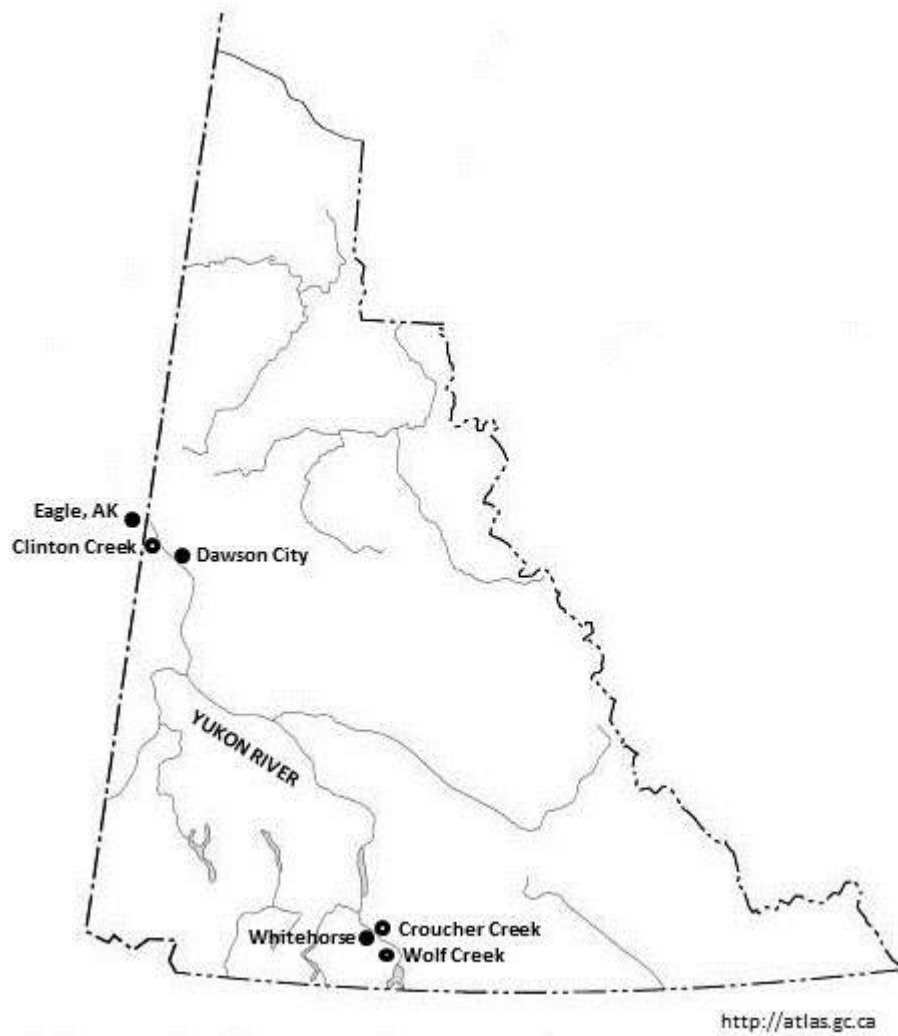


Figure 2. General location of the three study creeks in the 2010 Yukon slimy sculpin health project.

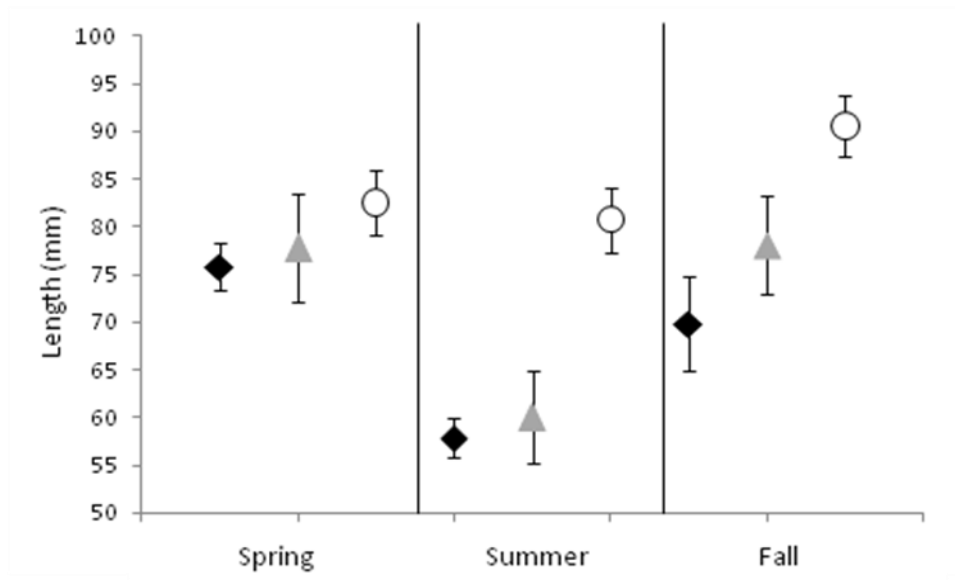


Figure 3. Slimy sculpin sample mean length and standard error in Croucher $\blacklozenge$, Wolf $\blacktriangle$, and Clinton $\bigcirc$ Creeks in 2010.

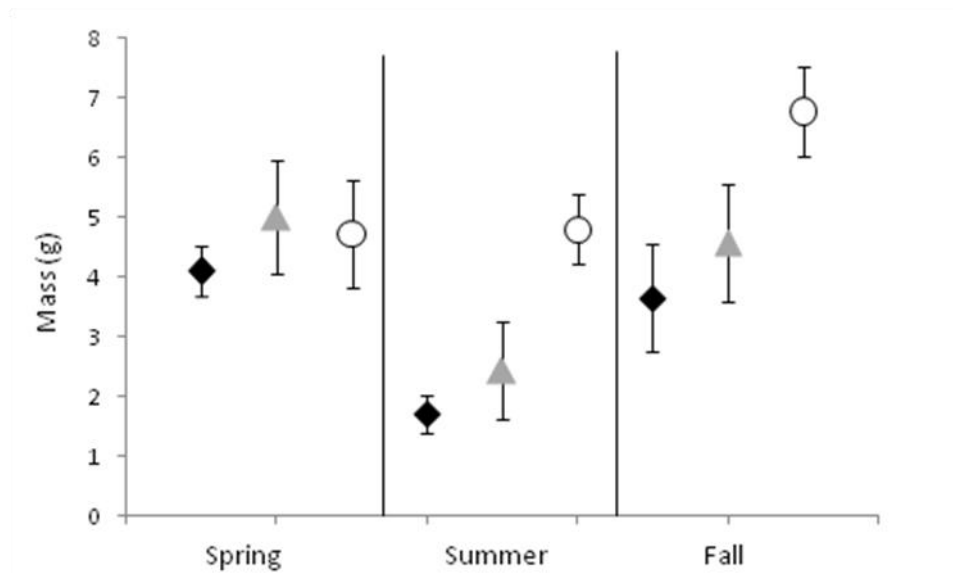


Figure 4. Slimy sculpin sample mean mass and standard error in Croucher $\blacklozenge$, Wolf $\blacktriangle$, and Clinton $\bigcirc$ Creeks in summer 2010 expressed as log length versus log mass.

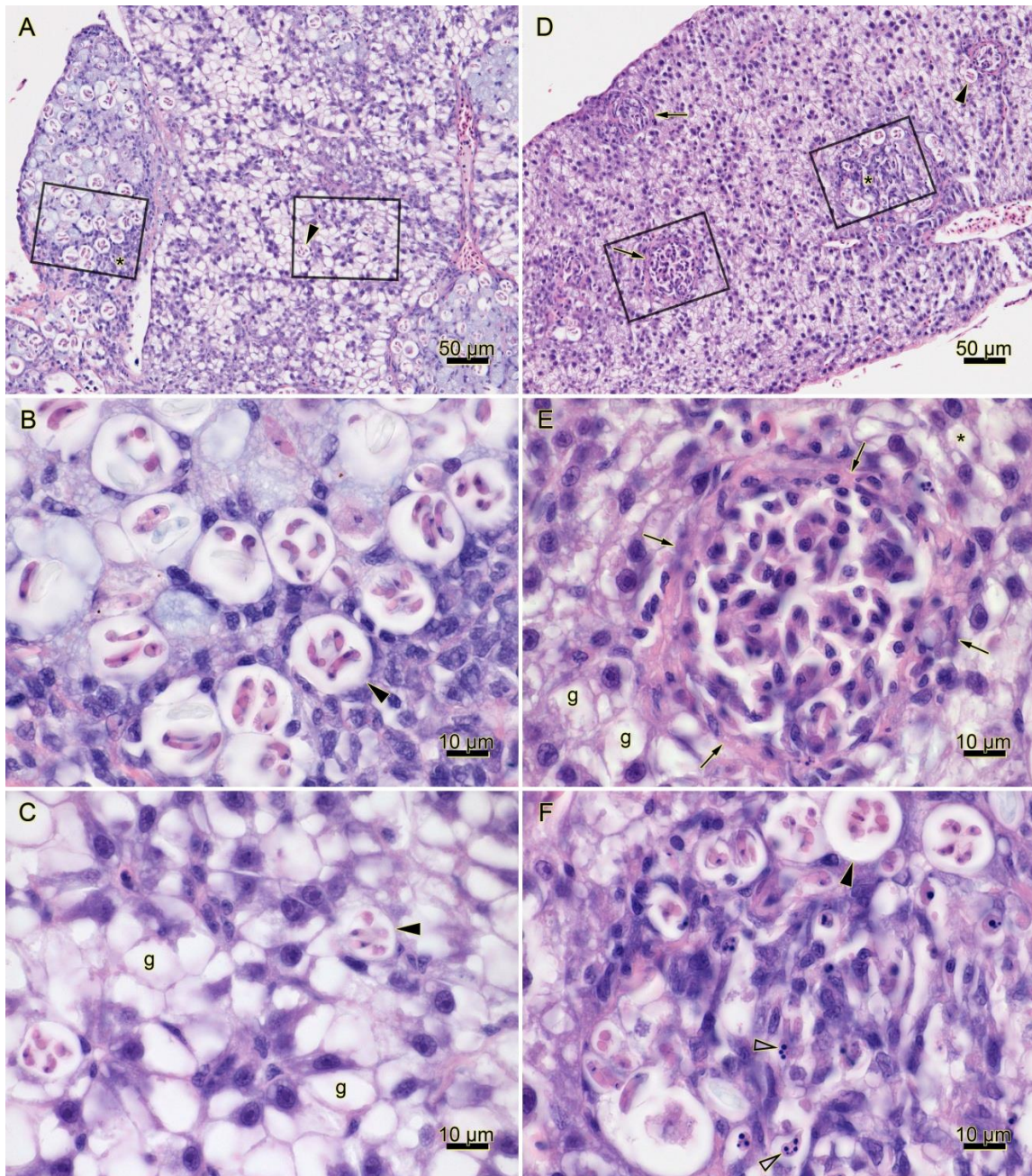


Figure 5. Photomicrographs of liver from a 3.8 g male (A, B, and C) and a 6.4 g male (D, E, and F) sampled from Croucher Creek in May. Both sections contain sporulated coccidian oocysts (solid arrowheads) with variable amounts of lymphohistiocytic inflammation (*). The oocysts in A, B, and C all seem viable, whereas foci of oocysts in D, E, and F are often degenerating, with pyknosis (open arrowheads in F) and reactive fibrosis (arrows). Both fish have hepatocytes with large cytoplasmic glycogen vacuoles (g). Areas outlined by black boxes in A are shown at higher magnification in B (left box) and C (right box). Areas outlined by black boxes in D are shown at higher magnification in E (left box) and F (right box). H&E stain.

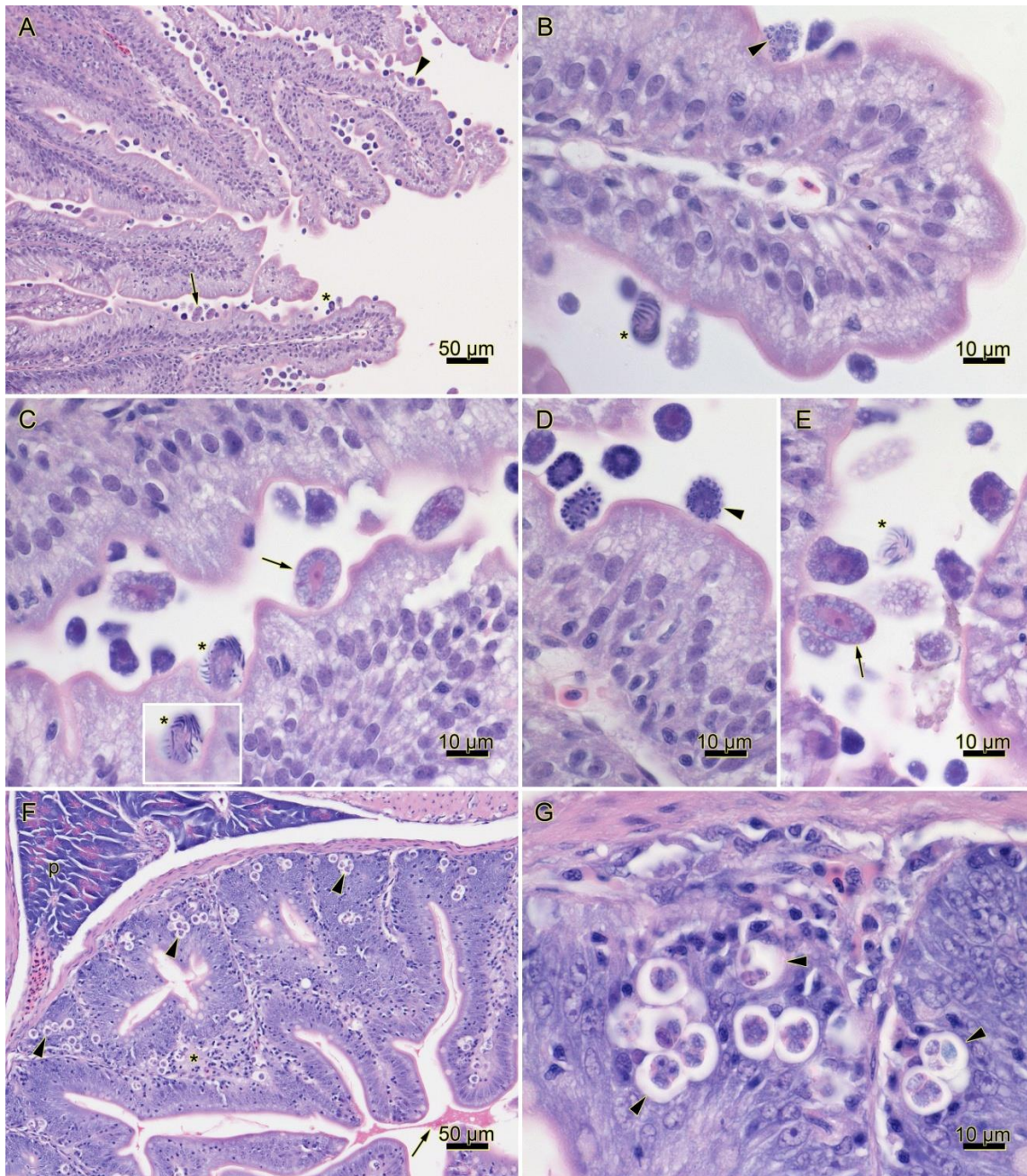


Figure 6. Photomicrographs of intestinal coccidian parasites; H&E stain. A – E. Intestine of a 6.2 g male sampled from Wolf Creek in early October. The epithelium contains several different epicytoplasmic presporogonic stages of coccidian development, including trophozoites or macrogamonts (arrows), microgamonts with abundant immature microgametes (arrowhead), and microgamonts with abundant elongate basophilic mature microgametes (*). Inset in C shows a microgamont (*) in a different focal plane. F and G. Intestine and exocrine pancreas (p) of a 1.4 g juvenile female sampled from Wolf Creek in June. Sporulating oocysts are most common in the intestinal epithelium (arrowheads) but some occur in the lamina propria (*). The intestinal lumen contains small amounts of proteinaceous material (arrow).

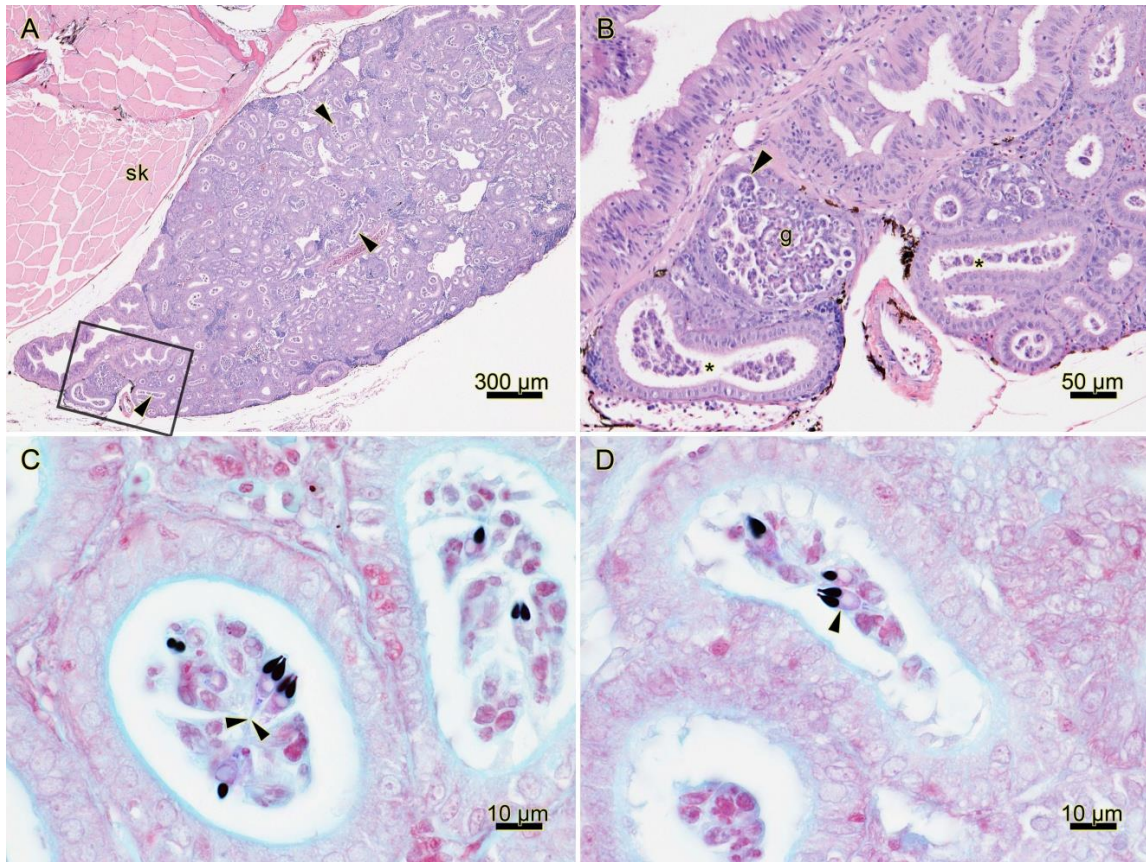


Figure 7. Photomicrographs of trunk kidney of a 4.6 g female sampled from Clinton Creek in May. **A.** Renal tubules contain myxosporean parasites (arrowheads); skeletal muscle (sk); H&E stain; black box demarcates area shown in **B.** **B.** Pansporoblasts and spores are associated with glomeruli (g and arrowhead) and tubules (*). **C** and **D.** Twort's Gram stain. Lenticular myxosporean spores characteristic of *Myxobilatus yukonensis* each have (i) two polar capsules that are symmetrical, pyriform, and Gram-positive, (ii) a vacuolated sporoplasm (arrowhead in **D**), and (iii) paired elongate caudal processes (arrowheads in **C**).

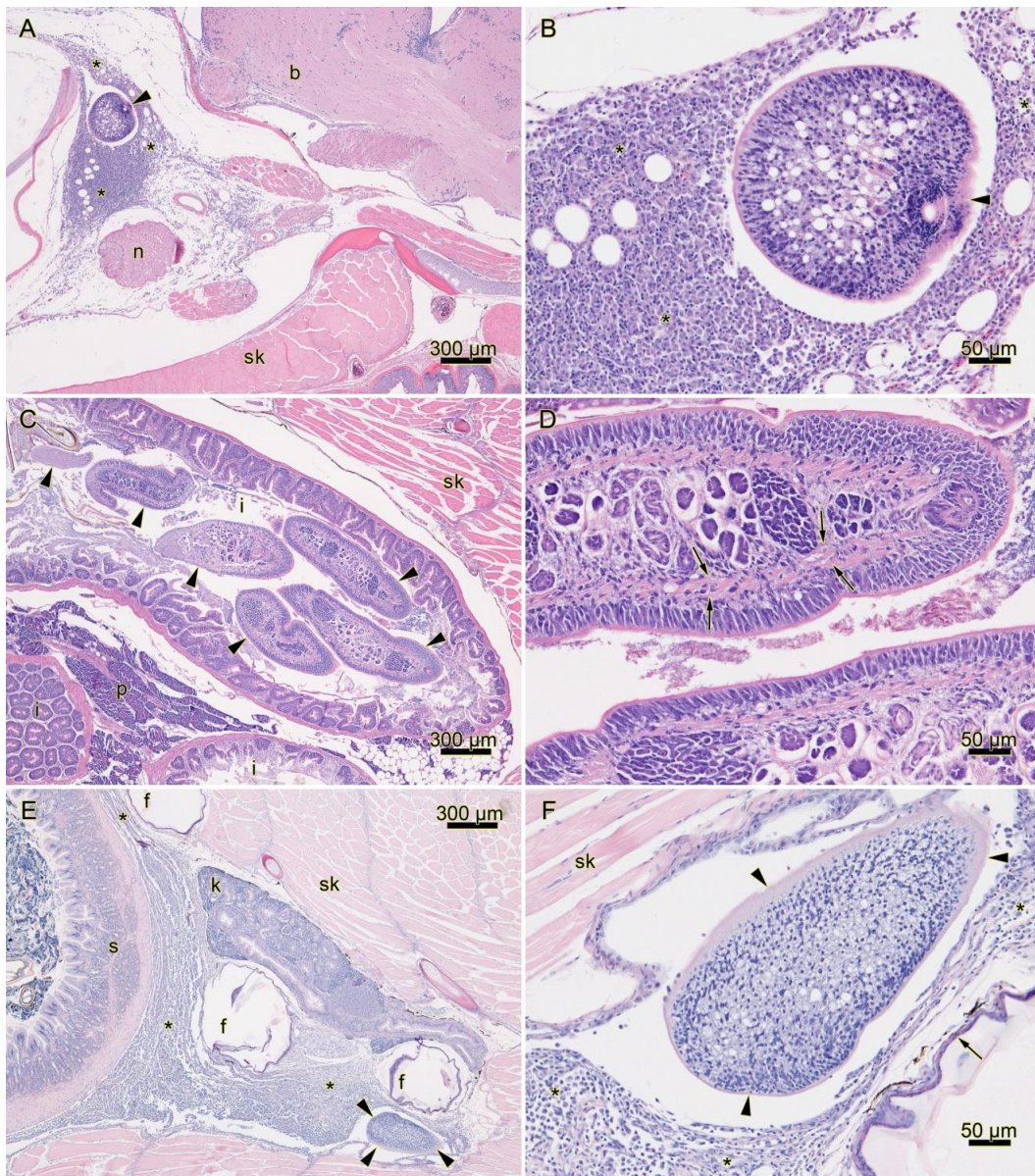


Figure 8. Photomicrographs of larval cestodes. H&E stain; within each row, the image on the right is a higher magnification view of part of the image on the left; skeletal muscle (sk). A and B. Section of the head from a 2.3 g male sampled from Croucher Creek in late September. A focus of granulomatous inflammation (*) in the loose connective tissue between the eye (rostral), brain (b), and cranial nerve (n) contains a larval cestode (arrowhead). C and D. Midsagittal section of the body of a 1.4 g juvenile male sampled from Wolf Creek in June; exocrine pancreas (p). The intestine (i) contains several maturing cestodes (arrowheads) but no associated inflammation; the cestodes have characteristic longitudinal muscles (arrows) beneath their cuticle. E and F. Parasagittal section of the body of a 0.6 g immature male sampled from Croucher Creek in late July. Granulomatous inflammation (*) bounded by the stomach (s) and trunk kidney (k) contains three fibrous capsule granulomas (f and arrow) and a larval cestode (arrowheads).

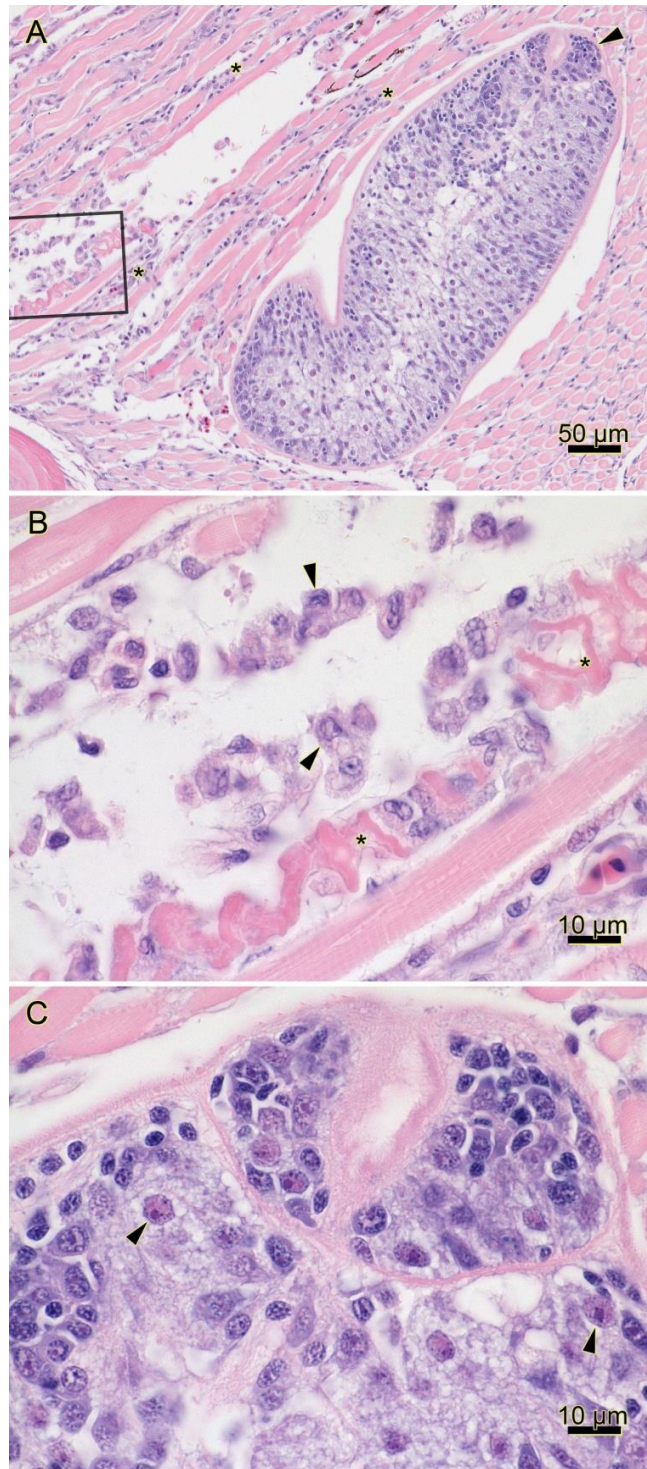


Figure 9. Photomicrographs of a larval cestode within skeletal muscle of the same fish as in Fig. 8 E and F; H&E stain. **A.** Overview. The cestode (arrowhead) splits myofibres, which contain small amounts of histiocytic inflammation (*). Black box in A outlines area shown at higher magnification in B. **B.** Inflammatory cells (arrowheads) are mixed with small numbers of necrotic myofibres (*). **C.** Distinctive eosinophilic nuclei (arrowheads) are scattered throughout the cestode parenchyma.

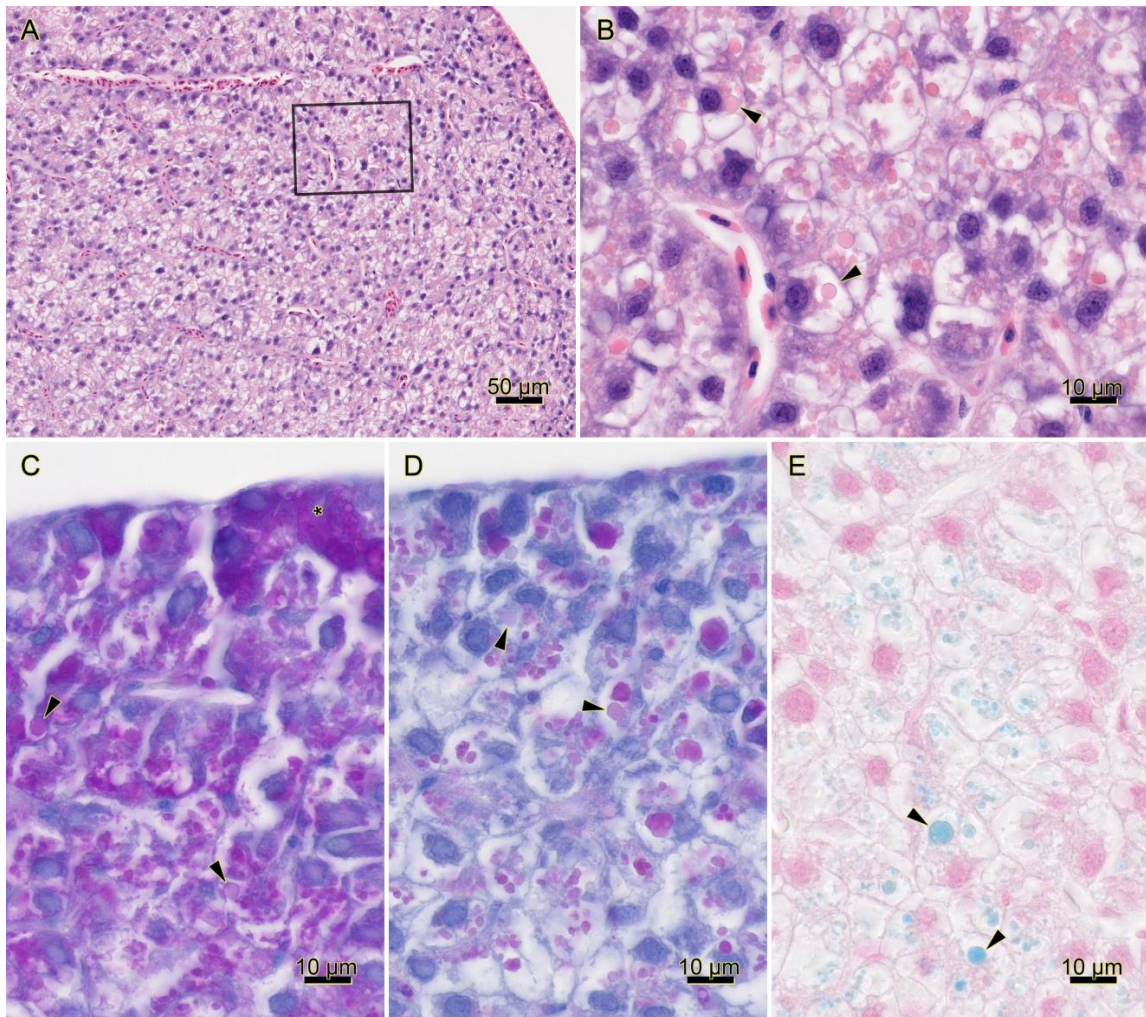


Figure 10. Photomicrographs of the liver from a 6.5 g male sampled from Wolf Creek in June. A and B. Most of the hepatocytes contain membrane-bound eosinophilic cytoplasmic droplets (arrowheads); H & E stain. Black box in A outlines area shown at higher magnification in B. C, D, and E were sectioned from the same paraffin block as A. C. Cytoplasmic glycogen stains diffusely with PAS (*); cytoplasmic droplets are also PAS-positive (arrowheads). D. With amylase digestion, PAS no longer stains glycogen, but cytoplasmic droplets (arrowheads) retain staining. E. Cytoplasmic droplets contain iron (arrowheads, Perl's iron stain).

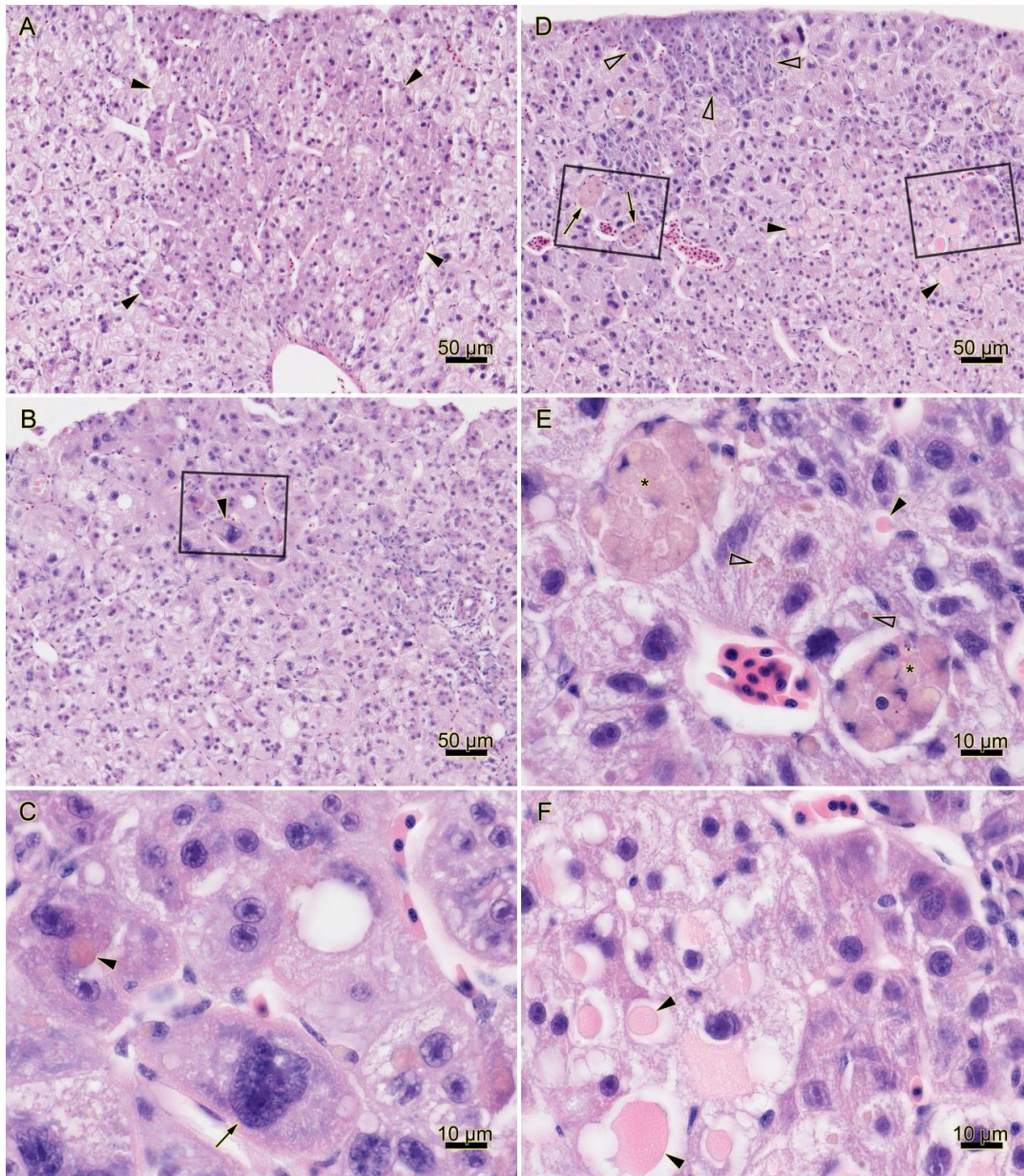


Figure 11. Photomicrographs of the liver from a 6.5 g male sampled from Clinton Creek in July; H & E stain. **A.** Eosinophilic focus of cellular alteration (arrowheads). **B.** Hepatocellular megalocytosis (arrowhead). Black box in B outlines area shown at higher magnification in C. Affected hepatocytes have enlarged nuclei (arrow) and membrane-bound eosinophilic cytoplasmic droplets (arrowhead). **D.** Basophilic focus of cellular alteration (open arrowheads), hepatocellular intracellular protein (arrowheads), and yellow-brown pigmented macrophage aggregates (arrows). Areas outlined by black boxes in D are shown at higher magnification in E (left box) and F (right box). **E.** Hepatocellular membrane-bound intracellular protein (arrowhead) and yellow-brown pigment (open arrowheads); pigmented macrophage aggregates (*). **F.** Hepatocellular membrane-bound intracellular protein (arrowhead).

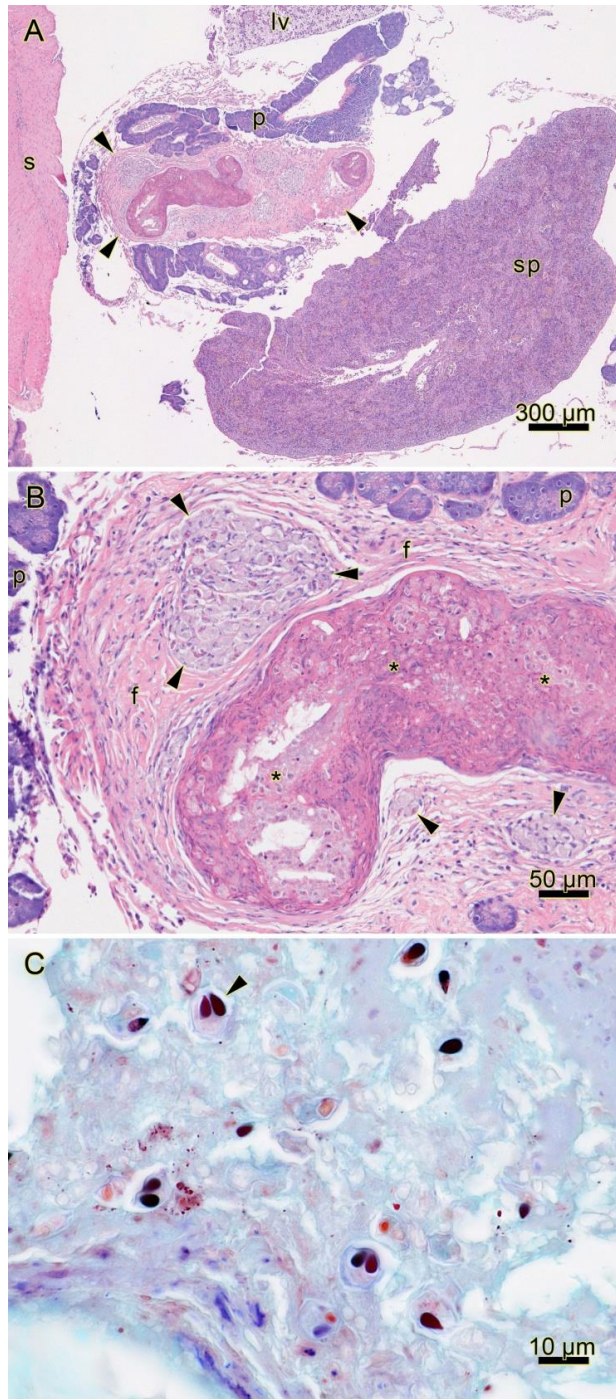


Figure 12. Photomicrographs of the viscera of a 4.8 g male sampled from Croucher Creek in May. **A.** A focus of granulomatous inflammation (arrowheads) expands the mesenteries between the stomach (s), spleen (sp), liver (lv), and endocrine pancreas (p); H & E stain. **B.** The focus of inflammation has a central core of necrosis (*) surrounded by foci of epithelioid macrophages (arrowheads) and fibrosis (f); H & E stain. **C.** Several intralesional myxosporean spores, each with two symmetrical, pyriform, polar capsules (arrowhead) consistent with a *Myxobolus* sp.; Twort's Gram stain.

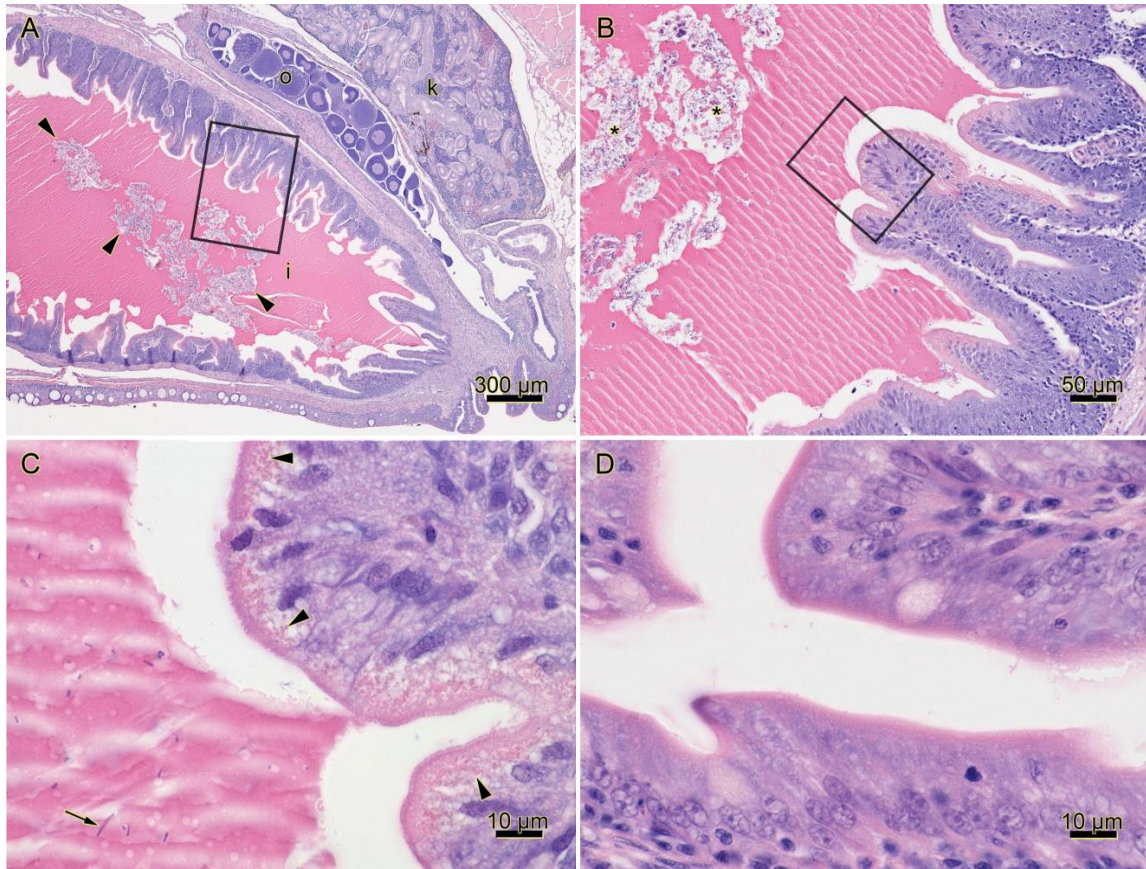


Figure 13. Photomicrographs of the caudal viscera of a 1.4 g juvenile female sampled from Wolf Creek in June (A – C); H & E stain. A. Overview of distal intestine (i), ovary (o), and trunk kidney (k); the intestinal lumen contains homogeneous protein with irregular colonies of bacteria (arrowheads); black box demarcates area shown in B. B. Higher magnification of the distal intestine, with colonies of bacterial rods (*) in the luminal protein; black box demarcates area shown in C. C. High magnification of apical epithelial protein droplets (arrowheads) and scattered bacterial rods (arrow) within the luminal protein. D. Intestinal epithelium from a 0.6 g juvenile male sampled from Croucher Creek in July. Neither the lumen nor the apical epithelium has brightly eosinophilic protein.

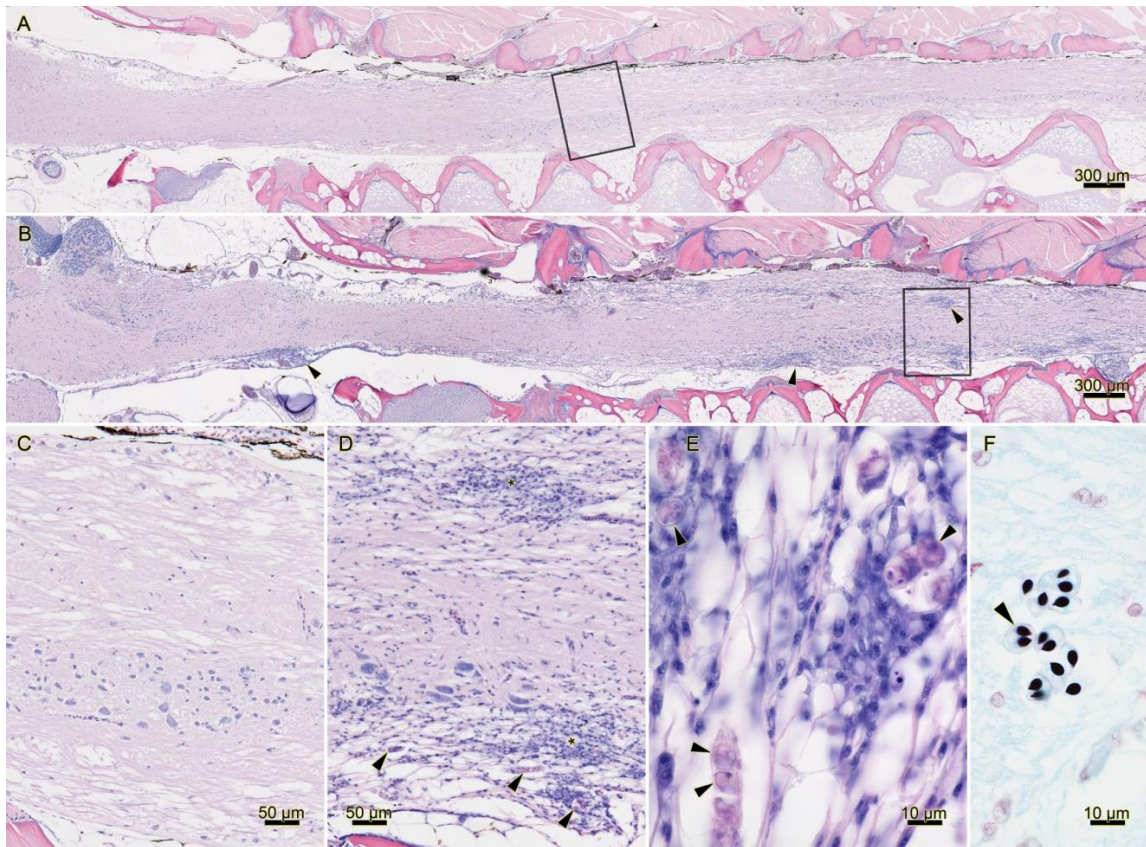


Figure 14. Photomicrographs of spinal cord (A – E); H & E stain. A. Overview of a normal spinal cord in a 1.9 g male sampled from Croucher Creek in July; black box demarcates area shown in C. B. Overview of an inflamed spinal cord (arrowheads) in a 2.6 g male sampled from Wolf Creek in July; black box demarcates area shown in D. D and E. Lymphohistiocytic inflammation (*) surrounds myxosporean parasites (arrowheads). F. Twort's Gram stain. Brainstem of a 7.0 g female sampled from Wolf Creek in June. Myxosporean spores (arrowhead) contain two symmetrical, goblet-shaped, polar capsules (arrowhead) consistent with a *Myxobolus* sp. Note that these spores are not associated with inflammation.

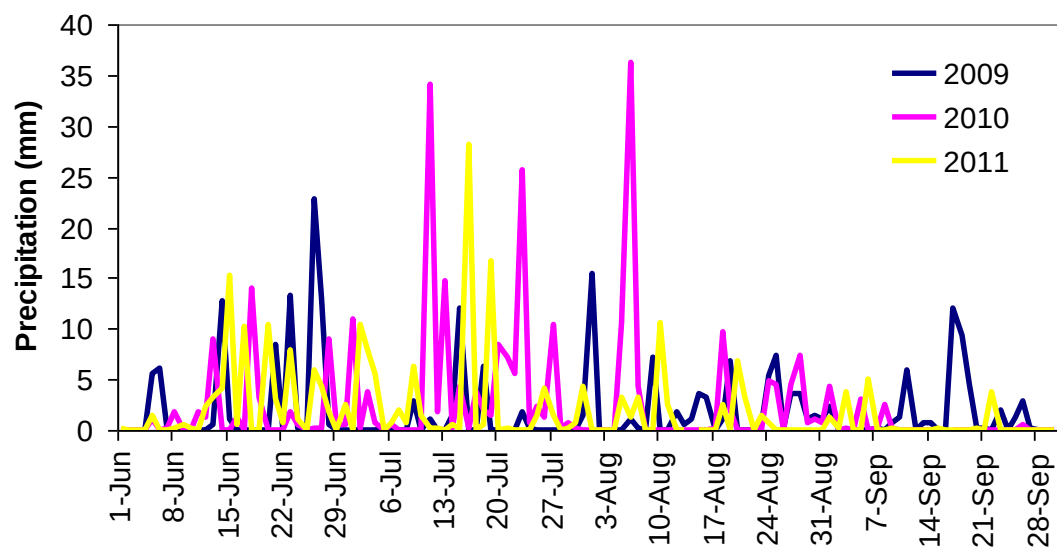


Figure 15. Comparison of precipitation recorded at Eagle, Alaska in 2009, 2010, and 2011. Record high precipitation was recorded in Eagle, AK in 2010. Only three years of data are shown to provide a general representation of precipitation variability.

Appendix 1. Compiled historical asbestos data for Clinton Creek

Location	Dates	Asbestos Concentration (x 10 ⁶ fb·L ⁻¹)	Source
Clinton Ck u/s of Hudgeon Lake	summers 1978-1980	nd - 14.2	Delaney et al 1981
Hudgeon Lake	Aug-95	0.198	Roach 1998
	Sep-98	<1.07 to 112.73	Royal Roads 1999
	Oct 1974 & March 1975	36.4 to 300	Landucci 1975
Clinton Ck u/s of Wolverine Ck	summer 2010	0.203 to 1.0554	LES 2011
	summers 1978-1980	nd to 64451	Delaney et al 1981
Clinton Creek at bridge	Aug-95	54.72	Roach 1998
Open Pit -porcupine pit	Aug-95	74.43	Roach 1998
Clinton Ck d/s Porcupine Ck u/s Wolverine Ck	summer 2010	0 to 4.3837	LES 2011
Clinton Creek at confluence with Wolverine Ck	Aug-95	142900	Roach 1998
Clinton Ck d/s Wolverine Ck u/s Eagle Ck	summer 2010	0 to 6.9007	LES 2011
Clinton Ck u/s Eagle Ck	Sep-98	4.29	Royal Roads 1999
	06-Sep-80	154.3	Delaney et al 1981
Clinton Ck d/s Eagle Ck	06-Sep-80	67.6	Delaney et al 1981
Clinton Ck near townsite	Jul-96	1.2	Roach 1998
	Sep-98	152.45	Royal Roads 1999
	summers 1978-1980	41.4 to 36024	Delaney et al 1981
Clinton Creek near confluence with Forty Mile	summer 2010	0 to 11.4495	LES 2011
Forty Mile River u/s of Clinton Creek	Oct-74	nd to 3	Landucci 1975
	Jul-96	0.718	Roach 1998
	Sep-98	1.07	Royal Roads 1999
	summers 1979-1980	nd to 43.6	Delaney et al 1981
Forty Mile River d/s of Clinton Creek	summer 2010	0	LES 2011
	Jul-96	0	Roach 1998
	06-Sep-80	2.7	Delaney et al 1981
Yukon River u/s of Forty Mile River	summer 1980	7.1 to 327.2	Delaney et al 1981
Yukon River d/s of Forty Mile River	Jul-96	33.51	Roach 1998
	summer 1980	0.5 to 6	Delaney et al 1981
Wolverine Creek u/s of culvert at road crossing	summer 2010	0 to 8.362	LES 2011
	summers 1978-1980	54.5 to 730676	Delaney et al 1981
Wolverine Creek at tailings slumps	Sep-98	22.62	Royal Roads 1999
	Sep-98	9.66	Royal Roads 1999
	Jul-96	0.167	Roach 1998
	Aug-95	1740	Roach 1998
Wolverine Creek u/s of tailings slumps	summers 1979-1980	nd to 38.5	Delaney et al 1981
Upstream of Porcupine Creek pond	summers 1978-1980	nd to 140.7	Delaney et al 1981
Eagle Creek u/s of confluence with Clinton	summer 1980	0.5 to 10.9	Delaney et al 1981
Bear Creek near Hudgeon Lake	summer 1980	3.3	Delaney et al 1981
nd: non-detect			
u/s: upstream			
d/s: downstream			

Appendix 2. Sampling, necropsy, and histopathology data for the 2010 Yukon Slimy sculpin health project.

Fall Slimy sculpin histopathology data

[illegible]

Fall Slimy sculpin histopathology data (con't)

				HEART -					SPLEEN -					STOMACH/INTESTINE -								GONAD -									
				ECD = epicarditis MKM = myocardial karyomegaly/megalocytosis END = endocarditis EEC = eosinophilic endocarditis GEC = granulomatous endocarditis HEC = histiocytic endocarditis LEC = lymphoplasmacytic endocarditis ENP = endocardial phagocytosis (atrium)					SMA = pigmented macrophage aggregates CON = congestion ELH = ellipsoid hypertrophy SGR = granulomatous inflammation					FAT = mesenteric adipose tissue FCG = fibrous capsule granuloma (serosal, includes specimens on margin of kidney; probable met NME = nematode with embryonated eggs (intestine) NMS = nematode (small) without eggs (intestine) EIM = interepithelial coccidian (<i>Eimeria</i> sp.?) GOU = superficial epithelial coccidian (<i>Goussia</i> sp.?) PER = peritonitis (includes margin of spleen and kidney)								Sex = male (M), female (F), immature male (IM), or immature female (IF) GFM = gonadal foamy macrophages OAM = oocyte atresia, mature follicles ORF = ovarian ruptured follicles ONG = oophoritis, nongranulomatous									
				coordinates in "Comments" (e.g., 35.4 x 112.3) = location of a specific finding on a slide; the structure/change can be found by placing the slide on the stage of the Nikon 50i microscope used by author G.D. Marty, with the frosted white part of the slide on the left.																											
#	Necropsy order	Slide #	Site	Heart					Heart Comments	Spleen					Spleen Comments	Stomach/Intestine/Intestinal ceca								Stomach/Intestine Comments	Gonad						Gonad Comments
				Atly	ECD	MKM	END	ENP		Atly	SMA	CON	ELH	SGR		Atly	FAT	FCG	NME	NMS	EIM	GOU	PER		Sex	Atly	GFM	OAM	ORF	ONG	
1	13	10-3728-15	Croucher Creek	0	0	0	0	0	part of heat on slide D2; LEC	0	2	2	0	0	0	0	0	0	0	1	M	0	0	NP	NP	NP	GFM has no organisms on Twort's Gram and Z-N Acid-fast stains;				
2	14	10-3728-2	Croucher Creek	0	0	0	0	0		0	2	1	0	0	0	0	0	1		M	0	3	NP	NP	NP						
3	15	10-3728-30	Croucher Creek	0	0	0	0	0		0	3	2	0	0	0	0	0	1		M	0	0	NP	NP	NP						
4	16	10-3728-18	Croucher Creek	0	0	0	0	0		1	2	1	0	0	0	0	0	0		M	0	0	NP	NP	NP						
5	17	10-3728-36	Croucher Creek	0	0	0	0	0	slide DI DL5 has normal heart anatomy;	1	2	1	0	0	0	0	0	0		F	0	1	1	0	0						
6	18	10-3728-13	Croucher Creek	0	0	0	0	0		1	1	2	0	0	0	0	0	0		M	0	0	NP	NP	NP						
7	19	10-3728-7	Croucher Creek	0	0	0	0	1	transverse septum contains granulomatous	0	0	3	0	0	0	0	0	0		M	0	0	NP	NP	NP						
8	20	10-3728-14	Croucher Creek	0	0	0	0	0		0	1	1	0	0	0	0	0	0		F	0	0	0	0	0						
9	21	10-3728-19	Croucher Creek	0	0	0	0	0		2	0	2	0	0	0	0	0	0		M	0	0	NP	NP	NP						
10	22	10-3728-5	Croucher Creek	0	0	0	0	0	DL2 has a pericardial granuloma, 220 µm in	1	0	1	0	0	0	0	0	0	1		IM	0	0	NP	NP	NP					
11	23	10-3728-35	Croucher Creek	0	0	0	0	0	D DL2 has a pericardial fibrous capsule gra	1	0	1	0	0	0	0	0	2		IM	0	0	NP	NP	NP						
12	24	10-3728-29	Croucher Creek	0	0	0	0	0		2	0	1	0	0	0	0	0	0		IM	0	0	NP	NP	NP						
13	25	10-3728-28	Wolf Creek	0	0	0	0	0		0	1	1	0	0	0	0	0	0		M	0	0	NP	NP	NP	GFM contains protein droplets (caudal piece in slide 6DL);					
14	26	10-3728-32	Wolf Creek	0	0	0	0	0		2	0	1	0	0	0	0	1	1	0		F	0	0	0	0		0				
15	27	10-3728-16	Wolf Creek	0	0	0	0	0		1	0	1	0	0	0	0	0	0		M	0	0	NP	NP	NP						
16	28	10-3728-27	Wolf Creek	0	0	0	0	0		0	1	3	0	0	0	0	1	0		M	0	0	NP	NP	NP						
17	29	10-3728-8	Wolf Creek	0	0	0	0	0		1	1	2	0	0	0	0	0	0		M	0	0	NP	NP	NP						
18	30	10-3728-24	Wolf Creek	0	0	0	0	0		2	0	2	0	0	0	0	0	0		M	0	0	NP	NP	NP						
19	31	10-3728-33	Wolf Creek	0	0	0	0	0		1	0	1	0	0	0	0	1	2	0		IM	0	0	NP	NP		NP				
20	32	10-3728-34	Wolf Creek	0	0	0	0	0		0	0	2	0	0	0	0	0	1		M	0	0	NP	NP	NP						
21	33	10-3728-12	Wolf Creek	0	0	0	0	0		0	1	1	0	0	0	0	0	0		F	0	0	0	0	0						
22	34	10-3728-3	Wolf Creek	0	0	0	0	0		0	0	2	0	0	0	0	2	0		M	0	0	NP	NP	NP						
23	35	10-3728-10	Wolf Creek	0	0	0	0	0		0	0	3	0	0	0	0	3	0		M	0	0	NP	NP	NP						
24	36	10-3728-6	Wolf Creek	0	0	0	0	0		1	1	1	0	0	0	0	0	0		F	0	1	0	0	0						
25	37	10-3728-37	Wolf Creek	0	0	0	0	0		0	0	3	0	0	0	0	0	0		M	0	0	NP	NP	NP						
26	38	10-3728-38	Wolf Creek	0	0	0	0	0		0	0	3	0	0	0	0	1	0	0		IF	0	0	NP	NP	0					
27	1	10-3728-25	Clinton Creek	0	0	0	0	1	LEC	0	1	3	0	0	0	0	0	0	0		M	0	0	NP	NP	NP	Count MIN MAX Prevalence				
28	2	10-3728-1	Clinton Creek	0	0	0	0	0		0	1	1	0	0	0	0	0	0		M	0	0	NP	NP	NP						
29	3	10-3728-26	Clinton Creek	0	0	0	0	0		0	1	3	0	0	0	0	0	1		M	0	0	NP	NP	NP						
30	4	10-3728-20	Clinton Creek	0	0	0	0	0		0	1	2	0	0	0	0	0	0		F	0	0	3	0	0						
31	5	10-3728-11	Clinton Creek	0	0	0	0	0		0	1	2	0	0	0	0	0	1		M	0	0	NP	NP	NP						
32	6	10-3728-17	Clinton Creek	0	0	0	0	0		0	2	2	0	0	0	0	0	0		M	0	0	NP	NP	NP						
33	7	10-3728-21	Clinton Creek	0	0	0	0	0		0	1	1	0	0	0	0	0	0		F	0	0	2	0	0						
34	8	10-3728-22	Clinton Creek	0	0	0	0	0		0	0	2	0	0	0	0	0	1		M	0	0	NP	NP	NP						
35	9	10-3728-31	Clinton Creek	0	0	0	0	0		1	1	0	0	0	0	0	0	0		M	0	0	NP	NP	NP						
36	10	10-3728-9	Clinton Creek	0	0	0	0	0		0	2	1	0	0	0	0	0	0		M	0	0	NP	NP	NP						
37	11	10-3728-4	Clinton Creek	0	0	0	0	0		0	0	1	0	0	0	0	0	0		F	0	2	1	0	0						
38	12	10-3728-23	Clinton Creek	0	0	0	0	0		0	0	2	0	0	0	0	0	0		M	0	0	NP	NP	NP						
		Count		38	38	38	38	38		38	38	38	38	38		38	38	38	38		# IM =	4	38	38	8	8	9	Count			
		MIN		0	0	0	0	0		0	0	0	0	0		0	0	0	0		# IF =	1	0	0	0	0	0	MIN			
		MAX		0	1	0	1	2		2	3	3	0	0		3	2	2	1	2	1	3	2	0	0	0	MAX				
		Prevalence		0.0	2.6	0.0	5.3	78.9		36.8	55.3	97.4	0.0	0.0		71.1	78.9	18.4	15.8	28.9	15.8	18.4	31.6	0.0	10.5	50.0	0.0	0.0	Prevalence		
												</																			

Fall Slimy sculpin histopathology data (con't)

KIDNEY -										Skin/skeletal muscle/spinal cord							GILL -								HEAD (brain and eye) - [although many head sections include gill; all gill findings are scored with GILL r]																		
ISH = interstitial (hematopoietic) cell hyperplasia TEP = tubular epithelial protein (intracytoplasmic) IPC = intratubular (luminal) protein casts GEP = golden epithelial pigment in tubules TEV = tubular epithelial vacuolation MYX = tubular or glomerular myxosporean (<i>Myxobilatus yukonensis</i>)										SEP = Epistylis sp. STR = <i>Trichodina</i> sp. SFC = fibrous capsule granuloma (probable metacercaria) MYO = myositis MML = meningomyelitis (inflammation of spinal cord, brainstem, and surrounding meninges)							GILL - (includes gill in A and D slides) BRS = branchitis (nonspecific) EPS = <i>Epistylis</i> sp. RFM = refractile material TRD = <i>Trichodina</i> sp. MON = monogenean parasite GLH = lamellar epithelial hyperplasia/hypertrophy								HEP = <i>Epistylis</i> sp. HTD = <i>Trichodina</i> sp. HMG = monogenean parasite HFC = fibrous capsule granuloma (probable metacercaria) ETT = eye trematode metacercariae (<i>Diplostomum</i> sp.? Location - lens or retina) MDN = myodegeneration and necrosis																		
Kidney										Skin/skeletal muscle/spinal cord							Gill								Head (brain and eye)								All organ										
Necropsy #	Slide #	Site	Atly	ISH	TEP	IPC	GEP	TEV	MYX	Trunk Kidney Comments	Atly	SEP	STR	SFC	MYO	MML	Skin/skeletal muscle/spinal cord Comments	Atly	BRS	EPS	RFM	TRD	MON	GLH	Gill Comments	Atly	HEP	HTD	HMG	HFC	ETT	MDN	HEAD (brain and eye) Comments	CES									
1	13	10-3728-15	Croucher Creek	0	0	0	0	1	0	0	pigmented macrophage aggregates contain slide DL DL3 contains 2 tubules with birefr	0	0	0	1	0	0	MYO is mostly granulomatous inflammation	0	0	0	1	2	0	0	TRD on slides A, B; and D1;	0	1	0	0	1	1	0	HFC on slide D2 (23 x 117); focus of granu	0								
2	14	10-3728-2	Croucher Creek	0	0	0	0	1	0	0		0	0	0	0	1	0		0	0	0	0	0	0	0		0	0	0	0	0	0	1		1	0	ETT in retina and lens (slide DL DL2)	1					
3	15	10-3728-30	Croucher Creek	0	0	0	0	1	0	0		0	0	0	0	1	0		0	0	0	0	1	0	0		0	0	0	0	0	0	1		0	focus of myositis contains the edge of a pa	1						
4	16	10-3728-18	Croucher Creek	0	0	0	0	1	0	0		0	1	0	0	2	0		0	0	0	0	1	0	0		0	0	0	0	0	0	1		0	ETT has one in left lens and 7 in right lens;	0						
5	17	10-3728-36	Croucher Creek	0	0	0	0	1	0	0		0	0	0	0	2	0		0	0	0	0	1	0	0		0	0	0	0	0	1	2		0	PHOTO - focus of granulomatous inflamma	1						
6	18	10-3728-13	Croucher Creek	0	0	0	0	0	0	0		0	0	0	0	1	0		0	0	0	1	1	0	0		0	0	0	0	0	0	0		1	0		0					
7	19	10-3728-7	Croucher Creek	0	0	0	0	0	0	0		0	0	0	0	0	0		0	0	0	1	0	0	0		0	0	0	0	1	0	0		0	0		0					
8	20	10-3728-14	Croucher Creek	0	0	0	0	0	0	0		0	0	0	0	0	0		0	0	0	1	1	0	0		0	0	0	0	1	0	0		1	0		0					
9	21	10-3728-19	Croucher Creek	0	0	0	0	1	0	0		0	1	0	0	1	0		0	0	0	1	1	0	0		0	0	0	0	1	0	0		1	0	0	HFC (retrobulbar) contains pieces of a trem	1				
10	22	10-3728-5	Croucher Creek	0	0	0	0	0	0	0		0	0	0	1	2	0		0	0	0	1	1	0	0		0	0	0	1	0	0	1		1	0	ETT in retina and lens (slide DL2)	1					
11	23	10-3728-35	Croucher Creek	0	0	0	0	0	0	0		0	0	1	0	0	0		0	0	0	0	0	0	0		0	0	0	1	0	0	0		0	0	0		1				
12	24	10-3728-29	Croucher Creek	0	0	0	0	0	0	0		0	0	0	0	0	0		0	spinal cord has two foci of granulomatous in	0	0	1	0	0		0	0	0	0	1	0	0		2	1	0	large focus of granulomatous inflammation	1				
13	25	10-3728-28	Wolf Creek	0	0	0	0	0	0	0	MYO includes myonecrosis; MYO in slide D DL2 (23 x 118); MML has a focus of subcutaneous granulomatous inflar MML has no organisms (slide D DL2, 22 x 3 granulomatous inflammation in adipose tiss MML in slide D1 only (19.5 x 111.5); GEP in slide D; MML has no organisms (slide D DL1, 12 x 3 MML has no organisms (slide D DL1);	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	fish probably 5 years old (bone for aging in	0		
14	26	10-3728-32	Wolf Creek	0	0	0	0	0	0	0		0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0		0							
15	27	10-3728-16	Wolf Creek	0	0	0	0	0	0	0		0	1	0	0	0	0	3	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0		1							
16	28	10-3728-27	Wolf Creek	0	1	0	0	0	0	0		0	0	0	0	1	2	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0		0							
17	29	10-3728-8	Wolf Creek	0	0	0	0	0	0	0		0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	pectoral girdle bone: osteomyelitis, granul	0							
18	30	10-3728-24	Wolf Creek	0	0	0	0	0	0	0		0	1	0	0	0	3	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0		0							
19	31	10-3728-33	Wolf Creek	0	0	0	0	0	0	0		0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0		0							
20	32	10-3728-34	Wolf Creek	0	2	0	0	0	0	0		0	1	0	0	2	1	0	0	0	0	1	1	0	1	0	0	0	1	0	0	0	0	0	granulomatous inflammation in skeletal mu	0							
21	33	10-3728-12	Wolf Creek	0	0	0	0	1	0	0		GEP in slide D; MML has no organisms (slide D DL1, 12 x 3 MML has no organisms (slide D DL1);	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	focus of myositis, mild;	0		
22	34	10-3728-3	Wolf Creek	0	0	0	0	0	0	0			0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0		0						
23	35	10-3728-10	Wolf Creek	0	0	0	0	1	0	0			0	1	0	0	0	2	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0		0						
24	36	10-3728-6	Wolf Creek	0	0	1	0	0	0	0			0	0	0	0	0	2	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0		0						
25	37	10-3728-37	Wolf Creek	0	0	0	0	0	0	0	0		1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0		0							
26	38	10-3728-38	Wolf Creek	0	0	0	0	0	0	0	0		0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0		0							
27	1	10-3728-25	Clinton Creek	0	0	0	0	1	0	0	Subcutaneous adipose tissue - steatitis, gra granulomatous inflammation of neurosensor MML has no organisms (slide DR DL1, 28.5		0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	ETT has 2 trematodes in one retina; 1 trem	0
28	2	10-3728-1	Clinton Creek	0	0	0	0	0	0	0			0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0		0						
29	3	10-3728-26	Clinton Creek	0	0	0	0	0	0	0			0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	ETT has 2 trematodes in one retina; 0 trem	0							
30	4	10-3728-20	Clinton Creek	0	0	0	0	1	0	0			0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0		0						
31	5	10-3728-11	Clinton Creek	0	0	0	0	0	0	0			0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0		0						
32	6	10-3728-17	Clinton Creek	0	1	0	0	1	0	0			0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	granulomatous inflammation of neurosenso	0						
33	7	10-3728-21	Clinton Creek	0	0	0	0	1	0	0		0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	2	0	ETT has 11 trematodes in one retina and 8	0							
34	8	10-3728-22	Clinton Creek	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	2	0	0	ETT has 14-15 trematodes in each retina;	0							
35	9	10-3728-31	Clinton Creek	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	HTD limited to pocket in dorsal lip (slide Df	0							
36	10	10-3728-9	Clinton Creek	0	0	0	0	1	0	0		0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	pectoral girdle bone: osteomyelitis, granul	0							
37	11	10-3728-4	Clinton Creek	0	0	0	0	1	0	0		0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0		0							
38	12	10-3728-23	Clinton Creek	0	0	0	0	1	0	0		0	1	0	0	0	1	0	0	0	1	1	1	1	0	0	0	0	1	0	0	0	0	0	slide DR DL1 has an 80-µm-diameter focus	0							
= site differences	Count			38	38	38	38	38	38		38	38	38	38	38	38		38	38	38	38	38	38	38		38	38	38	38	38	38	38		38									
	MIN			0	0	0	0	0	0		0	0	0	0	0	0		0	0	0	0	0	0	0		0	0	0	0	0	0	0		0									
	MAX			0	2	1	0	1	0		0	1	1	1	2	3		0	0	1	1	2	1	1		0	1	1	0	2	2	1		1									
	Prevalence			0.0	7.9	2.6	0.0	39.5	0.0	0.0	0.0	26.3	2.6	5.3	28.9	36.8		0.0	0.0	76.3	60.5	10.5	2.6	2.6		0.0	52.6	2.6	0.0	18.4	31.6	5.3		21.1									

Appendix 3. Supplemental photomicrograph plates of Slimy sculpin histopathology features from Croucher, Wolf, and Clinton Creeks, YT in 2010.

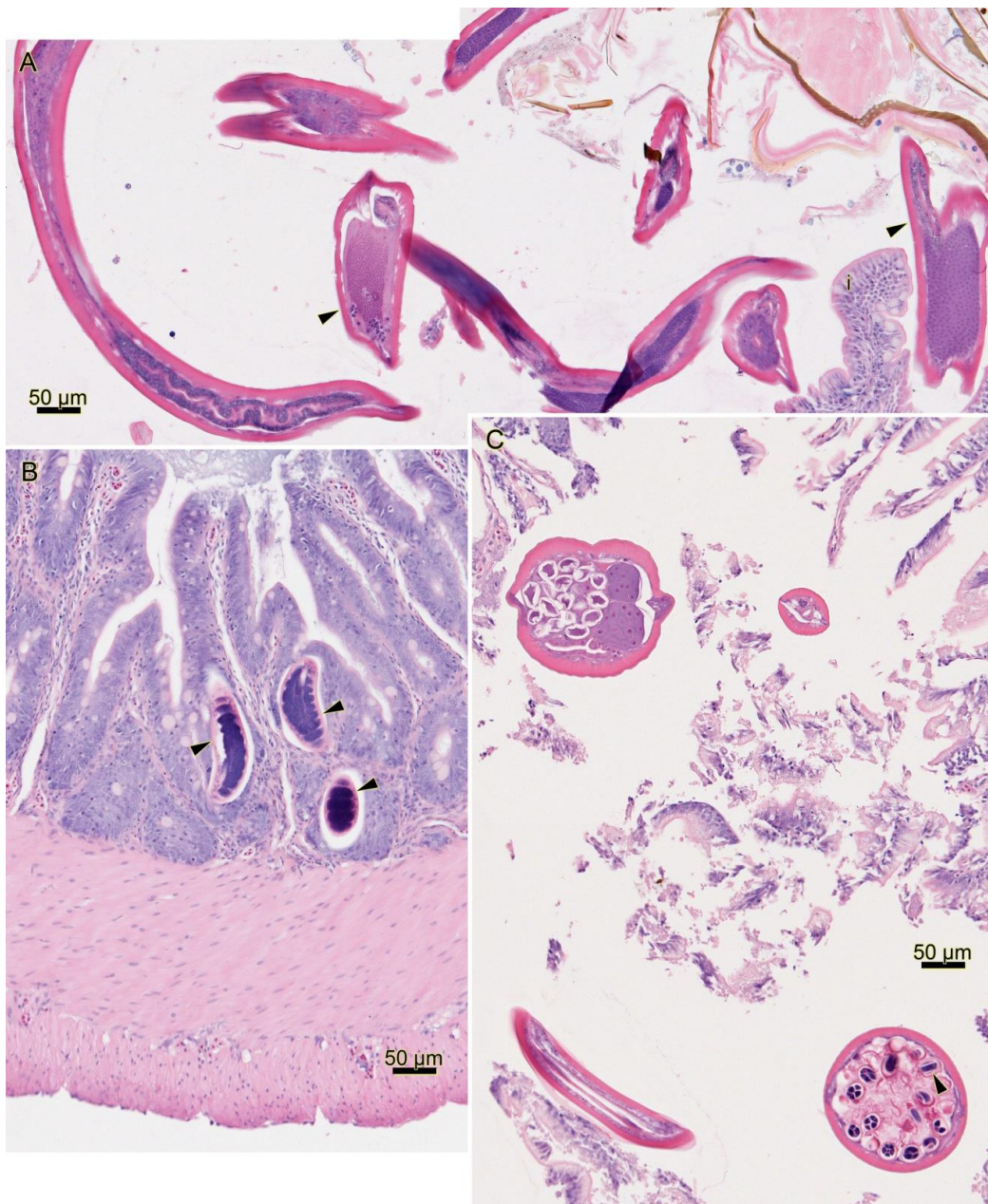


Plate A. Photomicrographs of nematode parasites within the intestinal lumen; H & E stain. A. An 8.4 g female sampled from Wolf Creek in early October. The diameter of the nematodes (arrowheads) is about the same as an intestinal villus (i). B. A 6.7 g male captured from Clinton Creek in July. Larval nematodes (arrowheads) are deep within the crypts. C. A 4.8 g female sampled from Wolf Creek in early October. Adult nematodes sometimes contain embryonated eggs (arrowhead).

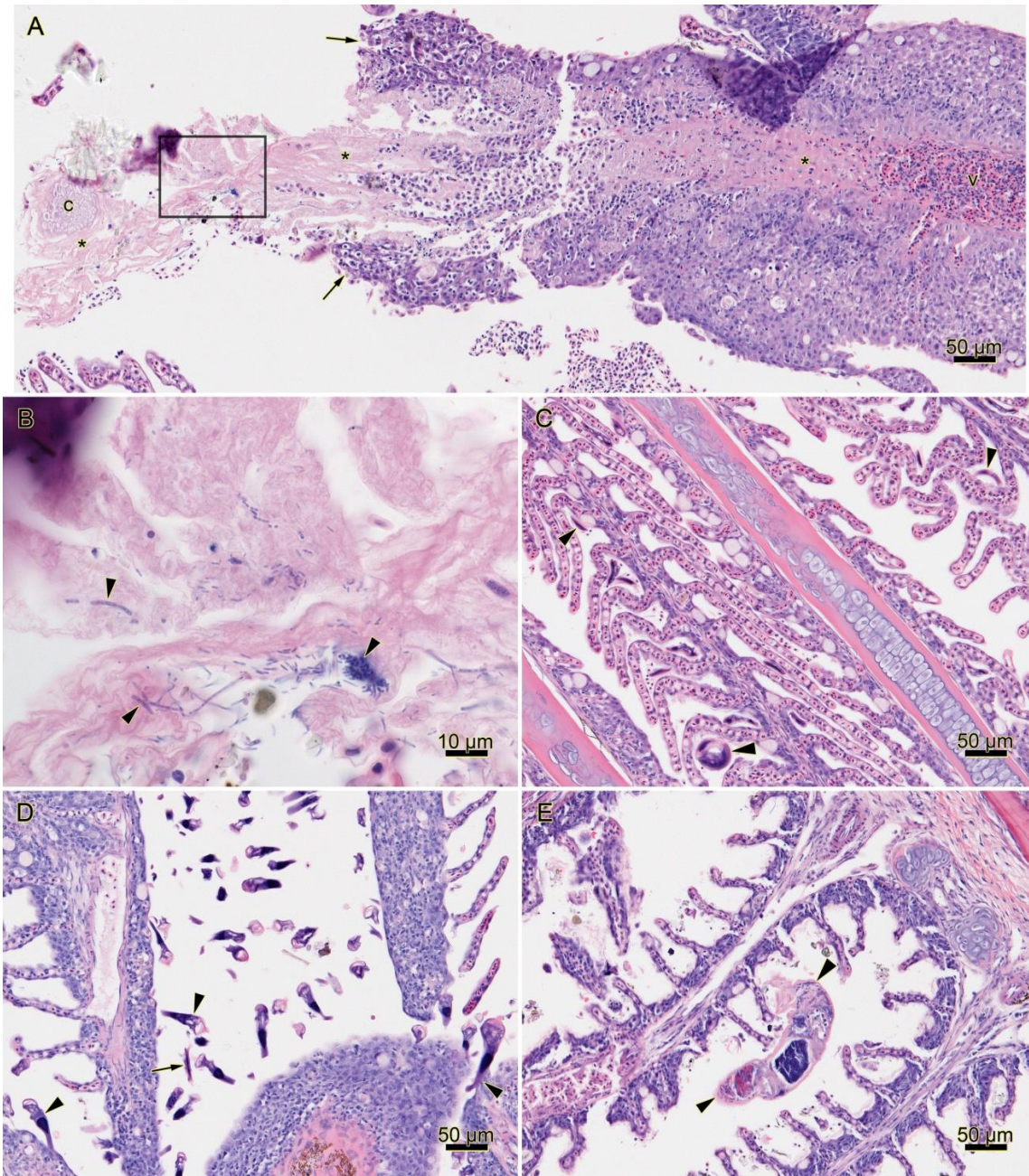


Plate B. Photomicrographs of gill lesions; H & E stain. A and B. A 10.2 g female sampled from Wolf Creek in June. An occlusive cellular thrombus in a filament blood vessel (v) has resulted in necrosis (*) of the distal filament with intralesional bacterial rods (arrowheads), crystals (c), and attempts at re-epithelialization (arrows). Black box in A outlines area shown at higher magnification in B. C. A 12.5 g male sampled from Clinton Creek in May. The interlamellar space contains abundant ciliated protozoa (*Trichodina* sp., arrowheads). D. A 4.9 g male sampled from Croucher Creek in May. Gill surfaces are covered by abundant sessile ciliates (*Epistylis* sp., arrowheads) and an occasional *Trichodina* sp. (arrow). E. A 3.5 g fish (sex not determined) sampled from Croucher Creek in May. A monogenean parasite (arrowheads) is attached to the surface of a lamella.

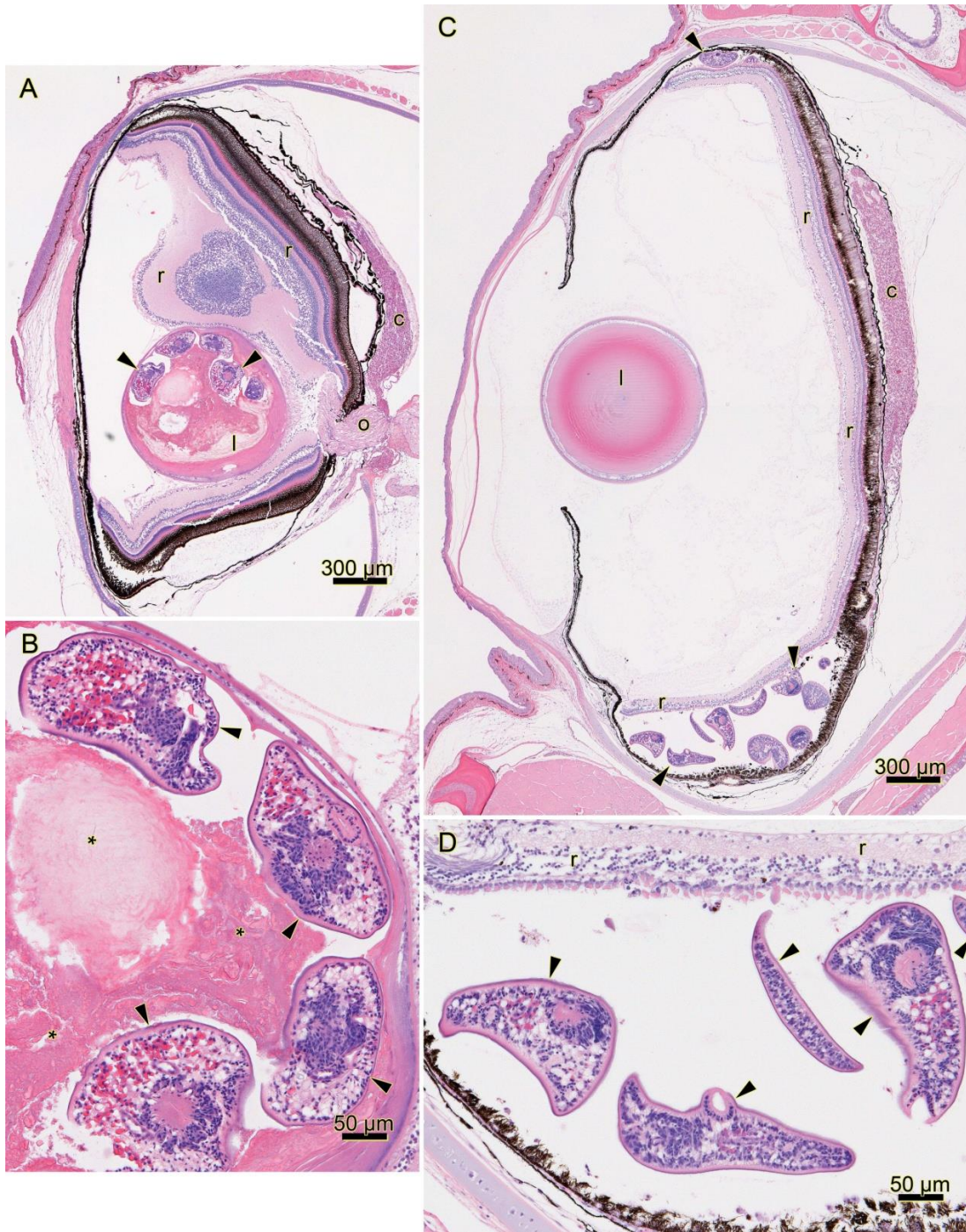


Plate C. Photomicrographs of eyes; H & E stain. A. Eye of a 4.8 g male sampled from Croucher Creek in May. The lens (l) contains 5 metacercariae (arrowheads); the retina (r), optic nerve (o), and choroid plexus (c) are intact. **B.** Higher magnification of the lens in A. Dissolution of the lens protein (*) is associated with the metacercariae (arrowheads). **C.** Eye of a 7.2 g male sampled from Clinton Creek in September. The lens (l) and choroid plexus (c) are normal, but the retina (r) contains several metacercariae (arrowheads). **D.** Higher magnification of the ventral retina in B. Metacercariae (arrowheads) separate the retina (r) between the layer of rods and cones and the layer of pigment epithelium.

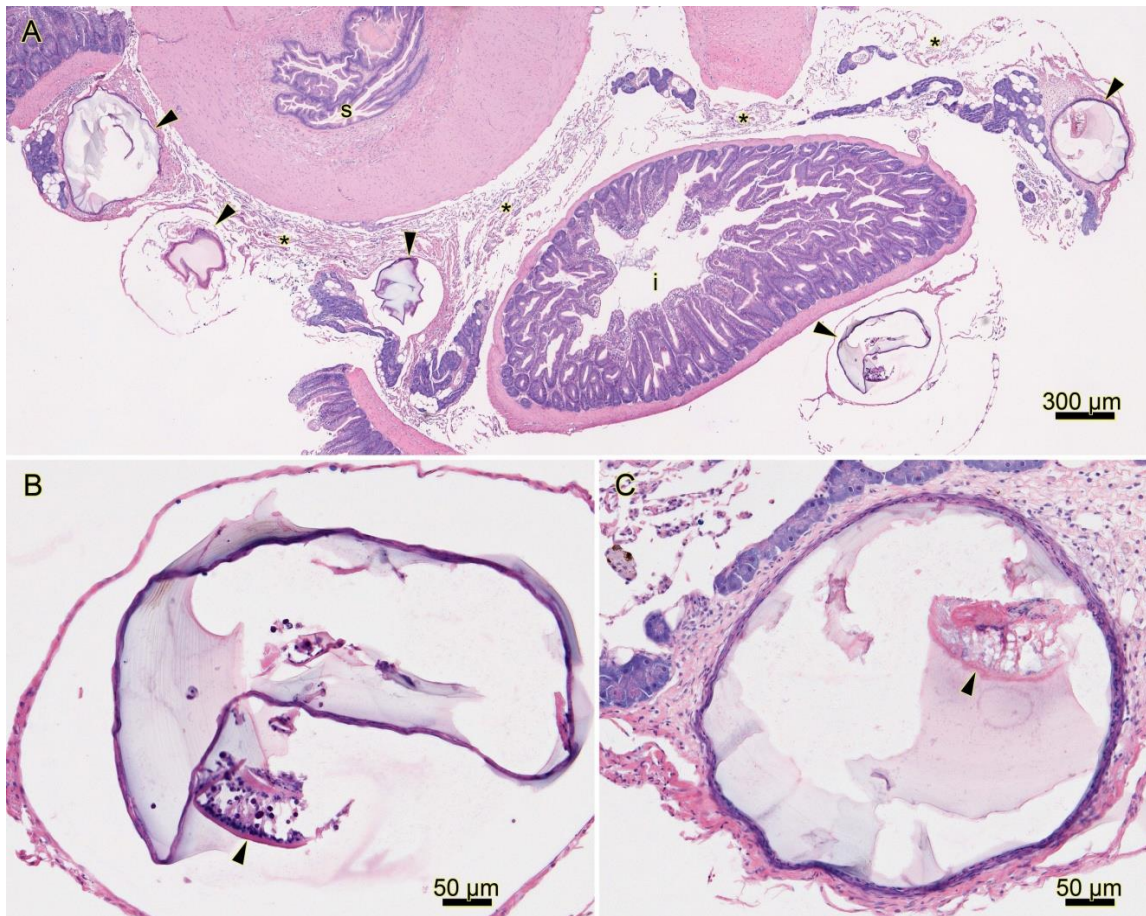


Plate D. Photomicrographs of viscera of a 4.8 g male sampled from Croucher Creek in May (the same fish as in Figure 12). H & E stain. A. Overview includes five fibrous capsule granulomas (arrowheads) partly surrounded by fibrillar inflammation (*); stomach (s), intestine (i). B and C. Higher magnification of fibrous capsule granulomas in A; degenerating metacercariae (arrowheads) are surrounded by a mucoid matrix.

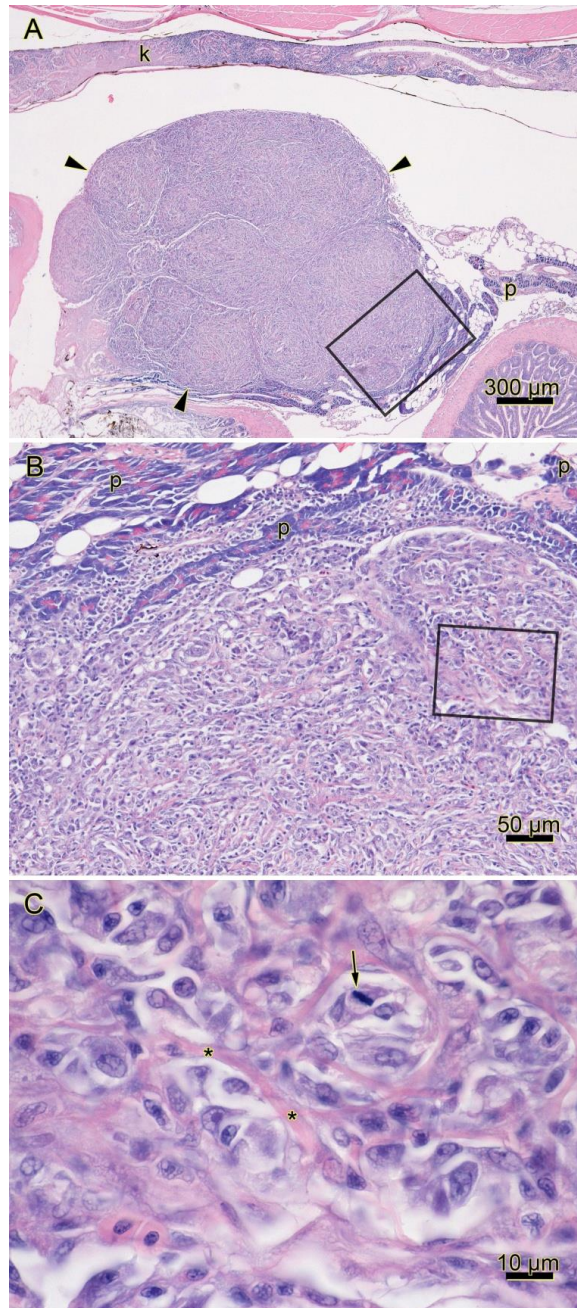


Plate E. Photomicrographs of midsagittal section of the viscera of a 1.8 g male sampled from Wolf Creek in early October. The mesenteries are expanded by a poorly differentiated tumour (arrowheads) that expands into the surrounding exocrine pancreas (p); trunk kidney (k). Tumour cells are arranged in irregular packets by thin bands of collagen (*), and mitotic figures (arrow) are uncommon. Black box in A outlines area shown at higher magnification in B. Black box in B outlines area shown at higher magnification in C. H & E stain.

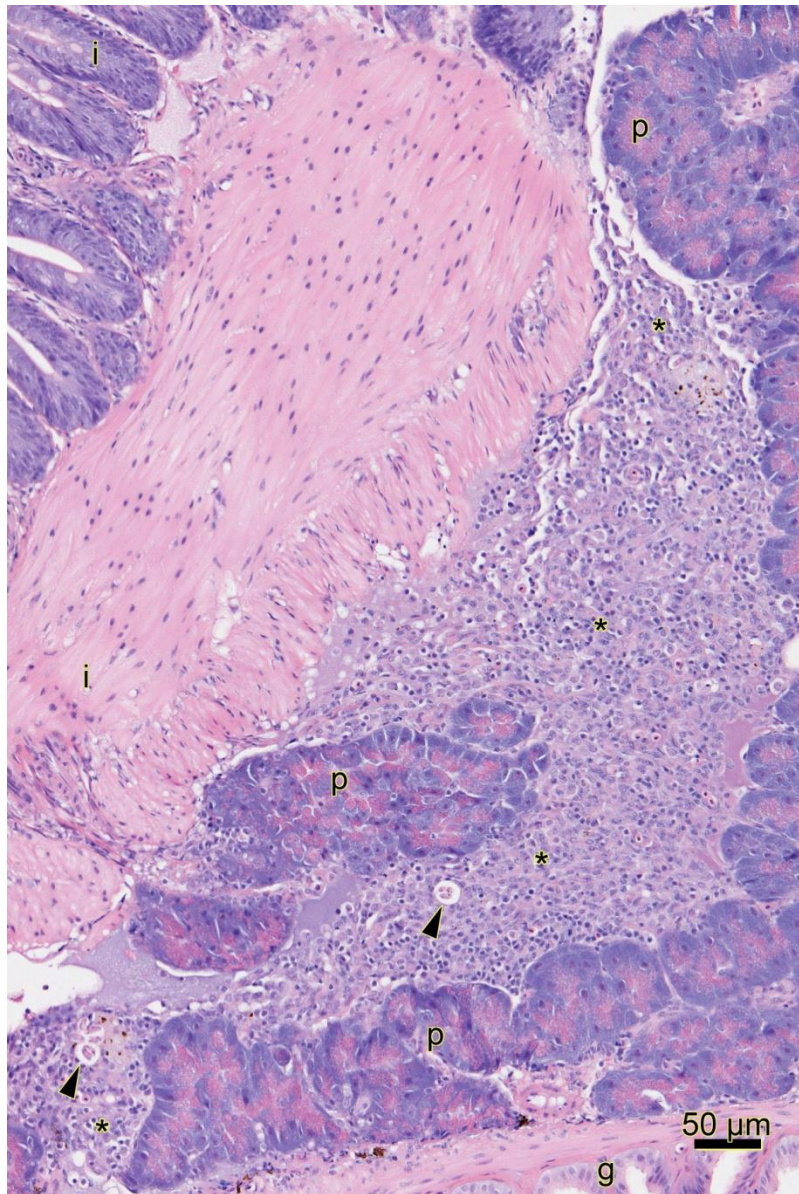


Plate F. Photomicrograph of section of viscera from a 3.2 g female sampled from Croucher Creek in May. Granulomatous inflammation (*) in the mesenteries between the intestine (i), gallbladder (g), and exocrine pancreas (p) contains small numbers of sporulated oocysts (arrowheads). H & E stain.

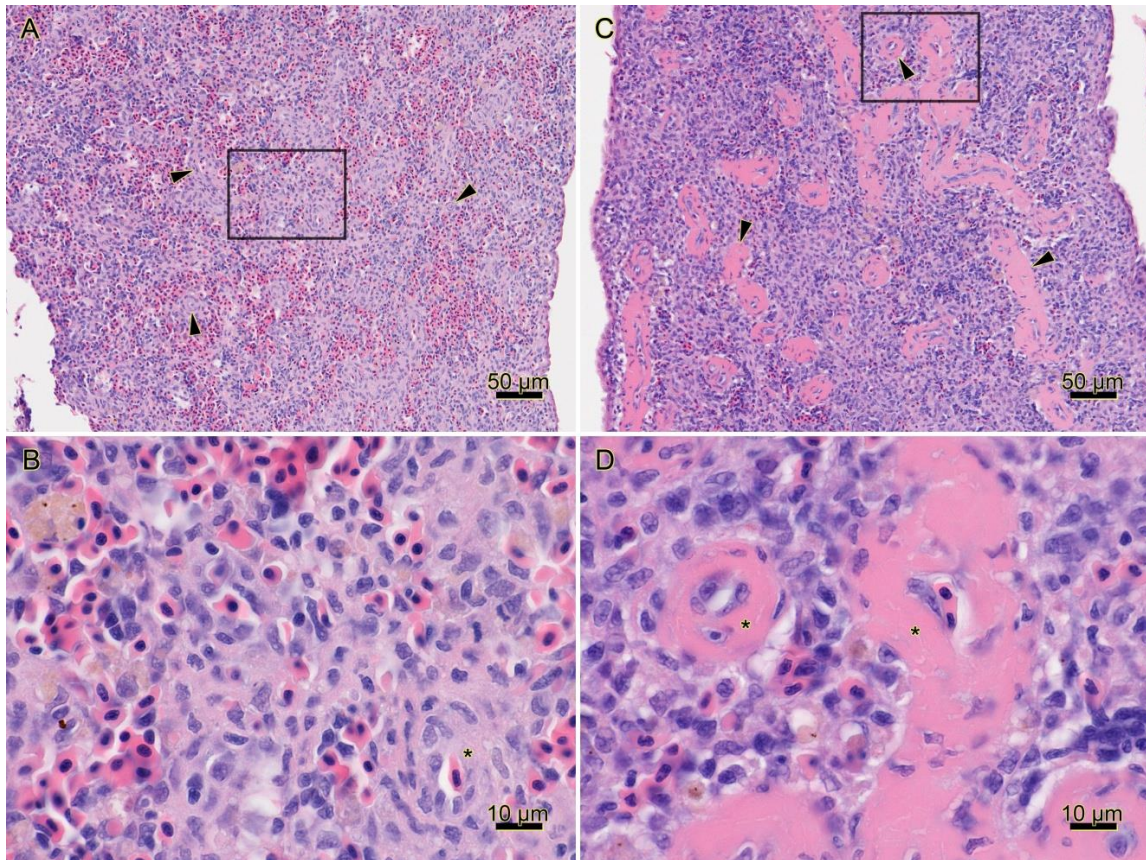


Plate G. Photomicrographs of postspawning sex differences in spleen morphology; H & E stain. A and B. A 3.1 g male sampled from Clinton Creek in May. Ellipsoids (arrowheads in A; * in B) are not prominent. Black box in A outlines area shown at higher magnification in B. C and D. A 7.6 g female sampled from Wolf Creek in June. Ellipsoids (arrowheads in C; * in D) are prominent and eosinophilic. Black box in C outlines area shown at higher magnification in D.

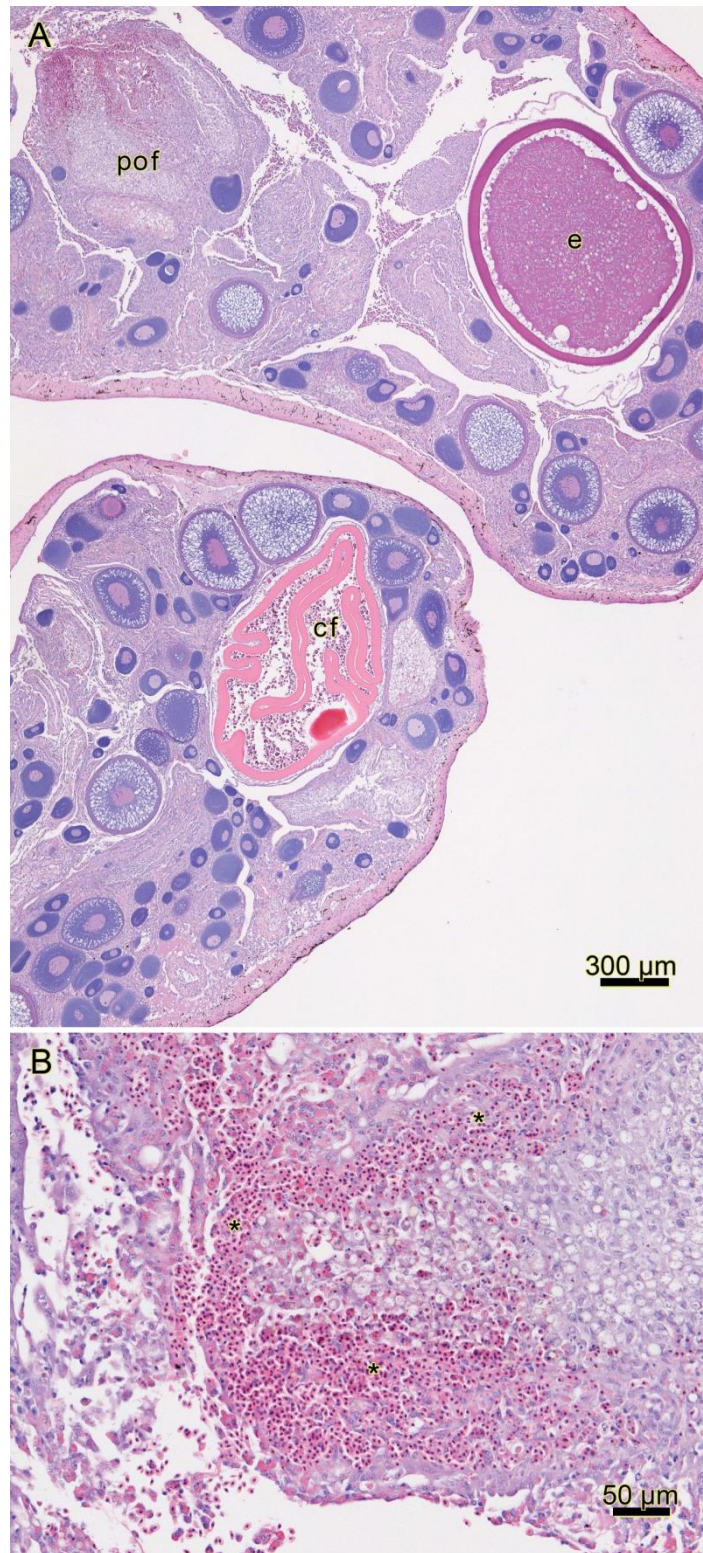


Plate H. Photomicrographs of postspawning ovary from a 4.6 g female sampled from Clinton Creek in May; H & E stain. **A.** Overview includes several small oögonia and medium-sized developing follicles, plus one each of a postovulatory follicle (pof), unspawned egg (e), and a collapsed mature follicle (cf). **B.** Higher magnification of the postovulatory follicle contains superficial hemorrhage (*) admixed with a few macrophages and plump follicular epithelial cells.

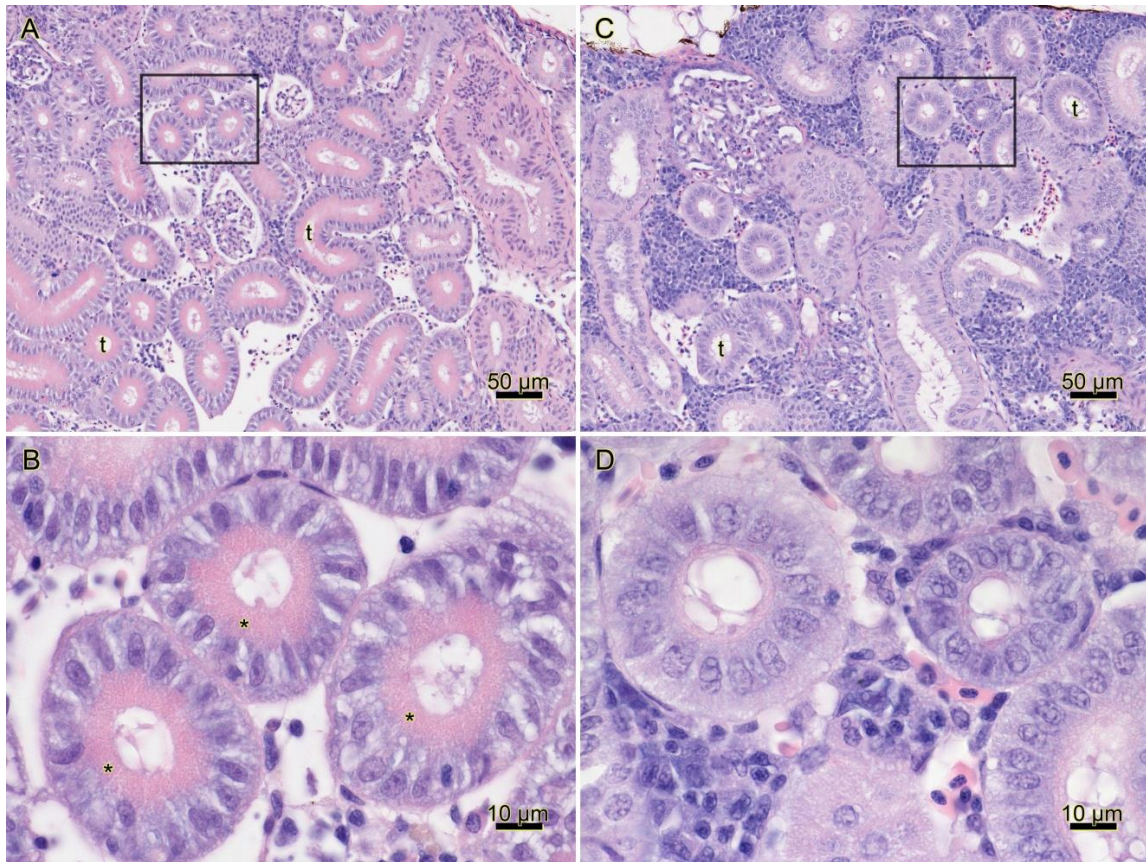


Plate I. Photomicrographs of postspawning differences in trunk kidney morphology; H & E stain. A and B. A 4.8 g male sampled from Croucher Creek in May. Apical epithelium of tubules (t) contains abundant, small, brightly eosinophilic protein droplets (*). Black box in A outlines area shown at higher magnification in B. C and D. A 4.9 g male sampled from Wolf Creek in June. Apical epithelium of tubules (t) does not contain eosinophilic protein droplets. Black box in C outlines area shown at higher magnification in D.

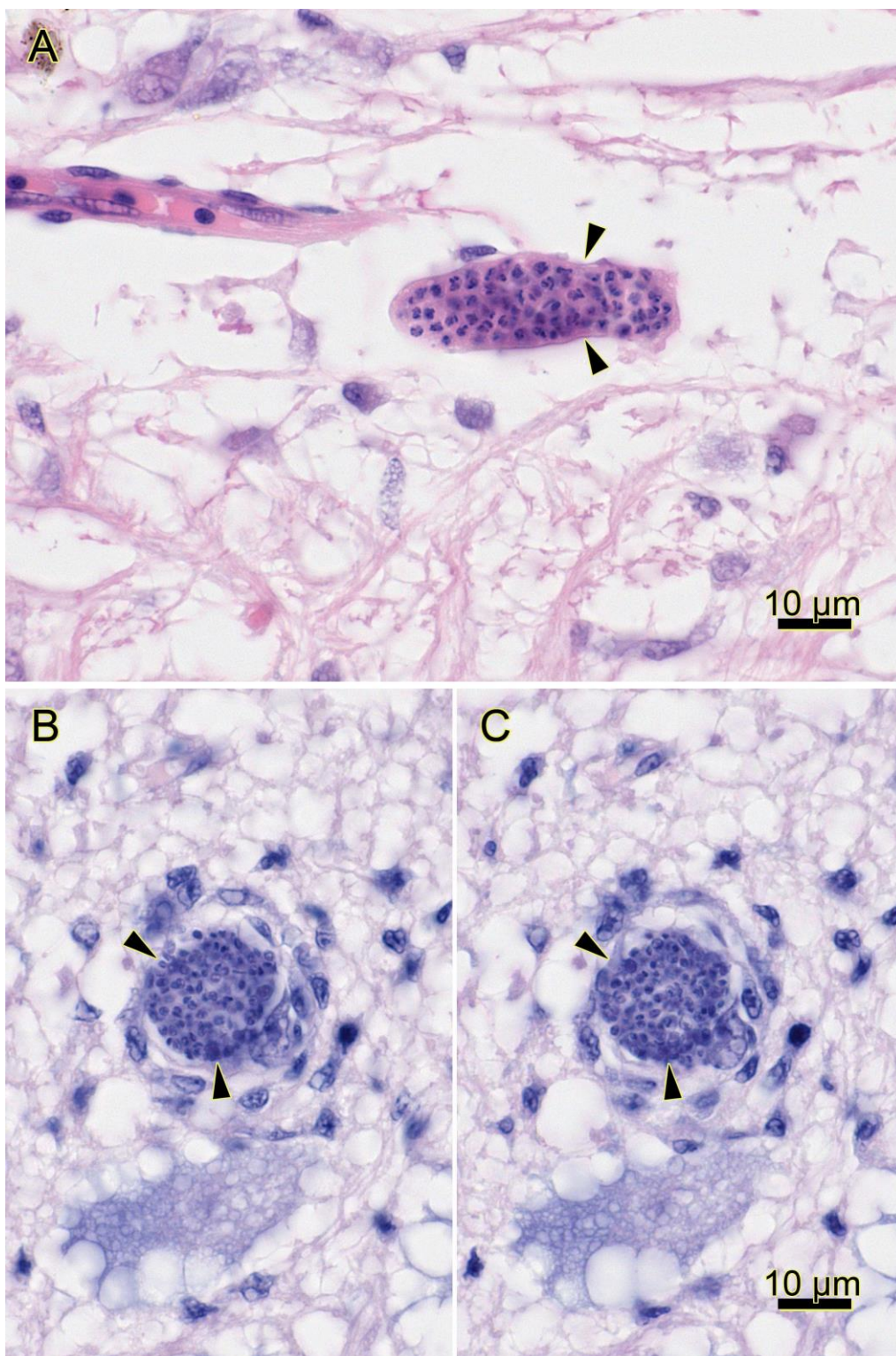


Plate J. Photomicrographs of multicellular parasite that might be a myxosporean trophozoite. A. A longitudinal section of the spinal cord of a 2.4 g male sampled from Clinton Creek in July. Longitudinal section through the parasite. B and C. A cross section of the spinal cord of a 8.0 g male sampled from Wolf Creek in July. Cross section of the parasite; images are from two different focal planes of the same section. H & E stain.

Appendix 4. Summary descriptions of tissue characteristics and lesions examined in the 2010 Yukon slimy sculpin health project.

Liver

The liver is, by far, the most studied organ in terms of toxic effects. The liver is the site of detoxification mechanisms such as the mixed function oxidase system and metallothionein; it produces many regulatory proteins; it is the site of energy storage – both lipid and glycogen; and it synthesizes bile. Present hepatic biomarkers include: hepatocellular coagulative necrosis, hyperplasia of regeneration, bile ductular/ductal hyperplasia, hepatocytomegaly (hepatocellular hypertrophy), foci of cellular alteration, and hepatic and bile duct neoplasms. These biomarkers and the contaminants that cause the documented changes have all been previously described by Hinton and Laurén (1990), Hinton et al. (1992), and Hinton (1993) (*in Blazer 2000*).

GLY: hepatocellular glycogen

Hepatocellular (liver) glycogen is best described as a measure of the readily available energy stores in the fish and is normally assessed by visual observation (pale to pink).

LIP: lipidosis

Lipidosis is a disorder of lipid metabolism, but in many species living in cold environments lipid in the liver is normal. Lipidosis can result from starvation or effects of toxic substances, for example. It manifests as lesions in the liver. If an otherwise healthy fish did not eat for an extended period of time (i.e., was starving), its own fat stores could be mobilized and could accumulate in the liver resulting in what appears as an excessively fatty liver.

COC: coccidians (in the liver)

Coccidians are protists (protozoans, single-celled organisms) where infection rarely leads to disease outbreaks. Some can cause disease, particularly in younger fish. An infected fish may become more prone to infection by other, more harmful, parasites and diseases (pathogens). In general, coccidians can be found in a number of tissues, including the intestine, swim bladder, liver, and reproductive structures. *Goussia sp.* and *Eimeria sp.* are common genera and can infect a variety of fish species. Coccidian infections in fish organs can prevent normal organ function because they destroy tissue (necrosis). For example, coccidian infection of the gut can reduce surface area making it harder for fish to absorb necessary nutrients resulting in a decrease in fish condition. Stress, crowding, poor water quality, and degraded habitat can increase the potential for infection by coccidians and other parasites alike. Many protozoans are free-living in the aquatic environment and often do not require an intermediate host for reproduction.

PMA: pigmented macrophage aggregates

Pigment-bearing macrophages are a prominent feature of fish spleen, liver and sometimes kidney and have been recognized as being normal components of these organs (Blazer and Dethloff 2000). Wolke et al. (1981, 1985a *in Blazer and Dethloff 2000*) first suggested pigmented macrophage accumulations as potential monitors of fish health because numerous studies had documented an increase in their number, size or hemosiderin (or other intracytoplasmic pigments like lipofuscin, melanin, ceroid; Harper and Wolf, 2009) content in fish collected at contaminated sites when compared to those

collected at reference sites (Blazer and Dethloff 2000). As a result, macrophage aggregates have been suggested as potentially sensitive biomarkers of contaminant exposure and/or stress.

PIG: *hepatocellular intracytoplasmic pigments*

Pigments in tissues are diverse and may or may not have health significance. Melanin accumulations, for example, are common at sites of tissue injury and may be found in kidney, spleen, liver, and other organs following various types of injury. Lipofuscin and ceroid are derived from cell membrane breakdown and disruption of lipid metabolism, and tend to increase with age. These pigments are often considered ‘wear and tear’ pigments and often accumulate in the liver as opposed to other organs (Heidel and Smith, 2007).

FPL: *focal/multifocal parenchymal leukocytes (hepatitis)*

FPL are foci of chronic immune stimulation (e.g., the result of a bacterial infection)

PVL: *perivascular lymphocytes/leukocytes*

The presence of perivascular lymphocytes/leukocytes could indicate chronic stimulation of the immune system (e.g., bacterial infection).

CPL: *cholangitis/pericholangial leukocytes*

Cholangitis/pericholangial leukocytes in the liver are evidence of chronic stimulation of the immune system (e.g., bacterial infection). It is an inflammatory condition which can result from bacteria ascending from the intestine to the liver through the biliary system.

LFN: *liver focal/multifocal necrosis*

Liver focal/multifocal necrosis can be the result of inadequate vascular perfusion (e.g., occurs with harmful algal blooms or hypoxia) or the result of direct cytotoxicity from viral or bacterial infections.

MEG: *hepatocellular megalocytosis/karyomegaly*

Hepatocellular megalocytosis/karyomegaly results from sub-lethal injury to hepatocytes via exposure of various types of toxins. Affected cells may be able to survive for several months.

SCN: *single cell necrosis & apoptosis*

Single cell necrosis is evidence of cellular damage in the liver which may result from exposure to toxins or viral infection. Apoptosis is a similar change also scored as SCN. Apoptosis occurs with remodelling of the liver in rapidly growing fish that suddenly go off feed about 24 hours before death. Apoptosis is the normal way in which hepatocyte numbers are decreased (i.e., the hepatocytes are not needed when growing fish stop feeding because few to no nutrients are being absorbed into the blood and entering the liver for processing)

Stomach/Intestine

FAT: *Mesenteric adipose tissue*

Mesenteric adipose tissue, located within the abdominal cavity, also provides some indication of the nutritional status of the fish.

FCG: *fibrous capsule granuloma*

Fibrous capsule granuloma in the stomach/intestinal region are evidence of foreign antigens. In wild fish, parasites are the most common cause of these lesions.

PER: *peritonitis* (includes margin of spleen & kidney)

Peritonitis may occur as a reaction to foreign material.

EIM: *Intrepthelial coccidians (Eimeria sp?)*

Intrepthelial coccidians are microscopic parasites in or on the intestinal lining.

NME: *nematodes with embryonated eggs* (intestine)

NMS: *nematodes (small) without eggs* (intestine)

GOU: *Superficial epithelial coccidians*

Superficial epithelial coccidians, identified as potentially being *Goussia sp*

Heart

ECD: *epicarditis*

Epicarditis is evidence of chronic immune stimulation.

MKM: *myocardial karyomegaly/megalocytosis*

MKM is likely an indication of multiple copies of DNA within the affected nuclei which could represent a developmental anomaly or a response to toxicant exposure.

Karyomegaly in other cell types has been associated with exposure to algal toxins.

END: *endocarditis*

Endocarditis is an inflammatory response (inflammation of the lining of the heart), and in some cases, can be evidence of chronic immune stimulation.

EEC: *eosinophilic endocarditis*

GEC: *granulomatous endocarditis*

HEC: *histiocytic endocarditis*

LEC: *lymphoplasmacytic endocarditis*

ENP: *endocardial phagocytosis* (atrium)

Spleen

In fish, the spleen has several functions: an accessory hematopoietic organ, a site for blood filtration, cell destruction, and for erythrocyte storage. It is a bag of reserve, immediate use, blood cells. The spleen is involved in all systemic inflammations,

generalized hematopoietic (related to formation of blood/blood cells) disorders, and metabolic disturbances. It is rarely the primary site of disease (Morrison 2007).

SMA: *macrophage aggregates* (spleen)

Macrophage aggregates are a normal feature of the fish spleen, liver and kidney (Blazer and Dethloff 2000), depending on the species. They have been found to play a role in the immune system by, essentially, engulfing foreign particles. One particular multi-organ stress response involves the formation of histologically evident pigmented macrophage aggregates (PMA) (Harper and Wolf, 2009). Alteration in their number, size, or both has been suggested as potential immunotoxicologic biomarkers. For example, larger fish, fish with nutritional deficiencies, or fish in poor health tend to have more or larger MAs. In addition, the number and/or size of MAs increase with age (Brown and George 1985; Blazer et al. 1987).

CON: *Congestion*

Congestion could probably be shaded as a measure of physiologic condition, related to the "bag of reserve, immediate use, blood cells" described above. A score of 0 probably represents a stressed fish that has contracted its spleen to increase circulating red blood cells. A score of 2 is probably closest to what a fish would naturally have in the wild (before capture and handling).

ELH: *ellipsoid hyalinization*

Ellipsoid hyalinization in the spleen occurred primarily in post-spawning females

Gill

EPS: *Epistylis*

Ectocommensal colonial ciliated protozoa like *Epistylis* or related species (*Heteropolaria*) attach to bony protuberances (e.g., fins, jaws, or operculum) with a stalk and feed on bacteria and other suspected organic debris in the water. Their attachment causes a small ulcer, and they are commonly associated mixed infections of gram-negative bacteria. They derive little nutrition directly from the fish. Their presence is usually an indicator of poor water quality (Noga 2000).

RFM: *refractile material in gills*

Small pieces of mineral between gill lamella appear with light microscopy as refractile material that is often birefringent under polarized light. We found no relation between asbestos content of the water and scores for refractile material.

TRD: *Trichodina sp.*

Trichodina sp is a ciliated protozoan that often occurs on skin and gills. Low numbers on fish are typically not harmful, however when conditions are suitable for multiplication of the parasite (e.g., fish are crowded in hatcheries, water quality is poor, etc.) it can cause fish to lose condition, become weak, and then become more susceptible to infection by bacterial pathogens in the water (Klinger and Floyd 1998).

MON: *Monogenean parasites*

Monogenean parasites are also known as flukes or flatworms. They commonly occur on the gills, skin or fins of fish and some aquatic invertebrates (Reed et al 2009). They have a series of hooks that enable them to attach while feeding. Most species are host- and site-specific, requiring only one host to complete an entire life cycle. They have a direct life cycle and do not require an intermediate host. Infected fish may become lethargic and in heavy infestations of the gills, for example, respiratory disease may result. Gills may become swollen and pale, respiration rate may be higher, and affected fish may be less tolerant to low oxygen conditions (Reed et al 2009). Secondary infection by bacteria and fungus is common on tissue that has been damaged by monogeneans (Reed et al 2009). Transmission of monogeneans from fish to fish is primarily by direct contact. Monogeneans can cause lesions and tissue damage as well as producing side effects such as hyperplasia of both skin and gill epithelium and creating entry sites for secondary infection.

GLH: *Lamellar epithelial hyperplasia/hypertrophy*

Lamellar epithelial hyperplasia/hypertrophy is nonspecific response to irritation. Inciting causes include parasites, bacteria, and toxins.

Gonads**GFM:** *Gonadal foamy macrophages*

Gonadal foamy macrophages function as scavengers of cellular debris, which is most common in post-spawning ovaries, but can also be a result of infections.

ORF: *Ovarian ruptured follicles***OAM:** *Oocyte atresia*

Oocyte atresia is degeneration and necrosis of developing ova. It is a normal physiological event in all fish to resorb a few developing oocytes, but it can become pathologic following exposure to certain environmental contaminants (McDonald et al in Schmitt and Dethoff 2000). McDonald et al (2000) suggest that the ability to detect increased degeneration or necrosis of developing oocytes by histological examination has inspired the use of oocyte atresia as a biomarker of reproductive impairment.

ONG: *Oophoritis (non-granulomatous)*

Oophoritis (non-granulomatous) is an inflammation of the ovaries; in slimy sculpin (this work) it was most commonly associated with post-spawning involution of the ovary.

Kidney

The kidney has varied functions among fish species; it filters large quantities of blood, produces urine, and is a major route of excretion for some xenobiotics (Blazer 2000). In at least some species of fish, as in mammals, the kidney can regenerate after sub-lethal toxic damage (Reimschuessel et al. 1990 in Blazer 2000).

TEP: *Tubular epithelial proteins* (intracytoplasmic)

Tubular epithelial proteins (intracytoplasmic) are normal in some species; in others they can be indicative of glomerular disease.

ISH: *Interstitial (hematopoietic) cell hyperplasia*

Interstitial (hematopoietic) cell hyperplasia can be indicative of anemia or inflammation where there is an increased demand for erythrocytes or white blood cells somewhere in the body.

IPC: *Intratubular (luminal) protein casts*

Intratubular (luminal) protein casts result from glomerular or tubular dysfunction; either excess protein leaks through glomeruli or tubules are unable to reabsorb protein.

GEP: *Golden epithelial pigment in tubules*

Golden epithelial pigment in tubules can result from poor feed (in the case of hatchery fish) or can be indicative of chronic infections and/or exposure to organic contaminants. When tubular epithelial cells are involved, variation in size of nuclei and cytoplasm is evidence of cellular degeneration and regeneration, and it is consistent with persistent damage to the tubules.

MYX: *Tubular or glomerular myxosporeans*

Tubular or glomerular myxosporeans were probably *Myxobilatus yukonensis* (Arthur and Margolis 1975).

TEV: *tubular epithelial vacuolation*

Tubular epithelial vacuolation can be indicative of kidney damage

Skin

SEP: *Epistylis sp*

(refer to summary in “Gill”)

STR: *Trichodina sp.*

(refer to summary in “Gill”)

SFC: *Fibrous tissue granuloma* (probable metacercaria)

MYO: *Myositis*

Myositis is an inflammation of the muscle.

MML: *Meningomyelitis*

Meningomyelitis is an inflammation of the spinal cord, brainstem, and surrounding meninges.

Head

HEP: *Epistylis sp*
(refer to summary in “Gill”)

HTD: *Trichodina sp*
(refer to summary in “Gill”)

HMG: *Monogenean parasites* (flukes/flatworms) in the head
(refer to summary in “Gill”)

HFC: *Fibrous capsule granuloma* (probable metacercaria)

LTT: *Lens/retina trematodes*

MDN: *Myodegeneration and necrosis*
Muscular degeneration and tissue death

CES: *larval cestodes*

Appendix 5. Compiled water quality data for Clinton, Croucher and Wolf Creeks 2010.

		Clinton Creek				Croucher Creek			Wolf Creek			CCME	Yukon CSR
Parameter	Units	26-May	26-May*	20-Jul	21-Sep	25-May	9-Aug	30-Sep	10-Jun	9-Aug	30-Sep	Aquatic Life	Aquatic Life
flow	m³/s	0.62		0.86	0.39	0.56	0.63	1.39	2.12	2.07	3.03		
field conductivity	uS/cm	287		311		123	160	105	100	85	75		
field DO	mg/L	9.34		9.27		8.99	8.17	10.75	10.23	10.68	12.14		
field ORP	mV	111.7		16.8	101.7	104.1	72.5	97.7	111.9	78.1	135.5		
field pH	pH Units	7.96		7.93	7.8	7.74	7.73	7.79	8.16	8.15	8.35	6.5 - 9.0	
field specific conductivity	uS/cm	368		396	679	172	203	184	137	126	127		
field TDS	g/L	0.239		0.258	0.422	0.112	0.132	0.119	0.089	0.082	0.083		
field temperature	°C	13.47		13.71	6.8	9.86	13.87	2.69	11.09	8.08	3.42		
total Suspended Solids (TSS)	mg/L	< 2		4	8	18	< 2	< 2	8	4	3		
field Turbidity	NTU	8.9		9.7		14	6.7	10.2	10.3	7.1	7.2		
Total Alkalinity	mg/L	95	95	104	154	99	152	136	69	83	83		
Hardness as CaCO3	mg/L	239	260	227	382	100	142	128	71	82	84		
Hardness Total diss. Hardness, mg CaCO3/l	mg CaCO3/l	240	238	232	393	107	146	135	74.8	105	85.3		
Hydroxide	mg/L	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5		
Nitrate and nitrite nitrogen	mg/L	0.03	0.03	0.24	0.14	0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01		400
pH	unitless	7.82	7.82	7.95	7.87	7.82	7.96	7.68	7.88	8.12	7.8		
Specific Conductivity	S/cm at 25	395	397	423	693	181	272	244	137	171	176		
Sulphate (SO4)	mg/L	98.4	98.6	108	208	1.76	1.8	3.23	7.09	9.52	9.7		1000
Ammonia (NH3)	mg/L	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05		1.31 - 18.4
Bicarbonate	mg/L	100	100	130	190	100	180	160	80	100	100		
Carbonate	mg/L	< 6	< 6	< 6	< 6	< 6	< 6	< 6	< 6	< 6	< 6		
Asbestos - length < 5 um	fb/L	ND		ND	0.81 x 10 ⁶	0.24 x 10 ⁶	ND	ND	ND	ND	ND		
Asbestos length > 5 um	fb/L	ND		ND	0.16 x 10 ⁶	ND	ND	ND	ND	ND	ND		
Dissolved Aluminum (Al)	mg/L	0.073	0.059	0.057	0.012	< 0.005	< 0.005	< 0.005	0.006	< 0.005	< 0.005		
Dissolved Antimony (Sb)	mg/L	0.0005	0.0006	0.0005	0.0007	< 0.0002	0.0002	0.0002	< 0.0002	0.0003	< 0.0002		0.2
Dissolved Arsenic (As)	mg/L	0.001	0.001	0.0012	0.0014	0.0004	0.0008	0.0003	0.0005	0.0005	0.0004		0.05 - 0.12
Dissolved Barium (Ba)	mg/L	0.038	0.038	0.039	0.059	0.026	0.038	0.028	0.07	0.062	0.066		5 - 10
Dissolved Beryllium (Be)	mg/L	< 0.00004	< 0.00004	0.00005	< 0.00004	< 0.00004	< 0.00004	< 0.00004	< 0.00004	< 0.00004	< 0.00004		0.053 - 1
Dissolved Bismuth (Bi)	mg/L	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001		
Dissolved Boron (B)	mg/L	0.039	0.039	0.041	0.062	< 0.004	< 0.004	< 0.004	< 0.004	< 0.004	< 0.004		50
Dissolved Cadmium (Cd)	mg/L	0.00005	0.00004	0.00002	0.00004	< 0.00001	< 0.00001	< 0.00001	< 0.00001	< 0.00001	< 0.00001		0.0001 - 0.001
Dissolved Calcium (Ca)	mg/L	44	47.6	42.7	71.6	33.7	46.8	41.8	20.4	23.4	23.9		
Dissolved Chromium (Cr)	mg/L	0.0016	0.0032	0.0014	0.0038	0.0004	0.0009	0.0004	<0.0004	0.0005	< 0.0004		
Dissolved Cobalt (Co)	mg/L	0.00043	0.00042	0.00035	0.00073	0.00005	0.00004	< 0.00002	0.00003	0.00003	< 0.00002		0.009
Dissolved Copper (Cu)	mg/L	0.003	0.003	0.003	0.003	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.001		0.02 - 0.09
Dissolved Iron (Fe)	mg/L	0.21	0.18	0.21	0.202	0.08	0.16	0.042	< 0.01	0.03	0.02		
Dissolved Lead (Pb)	mg/L	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0002		0.04 - 0.3
Dissolved Lithium (Li)	mg/L	0.004	0.004	0.005	0.011	0.001	0.001	0.001	< 0.001	< 0.001	< 0.001		
Dissolved Magnesium (Mg)	mg/L	31.4	34.3	29.2	49.4	4.8	6.2	5.9	4.8	5.7	5.9		
Dissolved Manganese (Mn)	mg/L	0.0671	0.0664	0.0549	0.222	0.0115	0.0051	0.004	0.0079	0.0054	< 0.001		
Dissolved Molybdenum (Mo)	mg/L	0.0008	0.0009	0.001	0.0017	0.0022	0.0022	0.0015	0.0005	0.0008	0.0006		10
Dissolved Nickel (Ni)	mg/L	0.012	0.012	0.01	0.014	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001		0.25 - 1.5
Dissolved Organic Carbon	mg/L	25.6	24.7	20.4	13.7	6.2	4.9	4.3	3.8	1.9	2.5		
Dissolved Orthophosphate-P	mg/L	0.06	0.06	0.04	0.05	0.07	0.06	0.05	0.05	0.05	0.04		
Dissolved Phosphorus (P)	mg/L	0.01	0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01		
Dissolved Potassium (K)	mg/L	0.9	1	0.5	0.9	0.8	0.4	0.6	0.7	0.6	0.7		
Dissolved Selenium (Se)	mg/L	< 0.0006	< 0.0006	< 0.0006	0.0014	< 0.0006	< 0.0006	< 0.0006	< 0.0006	< 0.0006	< 0.0006		0.01 - 0.54
Dissolved Silicon (Si)	mg/L	3.63	3.98	3.94	4.8	4.2	5.08	4.93	4.49	4.56	4.28		
Dissolved Silver (Ag)	mg/L	< 0.00001	< 0.00001	< 0.00001	< 0.00001	< 0.00001	0.00001	< 0.00001	< 0.00001	0.00001	< 0.00001		0.0005 - 0.015
Dissolved Sodium (Na)	mg/L	2.8	3	2.4	4.3	3	3.3	3.4	2.8	3.2	3.5		
Dissolved Strontium (Sr)	mg/L	0.232	0.232	0.254	0.427	0.14	0.231	0.182	0.195	0.199	0.202		
Dissolved Sulfur (S)	mg/L	36.3	39.4	36.2	74.4	0.9	1	1.3	2.8	3.4	3.7		
Dissolved Tellurium	mg/L	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
Dissolved Thallium (Tl)	mg/L	0.00001	0.00002	0.00001	0.00002	< 0.00001	< 0.00001	< 0.00001	< 0.00001	< 0.00001	< 0.00001		0.003
Dissolved Thorium	mg/L	< 0.0004	< 0.0004	< 0.0004	< 0.0004	< 0.0004	< 0.0004	< 0.0004	< 0.0004	< 0.0004	< 0.0004		
Dissolved Tin (Sn)	mg/L	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
Dissolved Titanium (Ti)	mg/L	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01		
Dissolved Trivalent Chromium	mg/L	0.0016	0.0032	0.0014	0.0038	0.0004	0.0009	0.0004	< 0.0004	0.0005	< 0.0004		0.09 - 0.56
Dissolved Uranium (U)	mg/L	0.0015	0.0016	0.0014	0.0019	0.0021	0.0028	0.0031	0.0005	0.0007	0.0007		1 - 3
Dissolved Vanadium (V)	mg/L	0.0004	0.0009	0.0005	0.0004	0.0006	0.0005	0.0003	0.0004	0.0003	0.0002		
Dissolved Zinc (Zn)	mg/L	0.004	0.001	< 0.001	0.004	0.002	0.002	0.002	< 0.001	< 0.001	0.002		0.075 - 2.4
Dissolved Zirconium	mg/L	0.0008	0.0009	0.0009	0.0008	0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
Total Aluminum (Al)	mg/L	0.126	0.124	0.131	0.089	0.233	0.009	0.039	0.224	0.033	0.207	0.1 (pH> 6.5)	
Total Antimony (Sb)	mg/L	0.0006	0.0006	0.0005	0.0005	< 0.0002	< 0.0002	< 0.0002	< 0.0002	0.0004	< 0.0002		
Total Arsenic (As)	mg/L	0.0011	0.0011	0.0013	0.0015	0.0006	0.0008	0.0004	0.0006	0.0006	0.0006	0.005	
Total Barium (Ba)	mg/L	0.041	0.04	0.041	0.064	0.033	0.039	0.029	0.076	0.065	0.069		
Total Beryllium (Be)	mg/L	< 0.00004	< 0.00004	< 0.00004	< 0.00004	< 0.00004	< 0.00004	< 0.00004	< 0.00004	< 0.00004	< 0.00004		
Total Bismuth (Bi)	mg/L	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001		
Total Boron (B)	mg/L	0.044	0.047	0.043	0.062	0.007	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	29 (short term) or 1.5 (long term)	
Total Cadmium (Cd)	mg/L	0.00005	0.00006	0.00004	0.00004	< 0.00001	< 0.00001	< 0.00001	< 0.00001	< 0.00001	< 0.00001	0.017	
Total Calcium (Ca)	mg/L	45.2	44.3	43.6	73.7	35	47.9	44.2	21.6	30	24.4		
Total Chromium (Cr)	mg/L	0.0021	0.0023	0.0021	0.0068	0.0009	0.0005	0.0005	0.0006	< 0.0004	0.0006	0.0089 (Cr3), 0.001 (Cr6)	
Total Cobalt (Co)	mg/L	0.00052	0.00051	0.00048	0.00083	0.00016	0.00005	0.00005	0.00015	0.00006	0.00014		
Total Copper (Cu)	mg/L	0.003	0.003	0.005	0.003	0.001	< 0.001	< 0.001	0.001	< 0.001	0.002	0.002 - 0.004	
Total Iron (Fe)	mg/L	0.38	0.369	0.432	0.48	0.424	0.284	0.142	0.275	0.128	0.304	0.3	
Total Kjeldahl Nitrogen	mg/L	0.86	0.72	0.3	0.48	0.31	0.22	0.24	0.16	0.13	0.15		
Total Lead (Pb)	mg/L	0.0002	0.0002	0.0002	0.0002	0.0002	< 0.0001	0.0001	0.0002	0.0001	0.0003	0.001 - 0.002 (hardness dependent)	
Total Lithium (Li)	mg/L	0.005	0.005	0.005	0.01	0.001	0.002	0.001	< 0.001	< 0.001	< 0.001		
Total Magnesium (Mg)	mg/L	30.9	30.8	30	50.7	4.68	6.34	6.08	5.07	7.3	5.92		
Total Manganese (Mn)	mg/L	0.0778	0.0762	0.0642	0.246	0.0219	0.0076	0.01	0.0293	0.0136	0.023		
Total Molybdenum (Mo)	mg/L	0.0009	0.0009	0.0011	0.0021	0.0024	0.0022	0.002	0.0006	0.0009	0.0009	0.073	
Total Nickel (Ni)	mg/L	0.013	0.014	0.012	0.016	0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.025 (min) - hardness dependent	
Total Phosphorus (P) ¹	mg/L	0.016	0.012	< 0.01	< 0.01	0.032	0.018	< 0.01	0.012	< 0.01	< 0.01		
Total Phosphorus (P) ²	mg/L	0.05	0.05	< 0.05	< 0.05	0.06	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05		
Total Potassium (K)	mg/L	0.7	0.6	0.1	0.9	0.6	0.5	0.8	0.7	0.9	0.8		
Total Selenium (Se)	mg/L	< 0.0006	< 0.0006	< 0.0006	0.0009	< 0.0006	< 0.0006	< 0.0006	< 0.0006	< 0.0006	< 0.0006	0.001	
Total Silicon (Si)	mg/L	3.72	3.64	4.25	3.91	4.31</							