TECHNICAL MEMORANDUM

Microcystin Bioaccumulation and Histopathology in Klamath River Salmonids; 2010 Study Results. **(Updated 6-5-2013).**

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EXECUTIVE SUMMARY

Copco and Iron Gate Reservoirs (the lowermost projects of PacifiCorp's Klamath Hydropower Project-- KHP) experience extensive blooms of toxigenic *Microcystis aeruginosa*. These blooms have been associated with high levels of the cyanotoxin microcystin, a potent hepatotoxin capable of causing chronic liver damage and acting as a tumor promoter. Previous bioaccumulation studies showed accumulation of microcystin toxin in muscle and/or liver tissues of yellow perch, Irongate hatchery salmon, and freshwater mussels, with microcystin levels in many of these samples exceeding public health threshold values for safe consumption.

Due to the overlap in timing of the toxic algal blooms and run-timing of salmonids (chiefly Chinook and Coho salmon and steelhead) that serve as a food source for the Karuk Tribe, the potential for bioaccumulation of microcystins exists both as a public health concern and as a contributor to fish stress and disease. In addition, previous histological examination of liver tissues determined that lesions were present in liver tissue from salmonid species. The purpose of this report is to present microcystin bioaccumulation and histopathology results for salmonids sampled during the fall of 2010.

Klamath River fish samples were collected at five locations during September through November; Orleans, Ishi Pishi Falls, Weitchpec, Happy Camp, and Irongate hatchery. Samples for *Microcystis* and microcystin toxin were also collected at a series of stations as part of the Karuk Tribe's public health monitoring program. Fillet and liver samples were sent to the California Department of Fish and Game (CDFG) lab for microcystin analysis and various organs were sent to the University of California, Davis and the Animal Health Centre, Abbotsford, British Columbia for processing and analysis for histopathological analysis.

Results from salmonid tissue samples collected by the Karuk Tribe in September, 2010 showed that 3 of 7 Chinook livers collected below Happy Camp at Ishi Pishi Falls had detectable levels of microcystin-RR. Samples collected in October showed that 1 of 7 Chinook livers had a high level of microcystin-RR (121 ppb), and 1 of 15 steelhead livers had a high level of microcystin-LR (152 ppb), both exceeding public health guideline levels. Microcystin was not detected in fish tissue samples during any of the other October or November samples. Aside from microcystin, none of the other measured algal toxins were detected in any of the Klamath River fish samples (i.e., anatoxin-a, domoic acid, or okadaic acid). Microcystin was not detected in any of the fillet samples.

A comparison to ambient toxin data indicates that congeners bioaccumulated in Klamath River salmonids do not match the ambient data with respect to detected microcystin congeners or variants. The reason for this difference between ambient and bioaccumulated microcystin is unclear. However, a similar trend was noted in Klamath River freshwater mussels, and a potential explanation is differential uptake, whereby concentrations of congeners that may have been present but below detection in ambient water, were then differentially accumulated through the bioaccumulation process.

Because concentrations of microcystin-RR in the September Chinook livers were below public health guideline values and livers are not typically consumed, those tested fish did not likely

pose a public health concern with respect to consumption. They did indicate that fish were exposed to microcystin, and that direct or indirect effects to fish health in terms of stress and/or disease were a possibility. However, histology results were unable to provide conclusive evidence of microcystin intoxication and there was no indication of cholestasis, megalocytosis, pseudoinclusions or other features recognized in fish with natural and experimental exposure to microcystin LR. However supervening or secondary infections and inflammation may have hampered microscopic assessment of the sectioned tissues, and the lack of pathognomonic lesions within the liver may be attributed to a number of factors, including sublethal toxin exposure, too short a time interval between bioaccumulation and subsequent fish sampling, intercurrent disease which may have obscured more significant or subtle histopathology and other factors. A broader suite of tissue samples with future harvests may provide additional insights into the overall health of the stock.

In summary, these results provide evidence that Klamath River salmonids were exposed to microcystin and that bioaccumulation in liver tissue occurred during the fall of 2010. Although, fish livers are not typically consumed, the levels of microcystin-RR in one of the October Chinook and –LR in the October steelhead exceeded public health guideline values. Fall 2010 sampling results point to the potential for recurring microcystin exposure and subsequent bioaccumulation of microcystins in Klamath River Salmonids. The lack of consistent microcystin bioaccumulation among the sampled fish likely reflects variable exposure time due to spatial differences in toxin distribution, as well as temporal and spatial differences with respect to migration timing and habitat use.

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INTRODUCTION

Copco and Iron Gate Reservoirs (the lowermost projects of PacifiCorp's Klamath Hydropower Project-- KHP) experienced extensive blooms of toxigenic *Microcystis aeruginosa* (MSAE) from 2004-2009 (Kann and Corum 2009; 2010; Jacoby and Kann 2007). These blooms were associated with high levels of the cyanotoxin microcystin, a potent hepatotoxin capable of causing chronic liver damage and acting as a tumor promoter (Carmichael 1995; Chorus et al. 1999; Chorus 2001).

The results of the 2005-2009 sampling program demonstrated widespread and abundant seasonal blooms of toxigenic MSAE in Copco and Iron Gate reservoirs as well as export of both MSAE and microcystin to the Klamath River downstream. These yearly bloom events consistently exceed World Health Organization (WHO 1999) and California State Water Resources Control Board (SWRCB 2010) public health guideline levels for both MSAE cell density and microcystin toxin by 10 to over 1000 times. The blooms vary in duration and severity in the free flowing-section of the River but have generally been present at some level during the August through October period (Kann and Corum 2009; Fetcho 2009).

Due to the overlap in timing of the toxic algal blooms and run-timing of salmonids that serve as a food source, the potential for bioaccumulation of microcystins exists both as a public health concern and as a contributor to fish stress and disease. For example, the Yurok Tribe fishes for fall Chinook starting in August, and fall Chinook reach the Karuk fishery in September. Fall steelhead enter the Klamath River in late summer; are in the mid-Klamath River by September or October, and reach Iron Gate hatchery by November. Salmonids are also caught and consumed by recreational fishermen and are sold in the Yurok commercial fishery.

Initial field sampling of salmonid fish tissue for public health was conducted by the Yurok Tribe in 2005, when a small number of fish livers and fillets were collected from the Klamath River between mid-September and early October (Fetcho 2006). Of the 5 Chinook livers, 4 Chinook fillets, 2 steelhead livers, and 2 steelhead fillets sampled, a trace amount of microcystin was detected in one steelhead liver, and 0.54 µg/g microcystin was found in the other steelhead liver (Fetcho 2006). In addition, bioaccumulation studies undertaken in 2007 showed accumulation of microcystin toxin in muscle and/or liver tissues of yellow perch, Irongate hatchery salmon, and freshwater mussels (Mekebri et al. 2009; Kann 2008; Kanz 2008). Microcystin levels in many of these samples exceeded public health threshold values for safe consumption (Kann 2008; OEHHA 2008; 2012).

Although other studies of Klamath River salmon and steelhead in 2007, and yellow perch in 2008 and 2009, did not show microcystin bioaccumulation in tissues (e.g., CH2MHILL 2009; Prendergast and Foster 2010), histological examination of liver tissues determined that lesions were present in liver tissue from both salmonid species (CH2M HILL 2009). Substantial bioaccumulation continued to be shown in freshwater mussels throughout the Klamath River below Irongate Dam in 2009 (Kann et al. 2010).

Given previous results showing the presence of microcystin in salmon and steelhead livers, and that in the mainstem of the Klamath River adult salmonids are an important subsistence food for Tribal people, additional salmonid sampling for microcystin bioaccumulation was conducted in 2010 by the Karuk Tribe. A secondary purpose of this investigation was to better define the

extent and severity of histologic abnormalities in sampled tissues and to assess the significance of findings to overall fish health. The previous iteration of this report provided results only for the presence of microcystin in salmonid tissues during the fall of 2010, and is updated here to include the histology results that were incomplete at that time.

METHODS

Klamath River fish samples were collected at five locations during September through November; Orleans, Ishi Pishi Falls, Weitchpec, Happy Camp, and Irongate hatchery (Figure 1). Fish were collected using hook and line at Weitchpec, Orleans, and Happy Camp and by traditional dip net at Ishi Pishi Falls. Fish from Irongate hatchery were collected after being spawned. Samples for *Microcystis* and microcystin toxin were also collected at a series of stations as part of the Karuk Tribe's public health monitoring program (Figure 2). In September, 10 livers and fillets were collected from fall steelhead and 7 livers and fillets were collected from fall Chinook (Figure 3). In October, 15 livers and fillets were collected from steelhead, and 7 livers and fillets from Chinook. In November, 6 liver and fillets were collected from Chinook and 3 livers and 2 fillets from Coho. Fillet and liver samples were sent to the California Department of Fish and Game (CDFG) lab for microcystin analysis and various organs were sent to the University of California, Davis for histological examination.

CDFG Protocol

Samples of five fish were collected per sample period-station combination. Fish tissue and liver samples consisted of 5-10 grams of tissue and 5-10grams of liver. Samples were placed in aluminum foil and Ziploc bags and then frozen. The samples were shipped with ice overnight to Dr. Abdou Mekebri at the Fish and Wildlife Water Pollution Control Lab (WPCL) in Rancho Cordova, CA for microcystin analysis by LCMS/MS (Mekebri et al. 2009). Chain of Custody forms are shown in Appendix I. Two of the September Chinook liver samples were split at the lab for quality assurance purposes and are labeled with "Dup" following the WPCL Lab number (Appendix II).

Histology Protocol

Tissue sampling for histopathology was incorporated into ongoing efforts to screen returning salmon stocks for microcystin analysis. At the time of collection by either fish line or dip net, fish were assigned a log number and the harvest site, species, and demographic data were recorded. The sample collection and processing dates were noted and gross observations of tissue abnormalities or other significant observations were recorded. For logistical reasons, collected fish were refrigerated at 4C for up to 3 days post collection prior to processing. Photos of the external aspect of intact whole fish and internal organs were obtained. Cytological examination of gill wet mounts and skin scrapes to screen for *Columnaris* sp. and other external pathogens was undertaken.

Fish dissection consisted of a midventral incision of the coelomic cavity, with en block removal of the internal viscera. Organs sampled for microscopic evaluation included the liver, spleen, pyloric caecae, pancreas, heart, gill arch, skin, skeletal muscle, spinal cord, vertebral column and

kidney. The tissue samples were rinsed with saline solution to minimize superficial blood. After the gill arches were removed, the head was transected and shipped fresh weekly on ice to Melissa A. Miller, DVM, PhD at the Marine Wildlife Veterinary Care and Research Center Department of Fish and Game and University of California, Davis in Santa Cruz, CA for dissection of the skull and removal of the brain (for September samples only). Preserved tissues were forwarded to the Animal Health Centre, Abbotsford, British Columbia for processing and analysis. Summary histological results are reported in the body of the report while complete results are found in Appendix III.

Figure 1. 2010 Fish sampling locations: Clockwise from top left; Iron Gate hatchery, Weitchpec, Ishi Pishi Falls, and Orleans (Happy Camp location not shown).

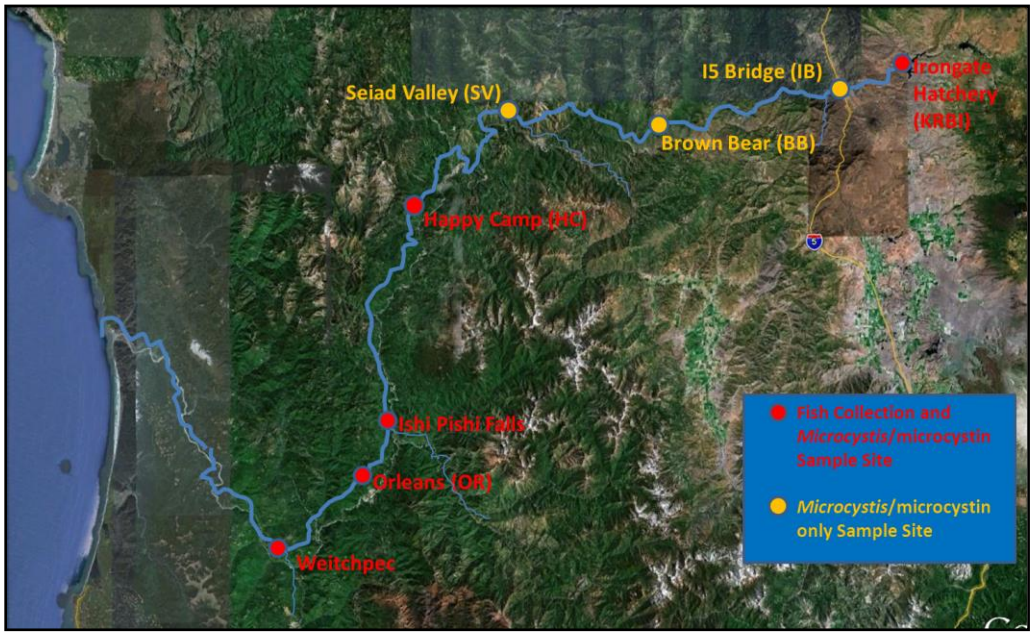

Figure 2. Klamath River fish and Microcystis sampling locations, 2010.

Figure 3. Example of 2010 Fish samples: clockwise from top left: Chinook from Ishi Pishi- IP092810_1C, Chinook liver from Ishi Pishi- IP092910_1C_Liver, Steelhead from Orleans- OR092310_1S and Chinook from Ishi Pishi- IP092910_1C.

RESULTS/DISCUSSION

Results from salmonid tissue samples collected by the Karuk Tribe in September, 2010 showed that 3 of 7 Chinook livers collected below Happy Camp at Ishi Pishi Falls had detectable levels of microcystin-RR (**Error! Reference source not found.**; Figure 4). Microcystin was not etected in any of the other September fish samples. Samples collected on the 14th and 15th of October showed that 1 of 7 Chinook livers had a high level of microcystin-RR (121 ppb), and 1 of 15 steelhead livers had a high level of microcystin-LR (152 ppb) , both exceeding public health guideline levels (Table 1; Figure 4). Microcystin was not detected in fish tissue samples during any of the other October or November samples. Aside from microcystin, none of the other measured algal toxins were detected in any of the Klamath River fish samples (Table 1; i.e., anatoxin-a, domoic acid, or okadaic acid). Microcystin was not detected in any of the fillet samples. Duplicate samples analyzed on two of the September fish showed good agreement (Table 1), and internal lab QA recoveries were generally 100% ±20%, although a few were closer to 75% (Figure 5; Appendix II).

During the period the Chinook were collected, the 2010 longitudinal microcystin (total microcystin as determined by ELISA) and *Microcystis* public health sampling data showed that ambient levels of both *Microcystis* cells and microcystin toxin increased in the Klamath River during mid-September, exceeding public heath guideline values at nearly all stations (Figures 6 and 7). Although microcystin values declined somewhat during the third week in September, they then rebounded in late September and into early October (Figures 6 and 7). These results indicate that microcystin was being transported downstream to areas where Chinook and steelhead were migrating upstream, and that fish collected during the September and October efforts were likely exposed to microcystin either prior to or during the collection period. Microcystin levels then declined to levels that were below detection during the November fish sampling period.

In addition to total microcystin as determined by ELISA during the course of regular public health sampling (e.g., Figure 7), samples were also periodically collected to specifically determine the presence of various microcystin congeners in Klamath River water samples (Table 2). These data show only two congeners were detected; microcystin-LR early in the season at station IB, and microcystin-LA during August through October at various stations (Figure 8).

These data indicate that congeners bioaccumulated in Klamath River salmonids did not match the ambient data with respect to detected microcystin congeners or variants. For example, of the five fish showing positive bioaccumulation, four showed the presence of microcystin-RR, and one microcystin-LR. Moreover, even though microcystin–LR was detected in ambient water earlier in the season, only –LA was detected during the period bracketing the October 15th steelhead that showed 152 ppb of microcystin –LR. The reason for this difference between ambient and bioaccumulated microcystin is unclear. However, a similar trend was noted in Klamath River freshwater mussels (Kann et al. 2010). A potential explanation is differential uptake, whereby concentrations of –RR and –LR that may have been present but below detection in ambient water, were then differentially accumulated through the bioaccumulation process.

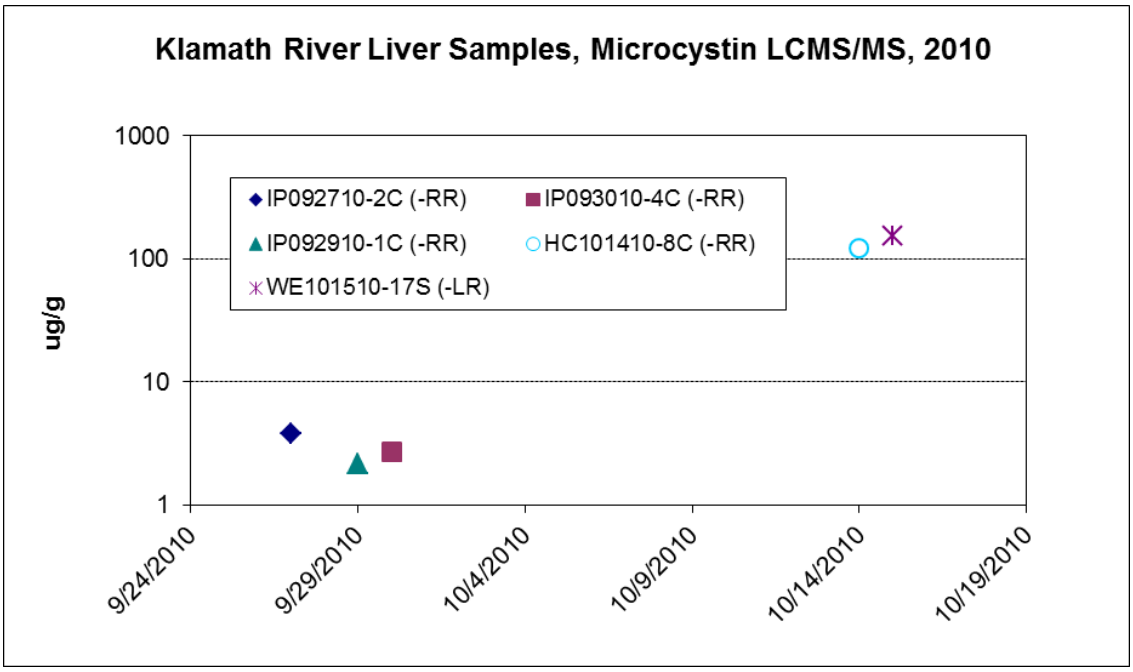

Figure 4. Microcystin concentration in Klamath River Salmonid Liver Samples, 2010 (showing occurrence of positive hits only—see data in Table 1).

Table 1. 2010 Fish and Wildlife Water Pollution Control Lab Toxin Results for microcystin bioaccumulation in Klamath River Salmonids. Red shaded cells show positive microcystin detections.

2010 Adult Salmonid Toxin Results			Estimated MDL (ppb)	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	5.00	2.00	1.00
			Reporting Limit (ppb)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	10.0	5.00	2.00
WPCL Lab#	Sample Identification	Date Collected	Matrix (Fresh Weight)	MC-RR (ppb)	MC-Desmethyl-RR* (ppb)	MC-LR (ppb)	MC-Desmethyl-LR (ppb)	MC-YR (ppb)	MC-LA (ppb)	MC-LW (ppb)	MC-LF (ppb)	MC-LY (ppb)	Anatoxin A (ppb)	Domoic acid (ppb)	Okadaic acid (ppb)
L-620-10-1	OR092310-1S	9/23/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-620-10-2	OR092310-1S	9/23/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-620-10-3	OR092310-2S	9/23/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-620-10-4	OR092310-2S	9/23/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-620-10-5	OR092610-3S	9/26/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-620-10-6	OR092610-3S	9/26/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-620-10-7	OR092610-4S	9/26/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-620-10-8	OR092610-4S	9/26/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-620-10-9	OR092610-5S	9/26/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-620-10-10	OR092610-5S	9/26/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-620-10-13	IP092710-2C	9/27/2010	fish liver	3.80	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-620-10-13Dup	IP092710-2C	9/27/2010	fish liver	2.93	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-620-10-14	IP092710-2C	9/27/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-620-10-15	IP092710-3C	9/27/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-620-10-16	IP092710-3C	9/27/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-620-10-11	IP092810-1C	9/28/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-620-10-12	IP092810-1C	9/28/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-641-10-1	IP093010-4C	9/30/2010	fish liver	2.71	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-641-10-2	IP093010-4C	9/30/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-641-10-3	IP092910-1C	9/30/2010	fish liver	2.17	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-641-10-3Dup	IP092910-1C	9/30/2010	fish liver	2.10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-641-10-4	IP092910-1C	9/30/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-641-10-5	WE092910-5S	9/30/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-641-10-6	WE092910-5S	9/30/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-641-10-7	IP093010-3C	9/30/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-641-10-8	IP093010-3C	9/30/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-641-10-9	IP092910-2C	9/30/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-641-10-10	IP092910-2C	9/30/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-641-10-11	WE092910-1S	9/30/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-641-10-12	WE092910-1S	9/30/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-641-10-13	WE092910-2S	9/30/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-641-10-14	WE092910-2S	9/30/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-641-10-15	WE092910-4S	9/30/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-641-10-16	WE092910-4S	9/30/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-641-10-17	WE092910-3S	9/30/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-641-10-18	WE092910-3S	9/30/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-1	HC101410-8C	10/14/2010	fish liver	121.20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-2	HC101410-8C	10/14/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

WPCL Lab#	Sample Identification	Date Collected	Matrix (Fresh Weight)	MC-RR (ppb)	MC-Desmethyl-RR* (ppb)	MC-LR (ppb)	MC-Desmethyl-LR (ppb)	MC-YR (ppb)	MC-LA (ppb)	MC-LW (ppb)	MC-LF (ppb)	MC-LY (ppb)	Anatoxin A (ppb)	Domoic acid (ppb)	Okadaic acid (ppb)
L-678-10-3	HC101410-11S	10/14/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-4	HC101410-11S	10/14/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-5	HC101410-12S	10/14/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-6	HC101410-12S	10/14/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-7	HC101410-13S	10/14/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-8	HC101410-13S	10/14/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-9	HC101410-14S	10/14/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-10	HC101410-14S	10/14/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-11	HC101410-15S	10/14/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-12	HC101410-15S	10/14/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-13	WE101510-16S	10/15/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-14	WE101510-16S	10/15/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-15	WE101510-17S	10/15/2010	fish liver	ND	ND	152.40	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-16	WE101510-17S	10/15/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-17	WE101510-18S	10/15/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-18	WE101510-18S	10/15/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-19	WE101510-19S	10/15/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-20	WE101510-19S	10/15/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-21	WE101510-20S	10/15/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-22	WE101510-20S	10/15/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-23	OR101710-21S	10/17/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-24	OR101710-21S	10/17/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-25	OR101710-22S	10/17/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-26	OR101710-22S	10/17/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-27	OR101710-23S	10/17/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-28	OR101710-23S	10/17/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-29	OR101710-24S	10/17/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-30	OR101710-24S	10/17/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-31	OR101710-25S	10/17/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-32	OR101710-25S	10/17/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-33	IG101810-9C	10/18/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-34	IG101810-9C	10/18/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-35	IG101810-10C	10/18/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-36	IG101810-10C	10/18/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-37	IG101810-11C	10/18/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-38	IG101810-11C	10/18/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-39	IG101810-12C	10/18/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-40	IG101810-12C	10/18/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-41	IG101810-13C	10/18/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-42	IG101810-13C	10/18/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-43	IG101810-14C	10/18/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-678-10-44	IG101810-14C	10/18/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-711-10-1	IG110110-15C	11/1/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-711-10-2	IG110110-15C	11/1/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-711-10-3	IG110110-16C	11/1/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-711-10-4	IG110110-16C	11/1/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-711-10-5	IG110110-17C	11/1/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-711-10-6	IG110110-17C	11/1/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-711-10-7	IG110110-18C	11/1/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-711-10-8	IG110110-18C	11/1/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-711-10-9	IG110110-19C	11/1/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-711-10-10	IG110110-19C	11/1/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-711-10-11	IG110110-20C	11/1/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-711-10-12	IG110110-20C	11/1/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-742-10-1	IG112910-1CO	11/29/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-742-10-2	IG112910-1CO	11/29/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-742-10-3	IG112910-2CO	11/29/2010	fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-742-10-4	IG112910-2CO	11/29/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L-742-10-5	IG112910-3CO	11/29/2010	fish liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

* Desmethyl-RR quantified as parent analog compound.

**Sample ID ending in 'S' denotes Steelhead , Sample ID ending in 'C' denotes Chinook and Sample ID ending in 'CO' denotes Coho.

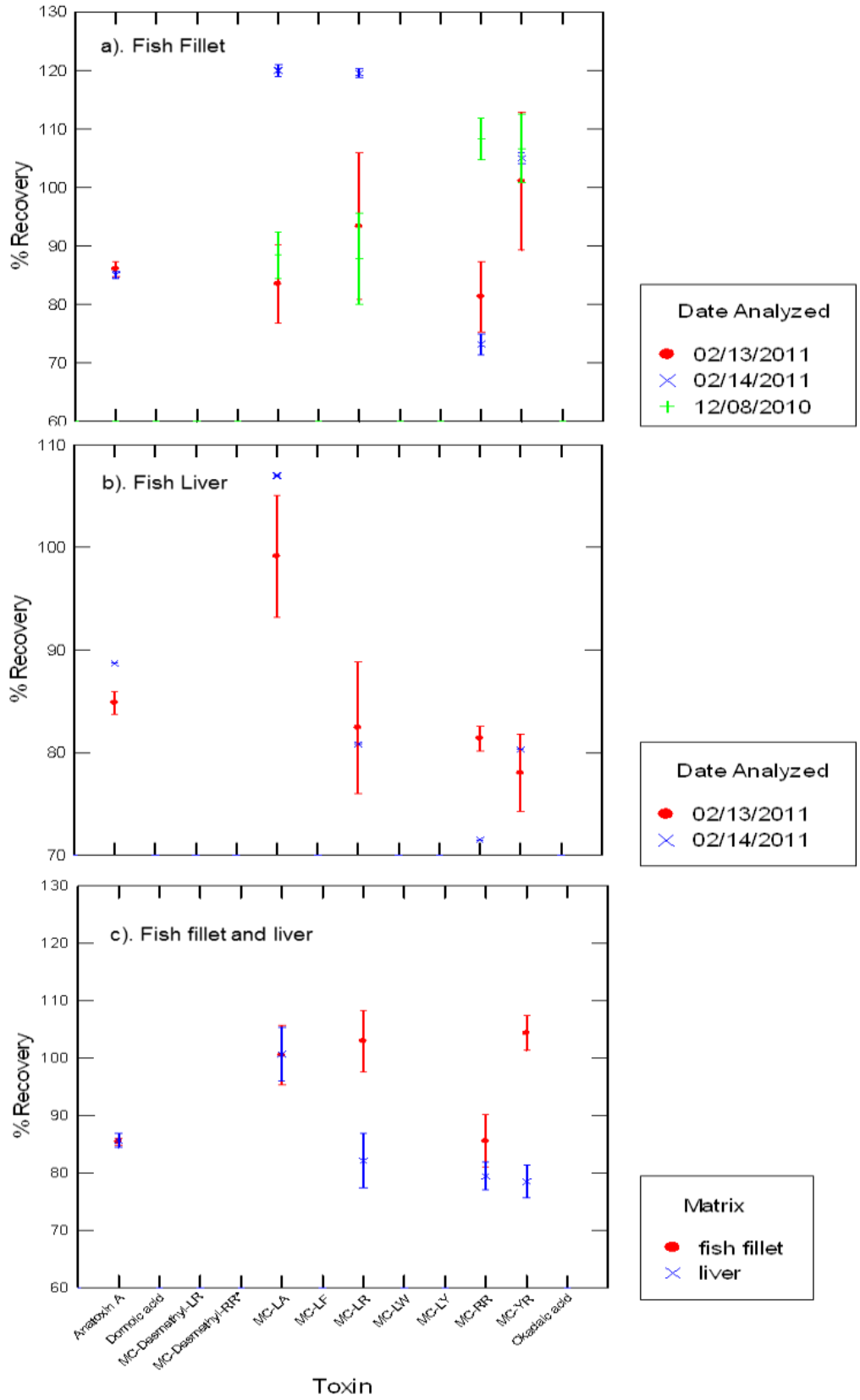

Figure 5. 2010 Fish and Wildlife Water Pollution Control Lab; Quality control analysis, percent recovery of spiked toxin in fish fillets (a), fish livers (b) and fillets and livers combined (c). Data plotted by date samples were analyzed; shown with mean and standard error bars.

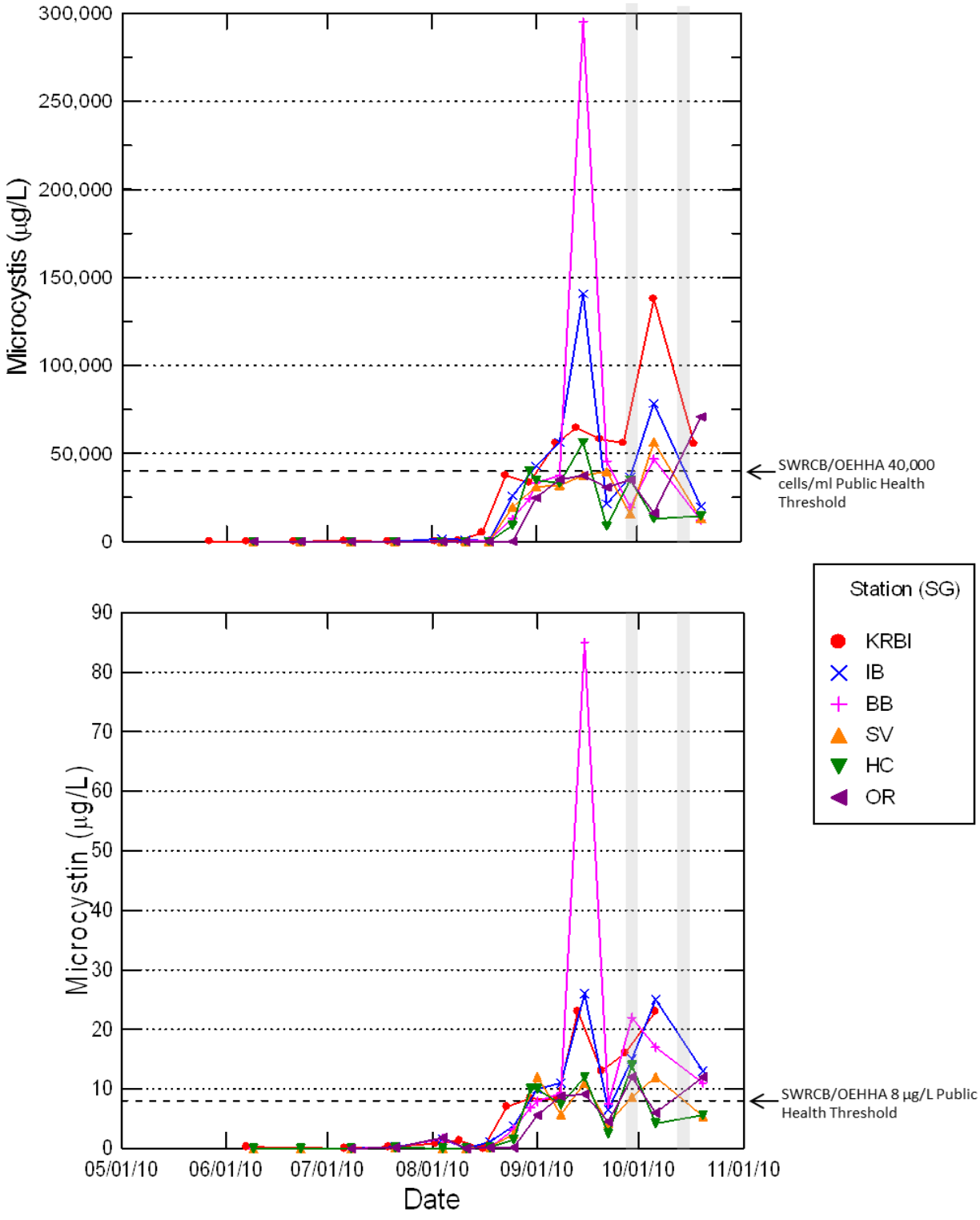

Figure 6. ***Microcystis aeruginosa*** **cell density (top panel) and microcystin toxin concentration (bottom panel) in Klamath River SG (surface grab near shoreline) water samples during 2010. Samples collected as part of the Karuk Tribes public health monitoring program; SWRCB/OEHHA limit line indicates the public health guideline value and the shaded bars indicate when fish samples with positive toxin results were taken.**

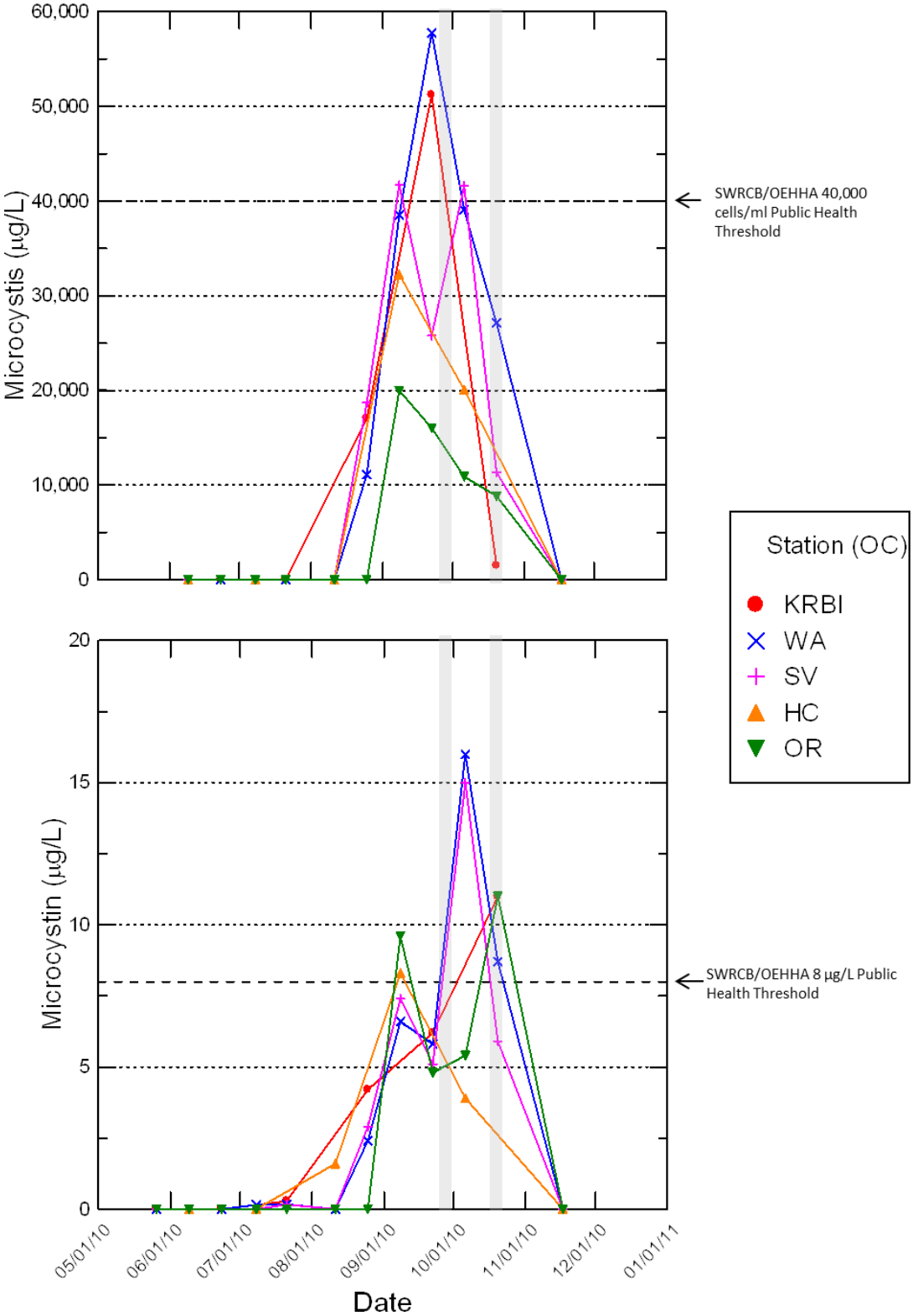

Figure 7. ***Microcystis aeruginosa*** **cell density (top panel) and microcystin toxin concentration (bottom panel) in Klamath River OC (near mid-channel) water samples during 2010. Samples collected as part of the Karuk Tribes public health monitoring program; SWRCB/OEHHA limit line indicates the public health guideline value and the shaded bars indicate when fish samples with positive toxin results were taken.**

Table 2. 2010 Fish and Wildlife Water Pollution Control Lab Ambient Microcystin Toxin Results (site codes as above in Figure 1, with the addition of LE and LES which are the Lower Estuary and the Lower Estuary Surface as collected by the Yurok Tribe).

Sample ID	Matrix	Date	Site Name	MC-RR	MC-Desmethyl-RR*	MC-LR	MC-Desmethyl-LR	MC-YR	MC-LA	MC-LW	MC-LF	MC-LY	Anatoxin A	Domoic acid	Okadaic acid
SV060910-SG	Water	6/9/2010	SV	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IB062310-SG	Water	6/23/2010	IB	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SV070810-SG	Water	7/8/2010	SV	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IG072110-SG	Water	7/21/2010	IG	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SV072110-SG	Water	7/21/2010	SV	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IB081110-SG	Water	8/11/2010	IB	ND	ND	0.0478	ND	ND	0.517	ND	ND	ND	ND	ND	ND
SV081110-OC	Water	8/11/2010	SV	ND	ND	ND	ND	ND	0.125	ND	ND	ND	ND	ND	ND
IB082510-SG	Water	8/25/2010	IB	ND	ND	0.467	ND	ND	1.82	ND	ND	ND	ND	ND	ND
SV090810-SG	Water	9/8/2010	SV	ND	ND	ND	ND	ND	2	ND	ND	ND	ND	ND	ND
SV090810-OC	Water	9/8/2010	SV	ND	ND	ND	ND	ND	1.95	ND	ND	ND	ND	ND	ND
WE090810-OC	Water	9/8/2010	WE						1.74						
LES090810-OC	Water	9/8/2010	LES						3.74						
BB092210-SG	Water	9/22/2010	BB	ND	ND	ND	ND	ND	2.58	ND	ND	ND	ND	ND	ND
SV092210-SG	Water	9/22/2010	SV	ND	ND	ND	ND	ND	1.8	ND	ND	ND	ND	ND	ND
LES092210-OC	Water	9/22/2010	LES						1.93						
HC100610-OC	Water	10/6/2010	HC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SV100610-SG	Water	10/6/2010	SV	ND	ND	ND	ND	ND	6.82	ND	ND	ND	ND	ND	ND
WE100610-OC	Water	10/6/2010	WE						2.24						
LES100610-OC	Water	10/6/2010	LES						3.26						
IG102010-OC	Water	10/20/2010	IG	ND	ND	ND	ND	ND	2.13	ND	ND	ND	ND	ND	ND
SV102010-SG	Water	10/20/2010	SV	ND	ND	ND	ND	ND	1.93	ND	ND	ND	ND	ND	ND

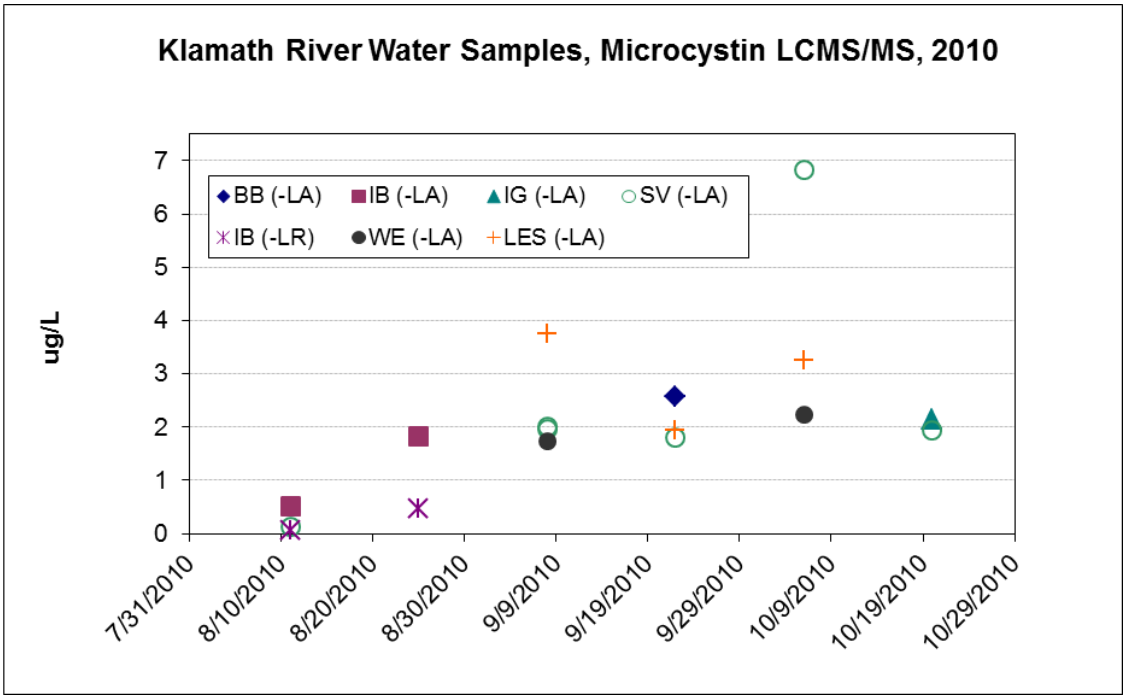

Figure 8. Microcystin congeners detected in Klamath River ambient water samples, 2010 (CFG Lab data shown in Table 2).

Because concentrations of microcystin-RR in the September Chinook livers were below public health guideline values (e.g. Kann 2008; OEHHA 2008) and livers are not typically consumed, those tested fish did not likely pose a public health concern with respect to consumption. They do, however, indicate that fish were exposed to microcystin, and that direct or indirect effects on fish health in terms of stress and/or disease are possible. The lack of consistent microcystin bioaccumulation among the sampled fish likely reflects variable exposure time due to spatial differences in toxin distribution, as well as temporal and spatial differences with respect to migration timing and habitat use

Although, as noted above, fish livers are not typically consumed, the level of microcystin-RR in the October Chinook and –LR in the October steelhead did exceed public health guideline values (e.g. OEHHA 2008; 2012; Mulvenna et al. 2012; Ibelings and Chorus 2007)[1]. The State of California recommends that internal organs should be removed from fish exposed to *Microcystis* blooms and specifically that the viscera (e.g., liver, kidney, etc.) of the fish should not be eaten. This would be especially important given the demonstrated exceedance of specific public health guideline values for microcystin in liver tissue of salmonids from the Klamath River.

Aside from fish consumption issues with respect to public health, the positive detection of microcystin in Klamath River Chinook and steelhead may indicate an impact to the health of these fish in terms of stress and/or disease. For example, fish exposed to typical microcystin producing blooms may experience sublethal toxic effects such as liver damage (OEHHA/CEP 2009). In addition, laboratory and field studies from elsewhere have also demonstrated the toxic effects of microcystin on salmonids (Anderson et al. 1993, Tencalla et al. 1994; Bury et al. 1997, Landsberg 2002) and other fish (Smith et al. 2008).

Although based on these literature studies and the documented presence of microcystin in the Klamath River and in Klamath River salmonid organs, the potential exists for sublethal (e.g., stress and disease) effects on salmonids from exposure to algal toxins, histology results were unable to provide conclusive evidence of microcystin intoxication (see Appendix III for complete analysis and discussion, including of parasites and other pathogens). As noted in Appendix III, there was no indication of microcystin intoxication; however, supervening or secondary infections and inflammation may have hampered microscopic assessment of the sectioned tissues. In addition, although biliary ductular hyperplasia and hepatocellular cytoplasmic vacuolation were evident in a small number of examined livers, there was no indication of cholestasis, megalocytosis, pseudoinclusions or other features recognized in fish with natural and experimental exposure to microcystin LR (Kent, 1990; Andersen et al, 1993).

The lack of pathognomonic lesions within the liver may be attributed to a number of factors, including sublethal toxin exposure, too short a time interval between bioaccumulation and subsequent fish sampling, intercurrent disease which may have obscured more significant or subtle histopathology and other factors. A broader suite of tissue samples with future harvests may provide additional insights into the overall health of the stock.

[1] Reported public health guideline values (concentration/wet-weight) are as follows for children: acute, seasonal, and lifetime TDI's of 250, 40, and 4 ppb were reported by Ibelings and Chorus (2007); a value of 40 ppb was reported by OEHHA (2008); a value of 24 ppb by Mulvenna et al. (2012); and an "action level" of 10 ppb by OEHHA (2012).

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APPENDIX I: Chain of custodies for Klamath River Tissue Study, 2010.

L-620-10

Chain of Custody for Klamath River Tissue Study

Karuk DNR	39051 Hwy 96
PHONE 530-469-3258	Orleans, CA 95556
CONTACT Grant Johnson	EMAIL cbowman@karuk.us, gjohnson@karuk.us
Collected By Grant Johnson	SIGNATURE

	Sample ID	Date	Time	Lab ID	Sample Description	Microcystin ELISA	Microcystin LCMS/MS	Anatoxin
1	OR092310-1S	09123	liver fillet	CDFG	liver & Fillet Klamath (Steelhead)		X	
2	OR092310-2S	09123					X	
3	OR092610-3S	09126					X	
4	OR092610-4S	1					X	
5	OR092610-5S	09126			liver. Fillet (Steelhead) Klamath		X	
6	IR092810-1C	09128			liver & fillet Klamath (Chinook)			
7	IR092710-2C	09127			\			
8	IR092710-3C	09127			liver & fillet Klamath (Chinook)			
9								
10								
11								
12								
13								
14								

Date Shipped: 09/29/10 Carrier/ Shipping # UPS

Date Received 9-30-10

Received by Jon Riley

Notes liver & fillet in seperate bags, Please return coolers & ice packs

Ship to:

For microcystin ELISA:
Sample Custodian
USEPA Region 9 Lab
1337 S. 46th Street
Building 201
Richmond, CA 94804
510-412-2389

For anatoxin-a and microcystin LCMS/MS:
Dr. Abdou Mekebri
Fish and Wildlife Water Pollution Control Lab
2005 Nimbus Road
Rancho Cordova, CA 95670
(916) 358-4396

Send Results To:

Grant Johnson
Karuk Tribe
Dept of Natural Resources
PO Box 282
Orleans, CA 95556
(530) 469-3258

Chain of Custody
Karuk Tribe Department of Natural Resources

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Chain of Custody for Klamath River Tissue Study L·6/1·10

Karuk DNR	39051 Hwy 96
PHONE 530-469-3258	Orleans, CA 95556
CONTACT Grant Johnson	EMAIL cbowman@karuk.us, gjohnson@karuk.us
Collected By Grant Johnson	SIGNATURE

	Sample ID	Date	Time	Lab ID	Sample Description	Microcystin ELISA	Microcystin LCMS/MS	Anatoxin
1	IP093010-4C	09/30		CDFG	Klamath River, Tissue Fillet & Liver		X	
2	IP092910-1C							
3	WE092910-6S							
4	IP093010-3C							
5	IP092910-2C							
6	WE092910-1S							
7	WE092910-2S							
8	WE092910-4S							
9	WE092910-3S	09/30		CDFG	Klamath River, Tissue Fillet & Liver		X	
10								
11								
12								
13								
14								

Date Shipped: 10-06-10 Carrier/ Shipping #

Date Received 10.7.10 0930

Received by

Notes

Ship to:

For microcystin ELISA:
Sample Custodian
USEPA Region 9 Lab
1337 S. 46th Street
Building 201
Richmond, CA 94804
510-412-2389

For anatoxin-a and microcystin LCMS/MS:
Dr. Abdou Mekebri
Fish and Wildlife Water Pollution Control Lab
2005 Nimbus Road
Rancho Cordova, CA 95670
(916) 358-4396

Send Results To:

Grant Johnson
Karuk Tribe
Dept of Natural Resources
PO Box 282
Orleans, CA 95556
(530) 469-3258

Chain of Custody for Klamath River Tissue Study L·678·10

Karuk DNR	39051 Hwy 96
PHONE 530-469-3258	Orleans, CA 95556
CONTACT Grant Johnson	EMAIL cbowman@karuk.us, gjohnson@karuk.us
Collected By Grant Johnson	SIGNATURE

		Sample ID	Date	Time	Lab ID	Sample Description	Microcystin ELISA	Microcystin LCMS/MS	Anatoxin
1	1	HC101410-8C	10/14		CDFG	Fish Tissue, Klamath		X	
2	2	HC101410-11S	"		"	"		"	
3	3	HC101410-12S	"		"	"		"	
4	4	HC101410-13S	"		"	"		"	
5	5	HC101410-14S	"		"	"		"	
6	6	HC101410-15S	10/14		"	"		"	
7	7	WE101510-16S	10/15		"	"		"	
8	8	WE101510-17S	"		"	"		"	
9	9	WE101510-18S	"		"	"		"	
10	10	WE101510-19S	"		"	"		"	
11	11	WE101510-20S	10/15		"	"		"	
12	12	OR101710-21S	10/17		"	"		"	
13	13	OR101710-22S	"		"	"		"	
14	14	OR101710-23S	10/17		CDFG	Fish Tissue, Klamath		X	

Date Shipped: 10/20/10 Carrier/ Shipping #

Date Received 10·21·10 0930

Received by Matt Hanks

Notes FROZEN 3.0°C

Ship to:

For microcystin ELISA:
Sample Custodian
USEPA Region 9 Lab
1337 S. 46th Street
Building 201
Richmond, CA 94804
510-412-2389

For anatoxin-a and microcystin LCMS/MS:
Dr. Abdou Mekebri
Fish and Wildlife Water Pollution Control Lab
2005 Nimbus Road
Rancho Cordova, CA 95670
(916) 358-4396

Send Results To:

Grant Johnson
Karuk Tribe
Dept of Natural Resources
PO Box 282
Orleans, CA 95556
(530) 469-3258

Chain of Custody
Karuk Tribe Department of Natural Resources

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Chain of Custody for Klamath River Tissue Study

L. 678. 10

Karuk DNR		39051 Hwy 96	
PHONE 530-469-3258		Orleans, CA 95556	
CONTACT Grant Johnson		EMAIL cbowman@karuk.us, gjohnson@karuk.us	
Collected By Grant Johnson		SIGNATURE	

		Sample ID	Date	Time	Lab ID	Sample Description	Microcystin ELISA	Microcystin LCMS/MS	Anatoxin
15	1	CR101710-2AS	10/17		CDFG	Fish Tissue, Klamath		X	
16	2	CR101710-2S	10/17						
17	3	IG101810-9C	10/18						
18	4	IG101810-10C							
19	5	IG101810-11C							
20	6	IG101810-12C							
21	7	IG101810-13C							
22	8	IG101810-14C	10/18		CDFG	Fish Tissue, Klamath		X	
	9								
	10								
	11								
	12								
	13								
	14								

Date Shipped: 10/20/10 Carrier/ Shipping #

Date Received 10-21-10 0930

Received by Scott Hamlin

Notes FROZEN 30°F

Ship to:

For microcystin ELISA:
Sample Custodian
USEPA Region 9 Lab
1337 S. 46th Street
Building 201
Richmond, CA 94804
510-412-2389

For anatoxin-a and microcystin LCMS/MS:
Dr. Abdou Mekebri
Fish and Wildlife Water Pollution Control Lab
2005 Nimbus Road
Rancho Cordova, CA 95670
(916) 358-4396

Send Results To:

Grant Johnson
Karuk Tribe
Dept of Natural Resources
PO Box 282
Orleans, CA 95556
(530) 469-3258

Chain of Custody
Karuk Tribe Department of Natural Resources

Page 2 of 2

Chain of Custody for Klamath River Tissue Study 2-711-10

Karuk DNR	39051 Hwy 96
PHONE 530-469-3258	Orleans, CA 95556
CONTACT Grant Johnson	EMAIL cbowman@karuk.us, gjohnson@karuk.us
Collected By Grant Johnson	SIGNATURE

	Sample ID	Date	Time	Lab ID	Sample Description	Microcystin ELISA	Microcystin LCMS/MS	Anatoxin
1	IG110110-15C	11/01		CDFG	Tissue, Liver & Fillet		X	
2	IG110110-16C	"		"	"		X	
3	IG110110-17C	"		"	"		X	
4	IG110110-18C	"		"	"		X	
5	IG110110-19C	"		"	"		X	
6	IG110110-20C	11/01		CDFG	Tissue, Liver & Fillet		X	
7								
8								
9								
10								
11								
12								
13								
14								

Date Shipped: 11/08/10 Carrier/ Shipping # _____

Date Received 11-9-10

Received by _____

Notes 7-20C PB

Ship to:

For microcystin ELISA:
Sample Custodian
USEPA Region 9 Lab
1337 S. 46th Street
Building 201
Richmond, CA 94804
510-412-2389

For anatoxin-a and microcystin LCMS/MS:
Dr. Abdou Mekebri
Fish and Wildlife Water Pollution Control Lab
2005 Nimbus Road
Rancho Cordova, CA 95670
(916) 358-4396

Send Results To:

Grant Johnson
Karuk Tribe
Dept of Natural Resources
PO Box 282
Orleans, CA 95556
(530) 469-3258

Chain of Custody
Karuk Tribe Department of Natural Resources

Page 1 of 1

Chain of Custody for Klamath River Tissue Study L·742.10

Karuk DNR	39051 Hwy 96
PHONE 530-469-3258	Orleans, CA 95556
CONTACT Grant Johnson	EMAIL cbowman@karuk.us, gjohnson@karuk.us
Collected By Grant Johnson	SIGNATURE

	Sample ID	Date	Time	Lab ID	Sample Description	Microcystin ELISA	Microcystin LCMS/MS	Anatoxin
1	IG112910-100	11/29		CDFG	Fish tissue, Fillet		X	
2	IG112910-100	"		"	Fish Tissue, Liver		"	
3	IG112910-200	"		"	Fish Tissue, Fillet		"	
4	IG112910-200	"		"	Fish Tissue, Liver		"	
5	IG112910-300	11/29		CDFG	Fish Tissue, Liver		X	
6								
7								
8								
9								
10								
11								
12								
13								
14								

Date Shipped: 12-06-10 Carrier/ Shipping # UPS

Date Received 12-7-10 1000

Received by Grant Hamm 41°F

Notes

Ship to:

For microcystin ELISA:
Sample Custodian
USEPA Region 9 Lab
1337 S. 46th Street
Building 201
Richmond, CA 94804
510-412-2389

For anatoxin-a and microcystin LCMS/MS:
Dr. Abdou Mekebri
Fish and Wildlife Water Pollution Control Lab
2005 Nimbus Road
Rancho Cordova, CA 95670
(916) 358-4396

Send Results To:

Grant Johnson
Karuk Tribe
Dept of Natural Resources
PO Box 282
Orleans, CA 95556
(530) 469-3258

Chain of Custody
Karuk Tribe Department of Natural Resources

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APPENDIX II: Fish and Wildlife Water Pollution Control Lab Sheets September, 2010.

WPCL Lab#	Estimated MDL	Reporting Limit	L-620-10-1	L-620-10-2	L-620-10-3	L-620-10-4	L-620-10-5	L-620-10-6	L-620-10-7	L-620-10-8	L-620-10-9	L-620-10-10	L-620-10-11	L-620-10-12	L-620-10-13	L-620-10-13Dup	L-620-10-14	L-620-10-15	L-620-10-16		L-620-10-MBLK	L-620-10-LCS	L-620-10-6MS	L-620-10-6MSD
Sample Identification			OR092310-1S	OR092310-1S	OR092310-2S	OR092310-2S	OR092610-3S	OR092610-3S	OR092610-4S	OR092610-4S	OR092610-5S	OR092610-5S	IP092810-1C	IP092810-1C	IP092710-2C	IP092710-2C	IP092710-2C	IP092710-3C	IP092710-3C				OR092610-3S	OR092610-3S
Date Collected			23/Sep/2010	23/Sep/2010	23/Sep/2010	23/Sep/2010	26/Sep/2010	26/Sep/2010	26/Sep/2010	26/Sep/2010	26/Sep/2010	26/Sep/2010	28/Sep/2010	28/Sep/2010	27/Sep/2010	27/Sep/2010	27/Sep/2010	27/Sep/2010	27/Sep/2010				26/Sep/2010	26/Sep/2010
Time Collected			NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA				NA	NA
Date Received			30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010				30/Sep/2010	30/Sep/2010
Date Extracted			04/Dec/2010	04/Dec/2010	04/Dec/2010	04/Dec/2010	04/Dec/2010	04/Dec/2010	04/Dec/2010	04/Dec/2010	04/Dec/2010	04/Dec/2010	04/Dec/2010	04/Dec/2010	04/Dec/2010	04/Dec/2010	04/Dec/2010	04/Dec/2010	04/Dec/2010		04/Dec/2010	04/Dec/2010	04/Dec/2010	04/Dec/2010
Date Analyzed			08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010		08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010
Matrix			fish liver	fish fillet	fish liver	fish fillet	fish liver	fish fillet	fish liver	fish fillet	fish liver	fish fillet	fish liver	fish fillet	fish liver	fish liver	fish fillet	fish liver	fish fillet		fish fillet	fish fillet	fish fillet	fish fillet
			fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight		fresh weight	fresh weight	fresh weight	fresh weight
Biotoxin Analytes	**ppb**	**ppb**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**		**ppb (ng/g)**	**% Recovery**	**% Recovery**	**% Recovery**
MC-RR	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.80	2.93	ND	ND	ND	Quality Control Results	ND	109	101	106
MC-Desmethyl-RR*	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
MC-LR	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	106	75.1	78.0
MC-Desmethyl-LR	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
MC-YR	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	117	99.3	99.4
MC-LA	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	112	81.4	84.4
MC-LW	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
MC-LF	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
MC-LY	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
Anatoxin A	5.00	10.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
Domoic acid	2.00	5.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
Okadaic acid	1.00	2.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA

* Desmethyl-RR quantified as parent analog compound.

WPCL Lab#	Estimated MDL	Reporting Limit	L-641-10-1	L-641-10-2	L-641-10-3	L-641-10-3Dup	L-641-10-4	L-641-10-5	L-641-10-6	L-641-10-7	L-641-10-8	L-641-10-9	L-641-10-10	L-641-10-11	L-641-10-12	L-641-10-13	L-641-10-14	L-641-10-15	L-641-10-16	L-641-10-17	L-641-10-18		L-641-10-MBLK	L-641-10-LCS	L-641-10-4MS	L-641-10-4MSD
Sample Identification			IP093010-4C	IP093010-4C	IP092910-1C	IP092910-1C	IP092910-1C	WE092910-5S	WE092910-5S	IP093010-3C	IP093010-3C	IP092910-2C	IP092910-2C	WE092910-1S	WE092910-1S	WE092910-2S	WE092910-2S	WE092910-4S	WE092910-4S	WE092910-3S	WE092910-3S				IP092910-1C	IP092910-1C
Date Collected			30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010	30/Sep/2010				30/Sep/2010	30/Sep/2010
Time Collected			NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA				NA	NA
Date Received			07/Oct/2010	07/Oct/2010	07/Oct/2010	07/Oct/2010	07/Oct/2010	07/Oct/2010	07/Oct/2010	07/Oct/2010	07/Oct/2010	07/Oct/2010	07/Oct/2010	07/Oct/2010	07/Oct/2010	07/Oct/2010	07/Oct/2010	07/Oct/2010	07/Oct/2010	07/Oct/2010	07/Oct/2010				07/Oct/2010	07/Oct/2010
Date Extracted			07/Dec/2010	07/Dec/2010	07/Dec/2010	07/Dec/2010	07/Dec/2010	07/Dec/2010	07/Dec/2010	07/Dec/2010	07/Dec/2010	07/Dec/2010	07/Dec/2010	07/Dec/2010	07/Dec/2010	07/Dec/2010	07/Dec/2010	07/Dec/2010	07/Dec/2010	07/Dec/2010	07/Dec/2010		07/Dec/2010	07/Dec/2010	07/Dec/2010	07/Dec/2010
Date Analyzed			08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010		08/Dec/2010	08/Dec/2010	08/Dec/2010	08/Dec/2010
Matrix			fish liver	fish fillet	fish liver	fish liver	fish fillet	fish liver	fish fillet	fish liver	fish fillet	fish liver	fish fillet	fish liver	fish fillet	fish liver	fish fillet	fish liver	fish fillet	fish liver	fish fillet		fish fillet	fish fillet	fish fillet	fish fillet
			fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight	fresh weight		fresh weight	fresh weight	fresh weight	fresh weight
Biotoxin Analytes	**ppb**	**ppb**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**		**ppb (ng/g)**	**% Recovery**	**% Recovery**	**% Recovery**
MC-RR	0.500	1.00	2.71	ND	2.17	2.10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	104	113	113
MC-Desmethyl-RR*	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
MC-LR	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	104	98.5	99.4
MC-Desmethyl-LR	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
MC-YR	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	125	120	108
MC-LA	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	107	92.6	95.3
MC-LW	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
MC-LF	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
MC-LY	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
Anatoxin A	5.00	10.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
Domoic acid	2.00	5.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
Okadaic acid	1.00	2.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA

Quality Control Results

* Desmethyl-RR quantified as parent analog compound.

WPCL Lab#	Estimated MDL	Reporting Limit	L-678-10-1	L-678-10-2	L-678-10-3	L-678-10-4	L-678-10-5	L-678-10-6	L-678-10-7	L-678-10-8	L-678-10-9	L-678-10-10	L-678-10-11
Sample Identification			HC101410-8C	HC101410-8C	HC101410-11S	HC101410-11S	HC101410-12S	HC101410-12S	HC101410-13S	HC101410-13S	HC101410-14S	HC101410-14S	HC101410-15S
Date Collected			14/Oct/2010	14/Oct/2010	14/Oct/2010	14/Oct/2010	14/Oct/2010	14/Oct/2010	14/Oct/2010	14/Oct/2010	14/Oct/2010	14/Oct/2010	14/Oct/2010
Time Collected			NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Date Received			21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010
Date Extracted			05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011
Date Analyzed			13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011
Matrix			liver	tissue	liver	tissue	liver	tissue	liver	tissue	liver	tissue	liver
			wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight
Biotoxin Analytes	ppb	ppb	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)
MC-RR	0.500	1.00	121	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-Desmethyl-RR*	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-LR	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-Desmethyl-LR	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-YR	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-LA	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-LW	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-LF	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-LY	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Anatoxin A	5.00	10.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Domoic acid	2.00	5.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Okadaic acid	1.00	2.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

WPCL Lab#	L-678-10-12	L-678-10-13	L-678-10-14	L-678-10-15	L-678-10-16	L-678-10-17	L-678-10-18	L-678-10-19	L-678-10-20	L-678-10-21	L-678-10-22
Sample Identification	HC101410-15S	WE101510-16S	WE101510-16S	WE101510-17S	WE101510-17S	WE101510-18S	WE101510-18S	WE101510-19S	WE101510-19S	WE101510-20S	WE101510-20S
Date Collected	14/Oct/2010	15/Oct/2010	15/Oct/2010	15/Oct/2010	15/Oct/2010	15/Oct/2010	15/Oct/2010	15/Oct/2010	15/Oct/2010	15/Oct/2010	15/Oct/2010
Time Collected	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Date Received	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010
Date Extracted	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011
Date Analyzed	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011
Matrix	tissue	liver	tissue	liver	tissue	liver	tissue	liver	tissue	tissue	liver
	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight
Biotoxin Analytes	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)
MC-RR	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-Desmethyl-RR*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-LR	ND	ND	ND	152	ND	ND	ND	ND	ND	ND	ND
MC-Desmethyl-LR	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-YR	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-LA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-LW	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-LF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-LY	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Anatoxin A	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Domoic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Okadaic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

WPCL Lab#	L-678-10-23	L-678-10-24	L-678-10-25	L-678-10-26	L-678-10-27	L-678-10-28	L-678-10-29	L-678-10-30	L-678-10-31	L-678-10-32	L-678-10-33
Sample Identification	OR101710-21S	OR101710-21S	OR101710-22S	OR101710-22S	OR101710-23S	OR101710-23S	OR101710-24S	OR101710-24S	OR101710-25S	OR101710-25S	IG101810-9C
Date Collected	17/Oct/2010	17/Oct/2010	17/Oct/2010	17/Oct/2010	17/Oct/2010	17/Oct/2010	17/Oct/2010	17/Oct/2010	17/Oct/2010	17/Oct/2010	18/Oct/2010
Time Collected	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Date Received	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010
Date Extracted	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011
Date Analyzed	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011
Matrix	tissue	liver	tissue	liver	tissue	liver	liver	tissue	liver	tissue	liver
	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight
Biotoxin Analytes	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)
MC-RR	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-Desmethyl-RR*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-LR	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-Desmethyl-LR	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-YR	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-LA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-LW	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-LF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-LY	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Anatoxin A	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Domoic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Okadaic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

WPCL Lab#	L-678-10-34	L-678-10-35	L-678-10-36	L-678-10-37	L-678-10-38	L-678-10-39	L-678-10-40	L-678-10-41	L-678-10-42	L-678-10-43	L-678-10-44
Sample Identification	IG101810-9C	IG101810-10C	IG101810-10C	IG101810-11C	IG101810-11C	IG101810-12C	IG101810-12C	IG101810-13C	IG101810-13C	IG101810-14C	IG101810-14C
Date Collected	18/Oct/2010	18/Oct/2010	18/Oct/2010	18/Oct/2010	18/Oct/2010	18/Oct/2010	18/Oct/2010	18/Oct/2010	18/Oct/2010	18/Oct/2010	18/Oct/2010
Time Collected	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Date Received	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010	21/Oct/2010
Date Extracted	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	06/Feb/2011	06/Feb/2011	06/Feb/2011	06/Feb/2011
Date Analyzed	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011
Matrix	tissue	tissue	liver	tissue	liver	tissue	liver	tissue	liver	tissue	liver
	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight
Biotoxin Analytes	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)	ppb (ng/g)
MC-RR	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-Desmethyl-RR*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-LR	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-Desmethyl-LR	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-YR	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-LA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-LW	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-LF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC-LY	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Anatoxin A	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Domoic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Okadaic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

* Desmethyl-RR quantified as parent analog compound.

	L-678-10-MBLK1	L-678-10-LCS1	L-678-10-2MS	L-678-10-2MSD	L-678-10-MBLK2	L-678-10-LCS2	L-678-10-23MS	L-678-10-23MSD	L-678-10-MBLK3	L-678-10-LCS3	L-711-10-4MS	L-711-10-4MSD
			HC101410-8C	HC101410-8C			OR101710-21S	OR101710-21S			IG110110-16C	IG110110-16C
			14/Oct/2010	14/Oct/2010			17/Oct/2010	17/Oct/2010			01/Nov/2010	01/Nov/2010
			NA	NA			NA	NA			NA	NA
			21/Oct/2010	21/Oct/2010			21/Oct/2010	21/Oct/2010			09/Nov/2010	09/Nov/2010
	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	05/Feb/2011	06/Feb/2011	06/Feb/2011	06/Feb/2011	06/Feb/2011
	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	13/Feb/2011	14/Feb/2011	14/Feb/2011
	liver	liver	tissue	tissue	liver	liver	tissue	tissue	liver	liver	tissue	tissue
	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight
	ppb (ng/g)	% Recovery	% Recovery	% Recovery	ppb (ng/g)	% Recovery	% Recovery	% Recovery	ppb (ng/g)	% Recovery	% Recovery	% Recovery
Quality Control Results	ND	80.0	89.7	87.8	ND	79.3	67.6	80.1	ND	83.1	76.7	69.7
	ND	NA	NA	NA	ND	NA	NA	NA	ND	NA	NA	NA
	ND	97.8	112	110	ND	81.5	69.7	81.7	ND	75.2	118	121
	ND	NA	NA	NA	ND	NA	NA	NA	ND	NA	NA	NA
	ND	86.7	117	119	ND	71.7	81.8	86.4	ND	76.8	103	107
	ND	84.5	98.2	84.7	ND	102	71.2	79.9	ND	105	118	122
	ND	NA	NA	NA	ND	NA	NA	NA	ND	NA	NA	NA
	ND	NA	NA	NA	ND	NA	NA	NA	ND	NA	NA	NA
	ND	NA	NA	NA	ND	NA	NA	NA	ND	NA	NA	NA
	ND	86.3	83.8	84.9	ND	82.1	86.9	88.6	ND	85.5	86.2	83.8
	ND	NA	NA	NA	ND	NA	NA	NA	ND	NA	NA	NA
	ND	NA	NA	NA	ND	NA	NA	NA	ND	NA	NA	NA

WPCL Lab#	Estimated MDL	Reporting Limit	L-711-10-1	L-711-10-2	L-711-10-3	L-711-10-4	L-711-10-5	L-711-10-6	L-711-10-7	L-711-10-8	L-711-10-9	L-711-10-10	L-711-10-11	L-711-10-12		L-678-10-MBLK3	L-678-10-LCS3	L-711-10-4MS	L-711-10-4MSD
Sample Identification			IG110110-15C	IG110110-15C	IG110110-16C	IG110110-16C	IG110110-17C	IG110110-17C	IG110110-18C	IG110110-18C	IG110110-19C	IG110110-19C	IG110110-20C	IG110110-20C				IG110110-16C	IG110110-16C
Date Collected			01/Nov/2010	01/Nov/2010	01/Nov/2010	01/Nov/2010	01/Nov/2010	01/Nov/2010	01/Nov/2010	01/Nov/2010	01/Nov/2010	01/Nov/2010	01/Nov/2010	01/Nov/2010				01/Nov/2010	01/Nov/2010
Time Collected			NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA				NA	NA
Date Received			09/Nov/2010	09/Nov/2010	09/Nov/2010	09/Nov/2010	09/Nov/2010	09/Nov/2010	09/Nov/2010	09/Nov/2010	09/Nov/2010	09/Nov/2010	09/Nov/2010	09/Nov/2010				09/Nov/2010	09/Nov/2010
Date Extracted			06/Feb/2011	06/Feb/2011	06/Feb/2011	06/Feb/2011	06/Feb/2011	06/Feb/2011	06/Feb/2011	06/Feb/2011	06/Feb/2011	06/Feb/2011	06/Feb/2011	06/Feb/2011		06/Feb/2011	06/Feb/2011	06/Feb/2011	06/Feb/2011
Date Analyzed			14/Feb/2011	14/Feb/2011	14/Feb/2011	14/Feb/2011	14/Feb/2011	14/Feb/2011	14/Feb/2011	14/Feb/2011	14/Feb/2011	14/Feb/2011	14/Feb/2011	14/Feb/2011		13/Feb/2011	13/Feb/2011	14/Feb/2011	14/Feb/2011
Matrix			**liver**	**tissue**	**liver**	**tissue**	**liver**	**tissue**	**liver**	**tissue**	**liver**	**tissue**	**liver**	**tissue**		**liver**	**liver**	**tissue**	**tissue**
			wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight	wet weight		wet weight	wet weight	wet weight	wet weight
Biotoxin Analytes	**ppb**	**ppb**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**		**ppb (ng/g)**	**% Recovery**	**% Recovery**	**% Recovery**
MC-RR	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	83.1	76.7	69.7
MC-Desmethyl-RR*	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
MC-LR	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	75.2	118	121
MC-Desmethyl-LR	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
MC-YR	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	76.8	103	107
MC-LA	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	105	118	122
MC-LW	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
MC-LF	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
MC-LY	0.500	1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
Anatoxin A	5.00	10.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	85.5	86.2	83.8
Domoic acid	2.00	5.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
Okadaic acid	1.00	2.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	NA	NA	NA
* Desmethyl-RR quantified as parent analog compound.																			

Quality Control Results

WPCL Lab#	Estimated MDL	Reporting Limit	L-742-10-1	L-742-10-2	L-742-10-3	L-742-10-4	L-742-10-5		L-742-10-MBLK	L-742-10-LCS	L-711-10-4MS	L-711-10-4MSD
Sample Identification			**IG112910-1CO**	**IG112910-1CO**	**IG112910-2CO**	**IG112910-2CO**	**IG112910-3CO**				**IG110110-16C**	**IG110110-16C**
Date Collected			29/Nov/2010	29/Nov/2010	29/Nov/2010	29/Nov/2010	29/Nov/2010				01/Nov/2010	01/Nov/2010
Time Collected			NA	NA	NA	NA	NA				NA	NA
Date Received			07/Dec/2010	07/Dec/2010	07/Dec/2010	07/Dec/2010	07/Dec/2010				09/Nov/2010	09/Nov/2010
Date Extracted			06/Feb/2011	06/Feb/2011	06/Feb/2011	06/Feb/2011	06/Feb/2011		06/Feb/2011	05/Feb/2011	06/Feb/2011	06/Feb/2011
Date Analyzed			14/Feb/2011	14/Feb/2011	14/Feb/2011	14/Feb/2011	14/Feb/2011		14/Feb/2011	14/Feb/2011	14/Feb/2011	14/Feb/2011
Matrix			**tissue**	**liver**	**tissue**	**liver**	**liver**		**liver**	**liver**	**tissue**	**tissue**
			wet weight	wet weight	wet weight	wet weight	wet weight		wet weight	wet weight	wet weight	wet weight
Biotoxin Analytes	**ppb**	**ppb**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**	**ppb (ng/g)**		**ppb (ng/g)**	**% Recovery**	**% Recovery**	**% Recovery**
MC-RR	0.500	1.00	ND	ND	ND	ND	ND		ND	71.5	76.7	69.7
MC-Desmethyl-RR*	0.500	1.00	ND	ND	ND	ND	ND		ND	NA	NA	NA
MC-LR	0.500	1.00	ND	ND	ND	ND	ND		ND	80.8	118	121
MC-Desmethyl-LR	0.500	1.00	ND	ND	ND	ND	ND		ND	NA	NA	NA
MC-YR	0.500	1.00	ND	ND	ND	ND	ND		ND	80.3	103	107
MC-LA	0.500	1.00	ND	ND	ND	ND	ND		ND	107	118	122
MC-LW	0.500	1.00	ND	ND	ND	ND	ND		ND	NA	NA	NA
MC-LF	0.500	1.00	ND	ND	ND	ND	ND		ND	NA	NA	NA
MC-LY	0.500	1.00	ND	ND	ND	ND	ND		ND	NA	NA	NA
Anatoxin A	5.00	10.0	ND	ND	ND	ND	ND		ND	88.7	86.2	83.8
Domoic acid	2.00	5.00	ND	ND	ND	ND	ND		ND	NA	NA	NA
Okadaic acid	1.00	2.00	ND	ND	ND	ND	ND		ND	NA	NA	NA
* Desmethyl-RR quantified as parent analog compound.												

Quality Control Results

APPENDIX III. Klamath River Salmonid Histopathology Results

Prepared by:
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Abbotsford, BC,
Canada, V3G 2M3

Histology Protocol

Tissue sampling for histopathology was incorporated into ongoing efforts to screen returning salmon stocks for microcystin analysis. At the time of collection by either fish line or dip net, fish were assigned a log number and the harvest site, species, and demographic data were recorded. The sample collection and processing dates were noted and gross observations of tissue abnormalities or other significant observations were recorded. For logistical reasons, collected fish were refrigerated at 4C for up to 3 days post collection prior to processing. Photos of the external aspect of intact whole fish and internal organs were obtained. Cytological examination of gill wet mounts and skin scrapes to screen for *Columnaris* sp. and other external pathogens was undertaken.

Fish dissection consisted of a midventral incision of the coelomic cavity, with en block removal of the internal viscera. Organs sampled for microscopic evaluation included the liver, spleen, pyloric caecae, pancreas, heart, gill arch, skin, skeletal muscle, spinal cord, vertebral column and kidney. The tissue samples were rinsed with saline solution to minimize superficial blood. After the gill arches were removed, the head was transected and shipped fresh weekly on ice to Melissa A. Miller, DVM, PhD at the Marine Wildlife Veterinary Care and Research Center Department of Fish and Game and University of California, Davis in Santa Cruz, CA for dissection of the skull and removal of the brain (for September samples only). Preserved tissues were forwarded to the Animal Health Centre, Abbotsford, British Columbia for processing and analysis.

Formalin fixed tissues were trimmed and placed into cassettes, then transferred by an automatic processor (Tissue-Tek VIP, Sakura) through a graded series of alcohols, xylene and then embedded in paraffin. Four to 6 *u*m sections were prepared and stained by an automatic stainer (Varistain, Gemini, Shandon) with hematoxylin and eosin. On review of the sections and when indicated, additional recuts and special stains including Ziehl-Neelsen (ZN) and periodic acid Schiff (PAS) (Prophet et al, 2002) were undertaken to screen for acid fast bacilli and fungal elements, respectively. Sections were reviewed blindly, lesions identified and scored by disease process, severity and extent of tissue involvement and morphologic diagnoses were tabulated in an excel spreadsheet. The disease process was scored as mild, moderate, marked, severe or no significant findings. Major diseases processed were identified and frequencies summarized. The tissue sections and blocks have been forwarded to Dr. Scott Foott, USFWS, CA-NV Fish Health Center for further review.

Results/Discussion

Histopathology was undertaken from harvested fish tissues. From the 50 sampled fish, 288 histologic slides were prepared and evaluated. Not all tissues were systematically collected from each harvested fish and in some instances, whole organs or samples greater than 1 cm in width were placed in formalin, which resulted in inadequate preservation and post mortem tissue degradation (autolysis). Sampled tissues included liver, small intestine, pyloric caecae, adipose tissue, pancreas, stomach, gills, posterior and anterior kidney, corpuscles of Stannius, skeletal muscle, spleen and skin. Those tissues with adequate fixation featured a number of pathologic processes (Appendix IV).

Morphologic findings in the liver included bile duct hyperplasia, biliary protozoa, flukes (trematodiasis), hepatocellular vacuolation, nodular hyperplasia and cholangitis or cholangiohepatitis (Appendix V). Cholangitis and cholangiohepatitis and biliary ductular hyperplasia (figures 9) were the most commonly observed processes (n=38 and n=30 fish, respectively), with fewer fish presenting with hepatocellular vacuolation (n=12), flukes (n=4), protozoa (n=3) or regenerative nodules (n=2) (figure 10). Within the gastrointestinal tract, the most common pathology was inflammation (enteritis or pyloric typhlitis), either associated with tapeworms (n=17) (figure 11 and 12) or more rarely, nematodes (n=3) and flukes (n=1). In many fish, the inflammation resulted in dilation of the intestinal lumina with mucosal erosions and ulcerations and in more severely affected animals; there was transmural extension of the granulomatous inflammatory infiltrate with secondary diverticulitis, peritonitis, steatitis and pancreatitis (figure 13). Low grade gastritis was noted in 6 animals and pancreatic Islet hyperplasia was apparent in 10 fish. Atrophy of the coelomic fat was apparent in 2 fish.

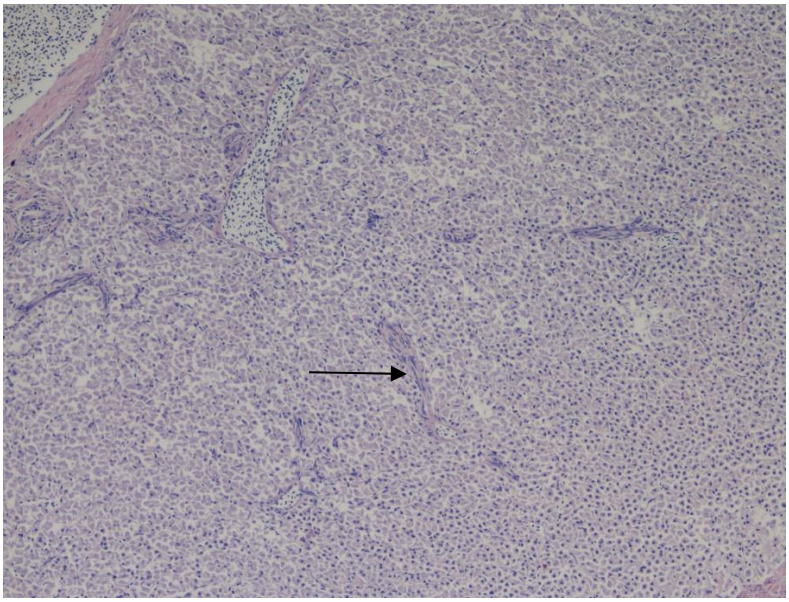

Figure 9: Liver with multifocal bile duct hyperplasia (arrow). Note the absence of megalocytosis and canulicular cholestasis.

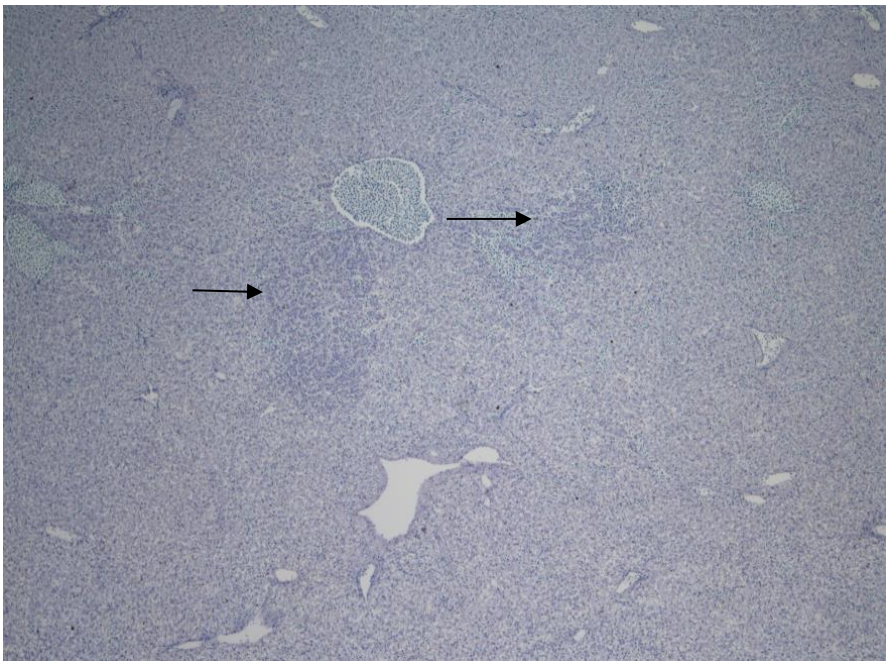

Figure 10. Basophilic foci within the liver parenchyma (arrows). These are focal regions of nodular regenerative hyperplasia. There is scattered biliary ductular hyperplasia within the intervening parenchyma with no evidence of megalocytosis.

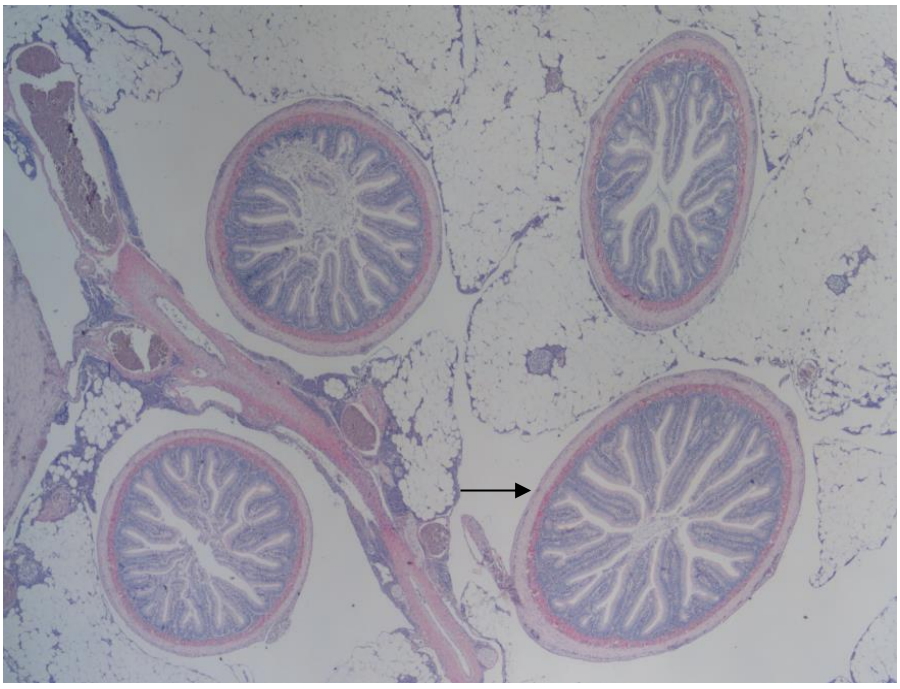

Figure 11. Image of normal pyloric caecae (arrow) with intervening adipose tissue and pancreatic acinar cells.

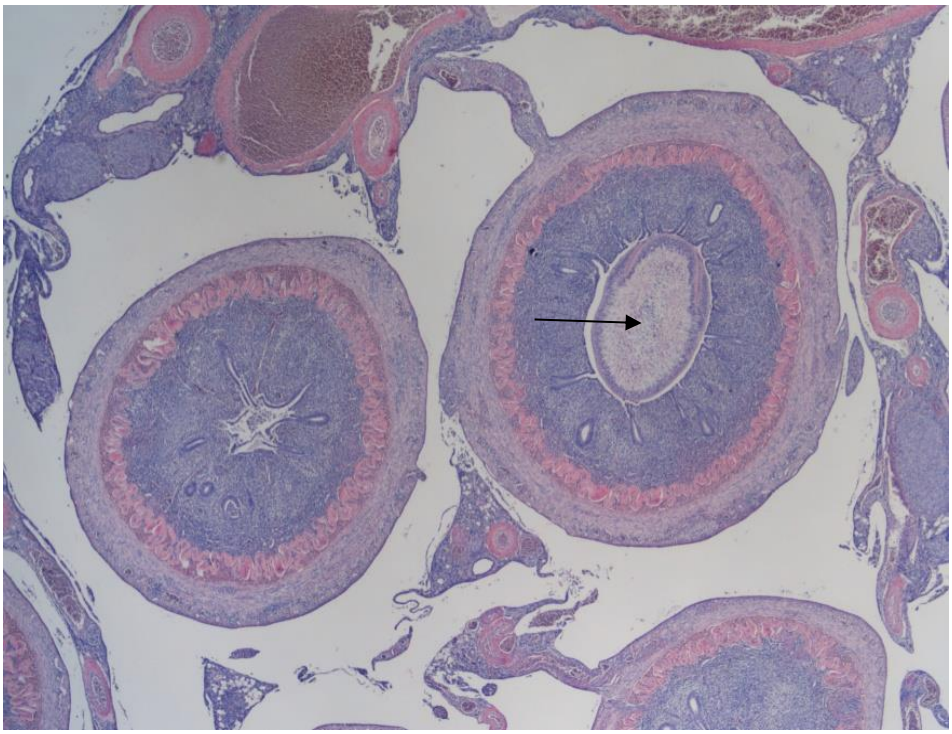

Figure 12. Pyloric enteritis, circumferential expansion of the lamina propria by inflammatory infiltrate with an intralesional cestode parasite (arrow).

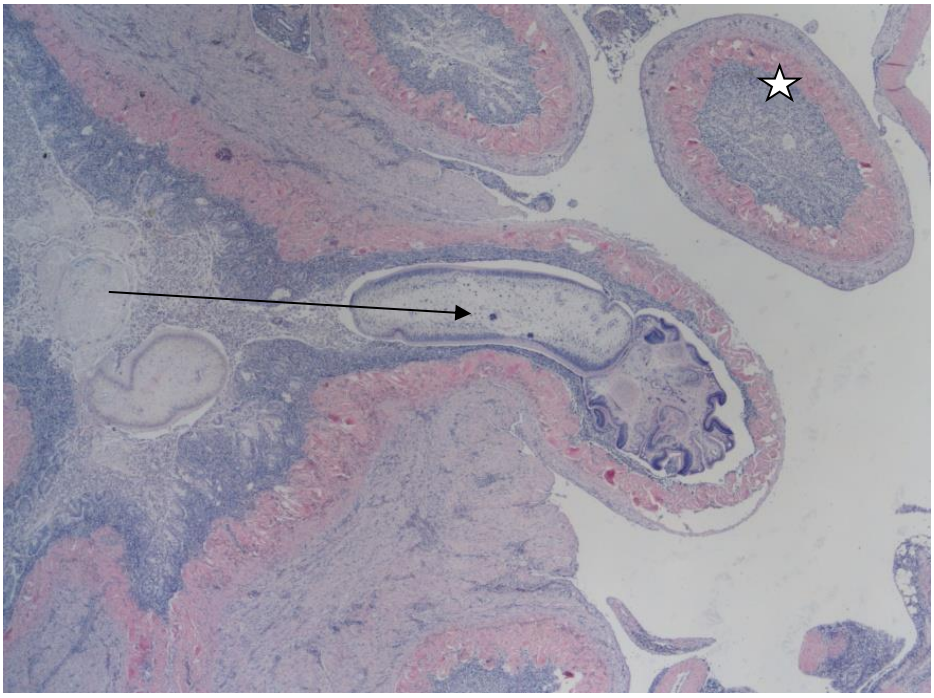

Figure 13. Focal diverticularization of the pyloric caecae with a large cestode parasite (arrow). Note the degree of inflammation within the mucosa (star).

Within the respiratory system, 46 fish featured branchitis and in 45 gill sections, there were variable numbers of trematodes or flukes (figure 14a and 14b), often associated with distortion of the primary lamella due to chondrodysplasia, respiratory epithelial hyperplasia and inflammatory infiltrate. In individual fish, there was mycosis morphologically consistent with saprolegniasis and protozoal parasitism. Two fish exhibited telangiectasia. The most significant finding in the examined kidneys was interstitial trematodes and glomerulonephritis. In 4 fish, there was membranous glomerulopathy and rare glomerular protozoa (figure 15). Renal tubular epithelial vacuolation and occasional tubuloproteinosis were also evident in 2 and 1 fish, respectively.

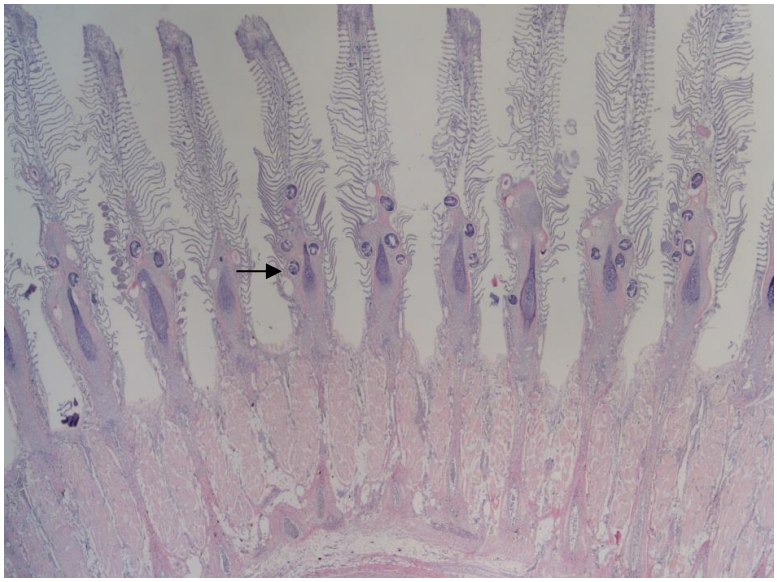

Figure 14a. Gill arch with severe segmental and laminar distortion of primary lamellae by chondrodysplasia, respiratory epithelial hyperplasia and numerous intralesional trematodes (arrow).

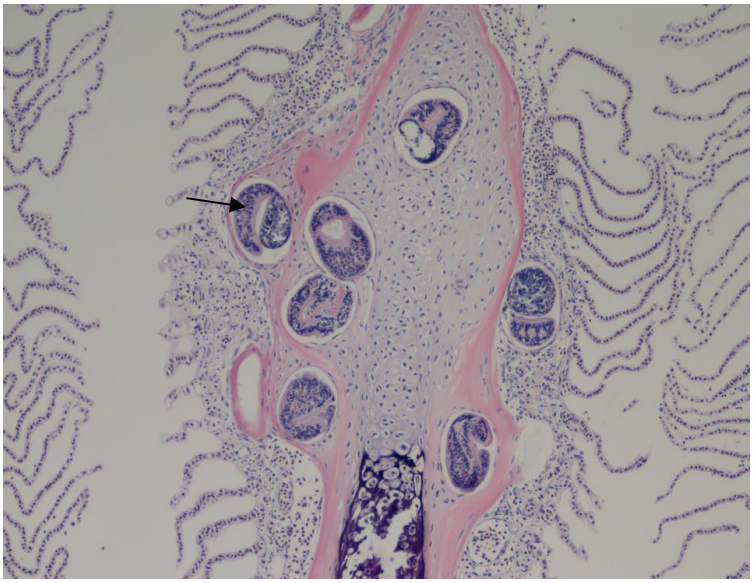

Figure 14b. Higher magnification of affected primary lamella. Note degree of chondrodysplasia and numerous intralesional trematodes (arrow).

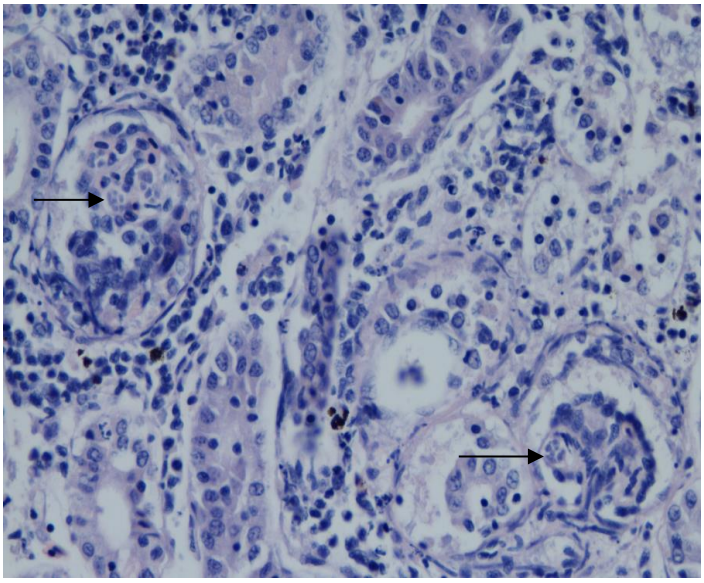

Figure 15. In a small number of fish, throughout the glomerular tufts, there are scattered aggregates of protozoal parasites. (arrows).

Throughout the ventricular myocardium, fluke infection (trematodiasis) was the most commonly identified pathologic process; 1 animal featured severe infection, 23 with marked, 3 with moderate and 16 with light burdens (figures 16a and 16b). Associated myocarditis was apparent in 43 fish; however, this infiltrate was generally sparse to mild, with occasional reactive endocardia and platelet aggregation. Nineteen animals presented with skeletal muscle degeneration, 6 with associated hemorrhage and individual fish featured nonspecific myositis and mild trematodiasis. In select fish, such as sample 1129103C, intracellular protozoa morphologically consistent with *Kudoa* spp were observed with no attend necrosis or inflammatory infiltrate (figure 17). Skin erosions and dermal fluke infections were observed in 12 and 2 fish, respectively and splenic congestion was apparent in 20 fish.

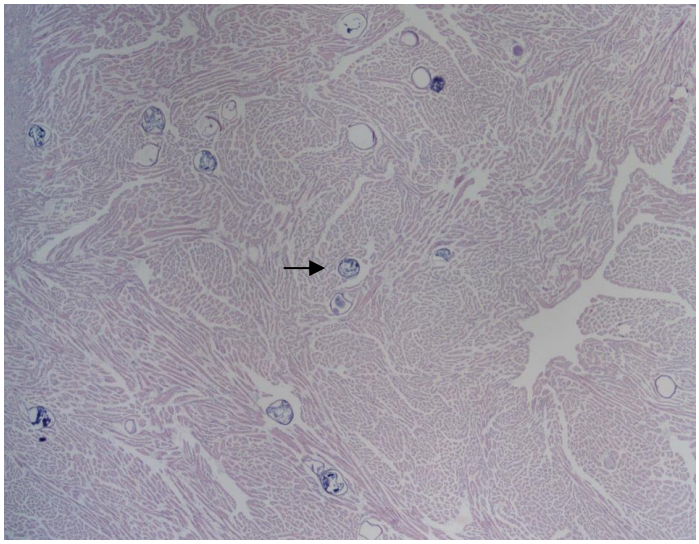

Figure 16a. Low power photomicrograph of the ventricle, note numerous punctate basophilic structures dispersed randomly throughout the myocardium (arrow).

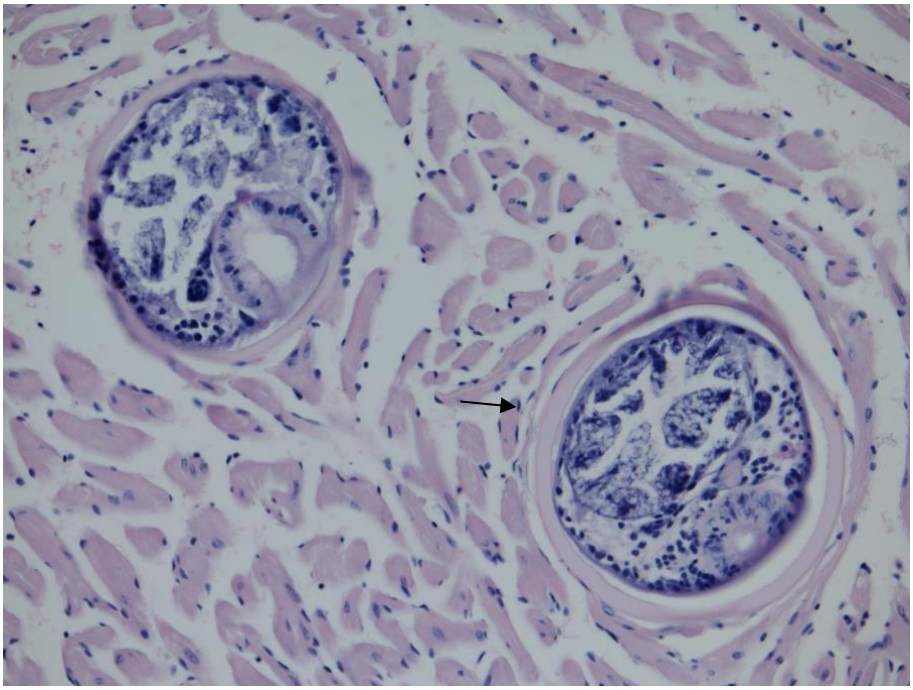

Figure 16b. Higher magnification image of figure 8a with 2 trematode parasites bound by a thin homogeneous and refractile margin (arrow).

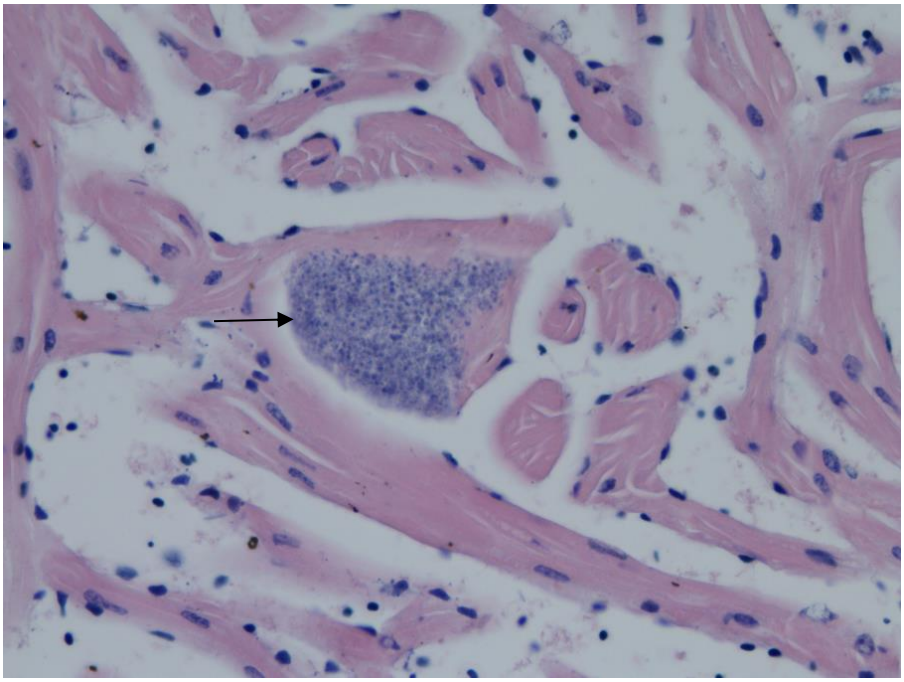

Figure 17. Within the ventricular spongy layer of the myocardium, there is focal intracellular accumulation of protozoa morphologically consistent with *Kudoa* spp (arrow). This parasite is distinct to the renal glomerular protozoa.

Histopathology of the sectioned tissues features multisystemic inflammation, often associated with intralesional pathogens, with no conclusive evidence of microcystin intoxication. Although biliary ductular hyperplasia and hepatocellular cytoplasmic vacuolation were evident in a small number of examined livers, there was no indication of cholestasis, megalocytosis, pseudoinclusions or other features recognized in fish with natural and experimental exposure to microcystin LR (Kent, 1990; Andersen et al, 1993). The lack of pathognomonic lesions within the liver may be attributed to a number of factors, including sublethal toxin exposure, too short a time interval between bioaccumulation and subsequent fish sampling, intercurrent disease which may have obscured more significant or subtle histopathology and other factors. In this case series, the observed liver lesions are most likely due to a combination of factors, such as disruption of the pyloric and intestinal mucosa by the cestodes with secondary ascending septicemia or bacteremia, regional peritonitis, suboptimal nutritional status associated with migration from salt to fresh water, or impaired respiratory function and possible ischemia due to the branchitis. With future investigations, more systemic sampling with a broader range of tissues may further resolve the pathogenesis and sequela associated with the parasitism.

In multiple fish, sections of pyloric caecae (small intestine) disclosed variably extensive ulcerative and granulomatous enteritis with numerous intralesional tapeworms and fewer nematodes in the gut lumen (Figures 12 and 13). In more severely affected fish the inflammation circumferentially expanded the lamina propria and submucosa, obliterated villi, occasionally extended across the intestinal wall and involved adjoining pancreatic and fat tissue. Cestodes have previously been documented in wild and farmed salmonids and are generally not considered highly pathogenic. In figure 13, an anterior scolex with bothria (suckers) is evident; the morphology is suggestive of *Eubothrium* spp, possibly *E salvelini* or *E crassum*, which have been associated with heavy infections in the pyloric caecae and intestines of salmonids in the Pacific Northwest, often with no untoward effect (Margolis, 1982; Kent, 1992). The lesions in this series of fish are more extensive than may typically be appreciated in sampled wild salmonids and may suggest a maladapted host parasite relationship, massive overwhelming environmental exposure to the infective stages of the parasites, immunosuppression due to the reproductive status of the fish, generalized debilitation associated with anadromous migration, malnutrition (catabolic state), suboptimal water quality, potential interactions with sub-lethal microcystin exposure or some other entity. As some cestodes in salmonids are zoonotic (transmission to people), collection of intestinal tracts and submission to a parasitologist for more precise speciation is recommended. In some histologic sections, the cestodes are larvae in profile and may represent *Diphyllobothrium* spp or other related tapeworm species.

Multisystemic trematode (fluke) parasitism involving the gills, bile duct system, and kidney tubules, in conjunction with microscopic lesions was evident in multiple fish and suggests population levels impacts. If further investigations are conducted into the health status of these animals, bacteriology, virology and possible heavy metal analysis may be considered. Cytology of gill wet mounts and skin scrapes did not appear to detect any significant external pathogens.

In a small number of examined hearts, there was variation and distortion of the compact layer of the myocardium, similar to malformation reported in farmed Atlantic salmon. The cause of these lesions is unknown; however, elevated water temperatures during egg and early larval development have been implicated. In this case series, large numbers of encapsulated or encysted trematodes (flukes) may have compromised the integrity of the spongy layer of the myocardium with

subsequent changes in conformation (for example sample 1014109C). Malformations were also apparent in examined hearts with light to moderate parasitism.

Protozoa were also apparent in the renal glomeruli, interspersed within the branchial cavity and myocardium. In rainbow trout *Chloromyxum majori* parasitemias (Yasutake and Wood, 1957) may localize to glomerular tufts with no apparent systemic effect on the infected fish; however, in other host species, protozoa may result in chronic obstructive glomerulonephritis with secondary osmoregulatory failure (Myers and McPherson, 1985). In this case series, the precise species of protozoa in the glomeruli is unknown and electron microscopy and ancillary molecular studies may be considered. The parasite and host response are distinct to proliferative kidney disease (PKD), due to *Tetracapsuloides bryosalmonae* (Canning et al, 1999) and consultation with Dr Foott may also provide valuable insights.

Although *Kudoa* spp has also been reported in renal glomeruli in some fish species (Paperna, 1982), in this case the renal protozoa appear morphologically distinct to this species. However, within sections of myocardium, there are rare intracellular protozoa consistent with *Kudoa* spp (fish 1129103C)(Kabata and Whitacker, 1981). This latter parasite has been associated with soft flesh or advanced liquifactive change in skeletal muscle post mortem; released proteolytic enzymes elaborated by the parasite are believed to contribute to the muscle degeneration. In multiple fish examined in this cohort, there was acute myocellular degeneration and necrosis characterised by varying degrees of loss of cytoplasmic striations, vacuolation, endomysial edema, hemorrhage and occasional subacute inflammation. These changes are distinct to the proteolytic myocytolysis and more likely due to capture myopathy and metabolic acidosis.

The multisystemic trematodiasis was profound and identified in numerous sampled fish. The morphology of the parasites appears consistent with each of the sectioned tissues (gills, heart, skin, skeletal muscle and kidney) and distinct to the cestodes and nematodes identified in the pyloric caecae and small intestine. Increased burdens and more widespread tissue dissemination may suggest debilitation or immunosuppression of host fish or massive environmental contamination of infectious stages; digenetic trematodes including *Phyllodistomum*, *Centrocestus*, and *Clonorchis* spp and monongenetic trematodes, such as *Acolpenteron*, *Kritskyia*, *Philureter* and *Urogyrus* spp (Ferguson, 2006). Submission of fresh tissues and histology sections for outside consultation and possible speciation may be considered. Based on the apparent lack of gross lesions, it is likely that the parasite burden did not impact overall homeostasis.

Despite the array of histopathologic findings, overall the fish presented largely in good health. There was no indication of microcystin intoxication; however, supervening or secondary infections and inflammation may have hampered microscopic assessment of the sectioned tissues. A broader suite of tissue samples with future harvests may provide additional insights into the overall health of the stock.

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APPENDIX IV. Morphologic Diagnoses of Sampled Fish.

MORPHOLOGIC DIAGNOSES:

Sample 1014108C:
Slide 1
1). Spleen: Congestion, moderate, diffuse
Slide 2
1). Liver: Hyperplasia, biliary ductular, mild, multifocal
2). Liver: Cholangiohepatitis, mild, multifocal, random, necrotising, subacute
Slide 3
1). Skeletal muscle: Myocellular degeneration, mild to moderate, multifocal, random with occasional endomysial hemorrhage
Slide 4
1). Kidney: Glomerulonephritis, mild to moderate, diffuse, multifocal, lymphohistiocytic with parietal cell hyperplasia and hypertrophy, synechiae and occasional periglomerular fibrosis
2). Kidney: Trematodes, encapsulated, moderate, multifocal, random with scattered tubuloproteinosis
3). Gills: Branchitis, interstitial, mild, multifocal, random, granulomatous with scattered respiratory epithelial hyperplasia, and intralesional trematode parasites
Slide 5
1). Liver: As in slide 2
2). Skeletal muscle: As in slide 3

Sample 1014109C:
Slide 1:
1). Heart: Myocarditis, mild, multifocal, random, subacute with numerous trematodes
There are no overt lesions within the posterior kidney.
Slide 2:
1). Small intestine: Enteritis, moderate, multifocal, subacute with villar blunting and fusion
2). Skeletal muscle: Hemorrhage, endomysial, mild, multifocal, acute
Slide 3:
3). Gills: Branchitis, interstitial, mild, multifocal, random, subacute, with scattered respiratory epithelial hyperplasia, and intralesional trematode parasites
Slide 4:
1). Heart: Myocarditis, mild, multifocal, random, subacute with numerous trematodes
2). Heart: Possible malformation, moderate, segmental
Slide 5:
1). Pyloric caeca and small intestine: Enteritis, moderate, multifocal, granulomatous, erosive and ulcerative, with glandular blunting, fusion and effacement and numerous intraluminal cestode parasites
2). Coelomic cavity: Peritonitis, moderate, multifocal, with mesothelial hypertrophy, hyperplasia and micropapillary proliferations
3). Pancreas: Pancreatitis, mild, multifocal, granulomatous with effacement and entrapment of acinar cells and diffuse islet hyperplasia

Sample 10141011S:
Slide 1
1). Kidney: Glomerulonephritis, mild, diffuse, multifocal, lymphohistiocytic with parietal cell hyperplasia and hypertrophy, synechiae and occasional periglomerular fibrosis
2). Skin: Erosions, mild, multifocal
3). Spleen: Congestion, moderate, diffuse, acute
There are significant lesions within the skeletal muscle.
Slide 2
1). Gills, primary lamellae and arch: Branchitis, interstitial, moderate, multifocal, random, granulomatous with chondrodysplasia, respiratory epithelial hyperplasia, and intralesional encapsulated trematode parasites
2). Heart: Myocarditis, mild, multifocal, random, subacute with myocardial degeneration and multiple intralesional trematodes
Slide 3
1). Small intestine: Enteritis, mild to moderate, multifocal with scattered intralesional cestodes and trematode parasites
There are no overt lesions within the pancreas or adipose tissue.
Slide 4
1). Liver: Hyperplasia, biliary ductular, mild to moderate, multifocal, random
Slide 5
Aside from post mortem change, there are no overt lesions within the pyloric caecae, pancreas or adipose tissue.

Sample 10141012S:
Slide 1
1). Heart: Myocarditis, mild, multifocal, random, subacute with occasional myocardial degeneration and multiple intralesional trematodes
2). Spleen: Congestion, moderate, multifocal
3). Skin: Erosion, moderate, diffuse
There are no overt lesions within the skeletal muscle.
Slide 2
1). Liver: Hyperplasia, biliary ductular, mild, multifocal
2). Liver: Hepatitis, mild, multifocal, random, nonsuppurative
3). Kidney: Glomerulonephritis, mild, multifocal, subacute with occasional periglomerular fibrosis
Slide 3
1). Gills, primary lamellae: Branchitis, interstitial, mild, multifocal, random, subacute with chondrodysplasia, respiratory epithelial hyperplasia, and

scattered intralesional trematode parasites
There are no overt lesions within the small intestine, pyloric caecae, pancreas or fat lobules.
Slide 4
Adipose tissue, pancreas, pyloric caecae and small intestine: As in slide 3.
Sample 10141013S:
Slide 1:
1). Gills, primary lamellae: Branchitis, interstitial, mild, multifocal, random, subacute with chondrodysplasia, respiratory epithelial hyperplasia, and scattered intralesional trematode parasites
2). Posterior kidney: Glomerulopathy, membranous, moderate, diffuse, multifocal with scattered tubuloproteinosis
3). Spleen: Congestion, moderate, multifocal, acute
There are no overt lesions within the corpuscle of Stannius, skeletal muscle, skin, or lateral line.
Slide 2:
1). Liver: Hyperplasia, biliary ductular, mild, multifocal
2). Liver: Hepatitis, mild, multifocal, random, nonsuppurative
Slide 3:
1). Heart: Myocarditis, mild, multifocal, random, subacute with occasional myocardial degeneration and scattered intralesional trematodes
Aside from post mortem change, there are no significant lesions in the small intestine, pyloric caecae, pancreas or fat lobules
Slide 4:
1). Small intestine, pyloric caecae, pancreas and fat lobules, as in slide 3.
Sample 10141014S:
Slide 1:
1). Liver: Hyperplasia, biliary ductular, mild, multifocal
2). Liver: Cholangiohepatitis, mild, multifocal, random, nonsuppurative
Slide 2:
1). Heart: Myocarditis, mild, multifocal, random, subacute with occasional myocardial degeneration and numerous intralesional trematodes
2). Posterior kidney: Glomerulopathy, membranous, moderate, diffuse, multifocal
There are no overt lesions within the skeletal musculature or skin.
Slide 3:
1). Stomach, laminar propria: Granuloma, parasitic, mild, focal with scattered submucosal mineral deposits
There are no overt lesions within the small intestine, pyloric caecae, pancreas and fat lobules,
Slide 4:
1). Gills, primary lamellae: Branchitis, interstitial, moderate, multifocal, random, subacute with chondrodysplasia, respiratory epithelial hyperplasia, and scattered intralesional trematode parasites
There are no overt lesions within the spleen or small intestine
Sample 10141015S:
Slide 1
1). Gills, primary lamellae and gill arch: Branchitis, interstitial, moderate, multifocal, random, granulomatous with chondrodysplasia, respiratory epithelial hyperplasia, and intralesional encapsulated trematode parasites
Aside from post mortem change, there are no overt lesions in the small intestine.
Slide 2
1). Liver: Hyperplasia, biliary ductular, mild to moderate, multifocal
2). Skin: Erosions, mild to moderate, multifocal
3). Skeletal muscle: Myocellular degeneration, mild, multifocal, random
Slide 3
1). Heart: Myocarditis, mild, multifocal, random, subacute with occasional myocardial degeneration and multiple intralesional trematodes
There are no significant lesions within the anterior kidney, posterior kidney or corpuscle of Stannius.
Slide 4
1). Liver: Hyperplasia, biliary ductular, mild to moderate, multifocal
2). Liver: Cholangiohepatitis, mild, multifocal, random, nonsuppurative
Slide 5
Aside from post mortem change, there are no overt lesions within the pancreas, adipose tissue, pyloric caecae, small intestine or spleen.
Slide 6
As with slide 5, aside from post mortem change, there are no overt lesions within the pancreas, adipose tissue, skeletal muscle, stomach, pyloric caecae or small intestine.
Sample 10151016S:
Slide 1:
1). Liver: Hyperplasia, biliary ductular, mild, multifocal, random
Slide 2:
1). Gills, primary lamellae: Branchitis, interstitial, mild, multifocal, random, with chondrodysplasia, respiratory epithelial hyperplasia, and scattered intralesional trematode parasites
2). Heart: Myocarditis, mild, multifocal, random, with scattered intralesional trematodes
Aside from autolysis, there are no overt lesions within the posterior kidney.
Slide 3:
Aside from post mortem change, there are no significant lesions within the small intestine, adipose tissue, skeletal muscle or skin.
Slide 4:
Aside from post mortem change, there are no significant lesions within the small intestine, adipose tissue, pyloric caecae or pancreas.
Sample 10151017S:
Slide 1
1). Liver: Hyperplasia, biliary ductular, mild to moderate, multifocal with diffuse congestion
Slide 2
1). Heart: Myocarditis, mild, multifocal, random, subacute with scattered intralesional trematodes
Aside from post mortem change, there are no overt lesions within the kidney, corpuscle of Stannius, pancreas, adipose tissue, pyloric caecae or small

intestine.
Slide 3
1). Skin: Erosion, moderate, diffuse
2). Fat lobules: Cytoplasmic condensation, moderate, variably extensive (atrophy)
There are no significant lesions within the small intestine, skeletal muscle or pancreas.
Slide 4
1). Gills, gill arch and primary lamellae: Branchitis, interstitial, mild, multifocal, random, granulomatous with respiratory epithelial hyperplasia, and intralesional trematodes
2). Spleen: Congestion, moderate, diffuse
There are no overt lesions within the stomach.
Sample 10151018S:
Slide 1
1). Heart: Myocarditis, mild, multifocal, random, subacute with scattered intralesional trematodes
2). Spleen: Congestion, moderate, diffuse
3). Posterior kidney: Cytoplasmic vacuolation, tubular epithelia, mild to moderate, multifocal with scattered granulomas (post mortem)
Slide 2
1). Gills, primary lamellae: Branchitis, interstitial, mild, multifocal, random, granulomatous with respiratory epithelial hyperplasia, and scattered intralesional trematodes
2). Skin, hypodermis: Encapsulated trematode, mild, focal
3). Skin: Erosions, moderate, multifocal
There are no overt lesions within the skeletal musculature.
Slide 3
1). Liver: Hyperplasia, biliary ductular, mild to moderate, multifocal with diffuse congestion
Aside from post mortem change, there are no significant lesions within the intestine or adipose tissue.
Slide 4
1). Small intestine, muscularis: Enteritis, mild, focal, subacute with an intralesional trematode
Aside from post mortem change, there are no significant lesions within the pyloric caecae, pancreas, or adipose tissue.
Sample 10151019S:
Slide 1:
1). Liver: Hyperplasia, biliary ductular, mild, multifocal with scattered nonsuppurative cholangiohepatitis
Slide 2:
1). Heart: Myocarditis, mild, multifocal, random, subacute with scattered intralesional trematodes
Aside from post mortem change, there are no overt lesions within the skin, skeletal muscle, posterior kidney, or adipose tissue.
Slide 3:
Aside from post mortem change, there are no significant lesions within the pyloric caecae, small intestine, pancreas, or adipose tissue.
Slide 4:
1). Gills, primary lamellae: Branchitis, interstitial, mild, multifocal, random, granulomatous with respiratory epithelial hyperplasia, and scattered intralesional trematodes
Pyloric caecae, small intestine, pancreas, and adipose tissue: As in slide 3
Sample 10151020S:
Slide 1:
1). Liver: Hyperplasia, biliary ductular, mild, multifocal with scattered nonsuppurative cholangiohepatitis
2). Spleen: Congestion, moderate, diffuse
Slide 2:
1). Gills, primary lamellae: Branchitis, interstitial, mild, multifocal, random, granulomatous with respiratory epithelial hyperplasia, and scattered intralesional trematodes
2). Small intestine: Enteritis, moderate, focal, transmural, subacute
3). Adipose tissue: Steatitis, mild, focal, plexiform, nonsuppurative
Slide 3:
1). Heart: Myocarditis, mild, multifocal, random, subacute with scattered degenerate intralesional trematodes
Aside from post mortem change, there are no significant lesions within the pyloric caecae, small intestine, pancreas, kidney, or adipose tissue.
Slide 4:
1). Pyloric caecae, small intestine, pancreas, and adipose tissue: As in slide 3.
There are no overt lesions within the skeletal musculature.
Sample 10171021S:
Slide 1:
1). Liver: Hyperplasia, biliary ductular, mild, multifocal
2). Liver: Hepatitis, mild, focal, nonsuppurative with an intralesional trematode
Slide 2:
1). Heart: Myocarditis, moderate, multifocal, random, subacute with numerous intralesional trematodes
2). Liver: As in slide 1.
3). Spleen: Congestion, moderate, diffuse
Slide 3:
1). Kidney: Trematodes, encapsulated, mild, multifocal, random with scattered tubuloproteinosis
There are no significant lesions within the swim bladder, skeletal muscle or skin.
Slide 4:
Aside from post mortem change, there are no significant lesions within the pyloric caecae, small intestine, pancreas, or adipose tissue.
Slide 5:
1). Gills, primary lamellae: Branchitis, interstitial, mild, multifocal, random, granulomatous with respiratory epithelial hyperplasia
2). Pyloric caecae, pancreas and adipose tissue: As in slide 4
Sample 10171022S:
Slide 1:

1). Gills, primary lamellae and gill arch: Branchitis, interstitial, mild, multifocal, random, granulomatous with scattered intralesional trematodes
2). Heart: Myocarditis, moderate, multifocal, random, subacute with numerous intralesional trematodes
3). Spleen: Congestion, moderate, diffuse
Slide 2:
1). Liver: Hyperplasia, biliary ductular, mild, multifocal
Slide 3:
Aside from post mortem change, there are no discernible lesions within the posterior kidney, small intestine, adipose tissue, skeletal muscle or skin.
Slide 4:
Aside from post mortem change, there are no significant lesions within the pyloric caecae, small intestine, pancreas, or adipose tissue.

Sample 10171023S:
Slide 1:
1). Liver: Hyperplasia, biliary ductular, mild, multifocal
2). Liver: Hepatitis, minimal, multifocal, nonsuppurative
Slide 2:
1). Heart: Myocarditis, mild, multifocal, random, subacute with multiple intralesional trematodes
There are no overt lesions within the spleen, corpuscles of Stannius or kidney.
Slide 3:
Aside from post mortem change, there are no significant lesions within the stomach, pancreas, pyloric caecae, small intestine, or adipose tissue.
Slide 4:
1). Gills, primary lamellae and gill arch: Branchitis, interstitial, moderate, multifocal, random, granulomatous with scattered intralesional trematodes
There are no overt lesions within the skeletal muscle or skin.

Sample 10171024S:
Slide 1:
1). Gills, primary lamellae: Branchitis, interstitial, moderate, multifocal, random, granulomatous with multiple intralesional trematodes
There are no overt lesions within the skeletal muscle or anterior kidney.
Slide 2:
1). Liver: Hepatitis, mild, multifocal, random, nonsuppurative
2). Liver: Hyperplasia, biliary ductular, mild, multifocal
Slide 3:
1). Liver: As in slide 2.
There are no overt lesions within the spleen.
Slide 4:
1). Heart: Myocarditis, moderate, multifocal, random, subacute with multiple intralesional trematodes
Aside from post mortem change, there are no apparent lesions in the small intestine or adipose tissue.
Slide 5:
Aside from post mortem change, there are no significant lesions within the pyloric caecae, small intestine, pancreas, or adipose tissue.

Sample 10171025S:
Slide 1
1). Gills, primary lamellae and arch: Branchitis, interstitial, marked, multifocal to segmental, random, granulomatous with respiratory epithelial hyperplasia, and scattered intralesional trematodes
2). Liver: Hyperplasia, biliary ductular, mild, multifocal
3). Liver: Cholangiohepatitis, mild, multifocal, random, nonsuppurative
4). Spleen: Congestion, moderate, diffuse, acute
There are no overt lesions within the pancreas or fat lobule.
Slide 2
Liver: As in slide 1
Aside from post mortem change, there are no significant lesions within the pyloric caecae, small intestine, pancreas or fat lobules
Slide 3
1). Heart: Myocarditis, mild, multifocal, random, subacute with scattered intralesional trematodes
2). Stomach: Trematodiasis, mild, focal
3). Pyloric caecae, small intestine, pancreas and fat lobules: As in slide 2
Slide 4
1). Skeletal muscle: Myocellular degeneration, moderate, multifocal to coalescing, with endomysial edema and scattered dystrophic sarcoplasmic calcification
There are no overt lesions within the anterior kidney or posterior kidney.

Sample 1050404:
Slide 2:
1). Gill, arch: Myositis, perivascular and interstitial, mild, multifocal, granulomatous with an intralesional trematode
There are no significant lesions within the spleen or peripheral vasculature.

Sample 1050473:
Slide 1
1). Heart: Myocarditis, moderate, multifocal, random, subacute with myocardial degeneration and necrosis and numerous intralesional trematodes
Slide 2
1). Liver: Cholangiohepatitis, moderate, multifocal, granulomatous with multifocal biliary ductular hyperplasia
Slide 3
There are no overt lesions within the anterior kidney or inter-renal tissue.
Slide 4
1). Gills: Branchitis, interstitial, mild, multifocal, random, granulomatous with scattered respiratory epithelial hyperplasia, and intralesional encapsulated trematode parasites
There are no overt lesions within the spleen.
Slide 5
There are no significant lesions in the skeletal muscle.

Slide 6
1). Pyloric caeca and small intestine: Enteritis, marked, diffuse, granulomatous, erosive and ulcerative, with glandular blunting, fusion and effacement, edema fluid, multifocal disruption and dissolution of the stratum compactum with numerous intraluminal cestode parasites
2). Coelomic cavity: Peritonitis, moderate, multifocal, with mesothelial hypertrophy, hyperplasia and micropapillary proliferations
3). Pancreas: Pancreatitis, moderate, multifocal to coalescing, granulomatous with effacement and entrapment of acinar cells and diffuse islet hyperplasia
Slide 7
Pyloric caecae and small intestine: As in slide 6.
Slide 8
Pyloric caecae and small intestine: As in slide 6.
Sample 1050474:
Slide 1
1). Heart: Myocarditis, moderate, multifocal, random, subacute with myocardial degeneration and numerous intralesional encapsulated trematodes
Slide 2
1). Liver: Hepatitis, mild, multifocal, random, nonsuppurative with multifocal biliary ductular hyperplasia and scattered macrovesicular cytoplasmic vacuolation
Slide 3
1). Liver: Cholangiohepatitis, mild, multifocal, random, nonsuppurative with scattered macrovesicular cytoplasmic vacuolation
There are no overt lesions within the anterior kidney or inter-renal gland.
Slide 4
1). Gills: Branchitis, interstitial, mild, multifocal, random, granulomatous with scattered respiratory epithelial hyperplasia and blunting and fusion of secondary lamellae, and rare intralesional encapsulated trematode parasites
There are no overt lesions within the spleen.
Slide 5
1). Skeletal muscle: Myocellular degeneration, moderate, multifocal to coalescing with wavy and contraction bands and scattered endomysial edema fluid
Slide 6
1). Pyloric caeca and small intestine: Enteritis, marked, diffuse, granulomatous, erosive and ulcerative, with glandular blunting, fusion and effacement, multifocal calcification, disruption and dissolution of the stratum compactum, mineral deposition with numerous intraluminal cestode parasites
2). Pancreas: Islet hyperplasia, mild, multifocal
Slide 7
1). Pyloric caeca and small intestine, as in slide 6
2). Pancreas: as in slide 6, but moderate hyperplasia
Slide 8
1). Small intestine: As in slide 6 with multifocal to transmural extension of the inflammatory infiltrate
Sample 1050475:
Slide 1
1). Heart: Myocarditis, mild, multifocal, random, subacute with numerous encapsulated trematodes
Slide 2
1). Liver: Cytoplasmic vacuolation, hepatocellular, moderate, diffuse, macrovesicular with scattered biliary ductular hyperplasia
Slide 3
1). Gills, primary lamellae and gill arch: Branchitis, interstitial, mild, multifocal, random, granulomatous with scattered chondrodysplasia, respiratory epithelial hyperplasia, and intralesional encapsulated trematode parasites
There are no overt lesions within the spleen, anterior kidney or inter-renal tissue.
Slide 4
There are no overt lesions within the anterior kidney or inter-renal tissue.
Slide 5
1). Skeletal muscle: Sarcoplasmic vacuolation, moderate, multifocal
Slide 6
Aside from post mortem change, there are no overt lesions within the pancreas, adipose tissue, small intestine, pyloric caecae, peripheral vasculature or peripheral nerves.
Slide 7
1). Pyloric caeca: Enteritis, moderate, multifocal, diffuse, granulomatous, with glandular blunting and fusion and scattered intraluminal helminth
Aside from post mortem change, there are no overt lesions within the pancreas, adipose tissue, peripheral vasculature or peripheral nerves.
Slide 8
1). Pyloric caeca: As in slide 7
Sample 1050476:
Slide 1
1). Heart: Myocarditis, mild, multifocal, random, subacute with numerous encapsulated intralesional trematodes
Slide 2
1). Liver: Cholangiohepatitis, mild, multifocal, random, nonsuppurative with multifocal to coalescing macrovesicular hepatocellular cytoplasmic vacuolation, individualization and rare hypertrophy
Slide 3
1). Kidney: Trematodiasis, encapsulated, mild, multifocal
Slide 4
1). Gills: Branchitis, interstitial, mild, multifocal, random, granulomatous with scattered chondrodysplasia, respiratory epithelial hyperplasia, and intralesional encapsulated trematode parasites
There are no overt lesions within the pancreas or spleen.
Slide 5
1). Skeletal muscle: Myositis, mild, multifocal, subacute with scattered intralesional nematode parasites
2). Skeletal muscle: Myocellular degeneration, mild, multifocal, random, acute

Slide 6
1). Small intestine: Enteritis, moderate, multifocal, segmental, granulomatous with multifocal disruption and dystrophic mineral deposition of the stratum compactum with granulomatous infiltrate and transmural edema and scattered myocellular degeneration
2). Pancreas: Hyperplasia, islet, moderate, diffuse
Slide 7
1). Pyloric caeca: Enteritis, marked, diffuse, granulomatous, erosive and ulcerative, with glandular blunting, fusion and effacement, multifocal to transmural lymphohistiocytic infiltrates scattered Langhan's type multinucleated giant cells and edema fluid
2). Pancreas: Hyperplasia, islet, moderate, diffuse
3). Coelomic cavity: Mesothelial hyperplasia, micropapillary, moderate, multifocal with variably extensive hypertrophy (reactive change)
Slide 8
1). Pyloric caecae: As in slide 7, but with scattered intraluminal cestode parasites
Sample 1050477:
Slide 1
1). Heart: Myocarditis, mild, multifocal, random, subacute with numerous encapsulated intralesional trematodes
2). Liver: Hyperplasia, biliary ductular, moderate, diffuse*
3). Liver: Hepatitis, mild, multifocal, random, nonsuppurative
4). Spleen: Congestion, moderate, diffuse
There are no overt lesions in the gall bladder.
Slide 2
There are no overt lesions within the anterior kidney, inter-renal tissue, or skeletal muscle.
Slide 3
1). Gills, primary lamellae and gill arch: Branchitis, interstitial, mild to moderate, multifocal, granulomatous with scattered chondrodysplasia, respiratory epithelial hyperplasia, and intralesional trematode parasites
Slide 4
1). Intestine, select segments: Enteritis, marked, diffuse, granulomatous with glandular blunting, fusion, disruption and effacement of the stratum compactum and focal transmural lymphohistiocytic infiltrates
Aside from post mortem change, there are no overt lesions within the pyloric caecae and pancreas.
Sample 1050478:
Slide 1
1). Heart: Myocarditis, mild, multifocal, random, subacute with scattered intralesional trematodes
2). Liver: Hyperplasia, biliary ductular, mild to moderate, multifocal
3). Liver: Hepatitis, mild, multifocal, random, nonsuppurative
Slide 2
1). Spleen and kidney: Melanomacrophage hyperplasia, mild to moderate, multifocal with diffuse congestion
2). Kidney, posterior: Hypertrophy, renal tubular epithelia, mild to moderate, diffuse with scattered hyperplasia
There are no overt lesions within the swim bladder or adipose tissue.
Slide 3
1). Gills, primary lamellae: Branchitis, interstitial, moderate, multifocal, granulomatous with scattered chondrodysplasia, respiratory epithelial hyperplasia, and numerous intralesional trematode parasites
Slide 4
1). Skin, hypodermis: Granuloma, minimal, focal, with intralesional trematode parasite remnant
2). Skin: Erosion, moderate, diffuse
There are no significant lesions within the skeletal muscle.
Slide 5
1). Pyloric caeca and small intestine: Enteritis, moderate, multifocal, segmental, granulomatous with glandular blunting, fusion and effacement
Sample 1050479:
Slide 1
1). Kidney: Glomerulonephritis, mild to moderate, diffuse, multifocal, lymphohistiocytic with parietal cell hyperplasia and hypertrophy, synechiae and occasional periglomerular fibrosis
2). Kidney: Edema, sinusoidal, moderate, diffuse with variable congestion
3). Liver: Cholangiohepatitis, mild, multifocal, nonsuppurative with scattered biliary ductular hyperplasia
4). Pancreas: Hyperplasia, islets, mild, multifocal
There are no overt lesions within the peripheral nerves or peripheral vasculature.
Slide 2
1). Skin: Erosion, moderate, diffuse
2). Skeletal muscle: Hemorrhage, endomysial, mild, multifocal, random, acute
Slide 3
1). Gills, primary lamellae: Branchitis, interstitial, mild to moderate, multifocal, granulomatous with scattered chondrodysplasia, respiratory epithelial hyperplasia, and intralesional encapsulated trematode parasites
Slide 4
1). Spleen: Congestion, moderate, diffuse
Aside from post mortem change and bacterial overgrowth, there are no overt lesions within the stomach, pyloric caecae, adipose tissue or peripheral nerves.
Slide 5
There are no significant lesions within the small intestine, pyloric caecae, adipose tissue, pancreas, peripheral vasculature or peripheral nerves.
Slide 6
1). Heart: Myocarditis, mild, multifocal, random, granulomatous with scattered intralesional trematodes
Sample 1050480:
Slide 1
1). Liver: Cholangiohepatitis, mild, multifocal, random, nonsuppurative with scattered biliary ductular hyperplasia
2). Heart: Myocarditis, mild, multifocal, nonsuppurative with scattered encapsulated trematodes
Slide 2

1). Kidney: Glomerulonephritis, mild, multifocal, diffuse, lymphohistiocytic with dispersed karyorrhectic debris, synechiae, periglomerular fibrosis and occasional senescence
2). Spleen: Congestion, moderate, diffuse
Aside from post mortem change, there are no overt lesions within the adipose tissue, pancreas, intestine or pyloric caecae.
Slide 3
1). Gills, primary lamellae and gill arch: Branchitis, interstitial, moderate, multifocal, random, granulomatous with chondrodysplasia, respiratory epithelial hyperplasia, and numerous intralesional trematode parasites
Slide 4
1). Small intestine: Enteritis, mild, focal, granulomatous with an intralesional trematode
2). Skin: Erosion, mild, diffuse
3). Skeletal muscle, superficial: Myositis, endomysial, mild, multifocal, lymphohistiocytic
There are no significant lesions within the esophagus or swim bladder.
Slide 5
1). Small intestine, submucosa: Enteritis, moderate, focal, granulomatous with numerous multinucleated giant cells
Aside from post mortem change, there are no overt lesions within the ovary, pyloric caecae, adipose tissue or pancreas.
Sample 1050481:
Slide 1
1). Liver: Cholangiohepatitis, mild, multifocal, nonsuppurative with multifocal biliary ductular hyperplasia
2). Heart: Myocarditis, mild to moderate, multifocal, nonsuppurative with encapsulated trematodes
Slide 2
1). Skin: Erosion, moderate, multifocal
There are no overt lesions within the skeletal muscle, peripheral nerves, peripheral vasculature, anterior kidney, posterior kidney,
Slide 3
1). Gills, primary lamellae: Branchitis, interstitial, moderate, multifocal, random, granulomatous with chondrodysplasia, respiratory epithelial hyperplasia, and scattered intralesional trematode parasites
Slide 4
1). Spleen: Congestion, moderate, diffuse
There are no significant lesions within the small intestine, testes (quiescent), peripheral vasculature, peripheral nerves or adipose tissue.
Slide 5
There are no overt lesions within the pyloric caeca, small intestine, adipose tissue or pancreas.
Sample 1050485:
Slide 1
1). Liver: Cholangiohepatitis, mild, multifocal, random, nonsuppurative with scattered biliary ductular hyperplasia and hepatocellular cytoplasmic vacuolation
2). Heart: Myocarditis, mild, multifocal, nonsuppurative, with rare encapsulated trematodes
Slide 2
Aside from post mortem change, there are no overt lesions within the anterior kidney, posterior kidney, spleen, or swim bladder.
Slide 3
1). Gills, primary lamellae: Branchitis, interstitial, mild, multifocal, random, granulomatous with chondrodysplasia, respiratory epithelial hyperplasia, and florid intralesional trematode parasites
Slide 4
1). Skeletal muscle: Edema, endomysial, mild, multifocal with scattered acute myocellular degeneration
Slide 5
Aside from post mortem change, there are no overt lesions within the pyloric caeca, adipose tissue, pancreas, peripheral nerves or peripheral vasculature
Sample 1050486:
Slide 1
1). Heart: Trematodiasis, moderate, multifocal, random, with encapsulation and occasional degeneration and scattered reactive endocardia
2). Liver: Congestion, moderate, diffuse (autolyzed)
Slide 2
Aside from autolysis, there are no overt lesions within the skeletal muscle, spleen, anterior kidney, inter-renal tissue, adipose tissue, swim bladder, or pneumatic duct.
Slide 3
1). Gills, primary lamellae: Chondrodysplasia, severe, multifocal, segmental with multifocal granulomatous branchitis and respiratory epithelial hyperplasia, segmental laminar telangiectasia and a myriad of encapsulated trematode parasites
Slide 4
1). Skeletal muscle: Edema, endomysial, mild, multifocal with scattered acute myocellular degeneration
2). Skin: Erosions, mild, multifocal
Slide 5
1). Stomach: Mineral deposition, punctate, muscularis, mild, multifocal, random
2). Adipose tissue: Hemorrhage, mild, multifocal, random, acute
Aside from autolysis, there are no overt lesions within the pyloric caeca or pancreas.
Sample 1050487:
Slide 1
1). Liver: Hyperplasia, biliary ductular, mild, multifocal, random
2). Heart: Myocarditis, minimal, focal, subacute with an encapsulated trematode
Slide 2
1). Liver: As in slide 1
2). Spleen: Congestion, moderate, diffuse
Aside from post mortem change, there are no overt lesions within the anterior or posterior kidneys.
Slide 3
1). Gills, primary lamellae: Branchitis, interstitial, marked, multifocal to segmental, granulomatous with chondrodysplasia, respiratory epithelial

hyperplasia, and florid intralesional trematode parasites
Slide 4
1). Skeletal muscle: Myocellular degeneration, mild, multifocal, acute
There are no significant lesions within the skin.
Slide 5
1). Stomach: Mineral deposition, muscularis, subintimal, and perivascular, mild, multifocal
Aside from post mortem change, there are no significant lesions within the pyloric caecae, adipose tissue or pancreas.
Sample 1050488:
Slide 1
1). Liver: Hyperplasia, biliary ductular, moderate, multifocal, random
2). Liver: Cytoplasmic vacuolation, hepatocellular, macrovesicular, moderate, multifocal
3). Liver: Cholangiohepatitis, mild, multifocal, nonsuppurative
4). Heart: Myocarditis, mild, multifocal subacute with scattered encapsulated trematodes and reactive endocardia
Slide 2
1). Spleen: Congestion, moderate, diffuse
There are no significant lesions within the skeletal musculature or anterior kidney.
Slide 3
1). Gills, primary lamellae: Branchitis, interstitial, moderate, multifocal to segmental, granulomatous with chondrodysplasia, respiratory epithelial hyperplasia, and florid intralesional trematode parasites
Slide 4
There are no significant lesions within the skeletal musculature or skin.
Slide 5
1). Stomach: Mineral deposition, muscularis, subintimal, and perivascular, mild, multifocal
Aside from post mortem change, there are no significant lesions within the pyloric caecae, adipose tissue or pancreas.
Sample 1050489:
Slide 1
1). Liver: Hyperplasia, biliary ductular, mild to moderate, multifocal, random with scattered macrovesicular hepatocellular cytoplasmic vacuolation
2). Heart: Myocarditis, mild, multifocal, nonsuppurative with myocardial degeneration and necrosis
Slide 2
1). Liver: As in slide 1.
2). Spleen: Congestion, moderate, diffuse
Aside from post mortem change, there are no overt lesions within the swim bladder, pneumatic duct, skeletal muscle, anterior kidney or posterior kidney.
Slide 3
1). Gills, primary lamellae: Branchitis, interstitial, moderate, multifocal to segmental, granulomatous with chondrodysplasia, respiratory epithelial hyperplasia, and numerous intralesional trematode parasites
Slide 4:
1). Skin: Erosion, moderate, diffuse
There are no significant lesions within the skeletal musculature.
Slide 5:
1). Small intestine, 1 of multiple segments: Enteritis, moderate, multifocal, granulomatous
Aside from post mortem change, there are no overt lesions within the pyloric caecae, adipose tissue or pancreas.
Sample 1050490:
Slide 1A:
There are no overt lesions within the anterior kidney or inter-renal tissue.
Slide 1B:
1). Heart: Myocarditis, mild to moderate, multifocal, nonsuppurative with numerous encapsulated trematodes
Slide 2
1). Skeletal muscle: Hemorrhage, endomysial, mild, multifocal, with edema fluid and scattered acute myofiber degeneration
Slide 3
1). Gills, primary lamellae: Branchitis, interstitial, moderate, multifocal to segmental, granulomatous with chondrodysplasia, respiratory epithelial hyperplasia with blunting and fusion of secondary lamella, and scattered intralesional trematode parasites and rare ciliated protozoa
Slide 4
1). Liver: Cholangiohepatitis, moderate, multifocal, random, nonsuppurative
2). Liver: Cytoplasmic vacuolation, hepatocellular, macrovesicular, moderate, multifocal
Slide 5
1). Liver: Cholangiohepatitis, moderate, multifocal, random, nonsuppurative
2). Liver: Cytoplasmic vacuolation, hepatocellular, macrovesicular, moderate, multifocal
Slide 6A
1). Small intestine, muscularis: Enteritis, perivascular and interstitial, moderate, multifocal, transmural, subacute with moderate mineral deposition in the muscularis and submucosa
2). Small intestine, 1 segment: Hemorrhage, lamina propria, moderate, circumferential, acute with scattered submucosal mineral deposits
Slide 6B
1). Gills, primary lamellae: Branchitis, interstitial, mild to moderate, multifocal, granulomatous with chondrodysplasia, respiratory epithelial hyperplasia, and scattered intralesional trematode parasites
Slide 7A
1). Pyloric caeca: Enteritis, marked, multifocal, granulomatous, with glandular blunting, fusion and effacement, disruption of the stratum compactum, with numerous intraluminal cestode parasites
2). Coelomic cavity: Peritonitis, mild to moderate, multifocal, lymphohistiocytic, with mesothelia hypertrophy and occasional hyperplasia (reactive) and cytoplasmic condensation of adipocytes
3). Pancreas: Islet hyperplasia, moderate, multifocal
4). Pancreas: Pancreatitis, mild, multifocal, random, nodular, lymphohistiocytic with periductular fibrosis

Slide 8A
1). Pyloric caecae: As in slide 7A, but with florid intraluminal cestode parasites
2). Coelomic cavity and pancreas: As in slide 7A
Slide 8B
1). Intestine, muscularis: Enteritis, marked, segmental, granulomatous with cleft formation and numerous intralesional nematode parasites
2). Pyloric caecae, pancreas and intestine: As in slide 8A
3). Pancreas: Islet hyperplasia, marked, multifocal to confluent

Sample 1050492:
Slide 1
1). Heart: Myocarditis, moderate, multifocal, random, granulomatous with myocardial degeneration and necrosis, and numerous finely encapsulated trematode parasites
2). Heart: Possible malformation, ventricular compact layer hypertrophy, diffuse
Slide 2
1). Liver: Cholangiohepatitis, mild, multifocal, random, nonsuppurative
2). Liver: Cytoplasmic vacuolation, hepatocellular, macrovesicular, moderate, multifocal
Slide 3
1). Liver: As in slide 2
There are no significant lesions within the spleen.
Slide 4
1). Skeletal muscle: Myocellular degeneration, moderate, multifocal to coalescing, with variation in myofiber size and cytoplasmic vacuolation, fragmentation and hyalinization
Slide 5
1). Pyloric caeca: Enteritis, marked, multifocal, granulomatous, with glandular blunting, fusion and effacement, disruption of the stratum compactum, with numerous intraluminal cestode parasites
2). Kidney: Nephritis, interstitial, mild, multifocal, granulomatous, with scattered trematodes

Sample 1050493:
Slide 1
1). Liver: Cholangiohepatitis, moderate, multifocal, random, nonsuppurative, with biliary ductular hyperplasia and scattered trematode
2). Liver: Cytoplasmic vacuolation, hepatocellular, macrovesicular, moderate, multifocally extensive
There are no overt lesions within the spleen.
Slide 2
Tissues: Open
There are no overt lesions within the peripheral nerves, peripheral vasculature, inter-renal tissue, anterior kidney, or spleen.
Slide 3
1). Heart: Myocarditis, moderate, multifocal, random, granulomatous with myocardial degeneration and necrosis, and numerous finely encapsulated trematode parasites
Slide 4
1). Skeletal muscle: Myocellular degeneration, moderate, multifocal to coalescing, with variation in myofiber size and cytoplasmic vacuolation, fragmentation and loss of striations
2). Skin: Possible erosions and ulceration, moderate, multifocal
Slide 5
1). Gills: Branchitis, primary lamellae, mild, multifocal, random, lymphohistiocytic, with circumferential epithelioid macrophages, intralesional trematodes and reactive respiratory epithelial hyperplasia
2). Gills: Hyperplasia, respiratory epithelial, mild, multifocal, random, with blunting and fusion of secondary lamellae
3). Gills: Telangiectasia, mild, multifocal, random, acute
Slide 6
1). Pyloric caeca: Enteritis, marked, diffuse, granulomatous, erosive and ulcerative, with glandular blunting, fusion and effacement, multifocal calcification, disruption and dissolution of the stratum compactum, scattered mineral deposition of the microvasculature and eosinophilic granular cell layer, with numerous intraluminal cestode parasites
2). Stomach: Gastritis, mild, multifocal, lymphohistiocytic
3). Coelomic cavity: Peritonitis, mild to moderate, multifocal, lymphohistiocytic, with mesothelia hypertrophy and occasional hyperplasia (reactive) and cytoplasmic condensation of adipocytes
4). Liver: Cholangiohepatitis, moderate, multifocal, random, subacute with multifocal hepatocellular dissociation and degeneration, macrovesicular hepatocellular cytoplasmic vacuolation and biliary ductular hyperplasia
Slide 7
1). Pyloric caecae: As in slide 6, but with transmural to subserosal inflammation
2). Stomach: As in slide 6
3). Pancreas: Islet hyperplasia, moderate, multifocal
4). Pancreas: Pancreatitis, mild, multifocal, random, nodular, lymphohistiocytic with periductular fibrosis
5). Coelomic cavity: As in slide 6
Slide 8
1). As in slide 7.

Sample 1018109C:
Slide 1:
1). Spleen: Congestion, moderate, diffuse with lymphoid depletion
Slide 2:
1). Liver: Hyperplasia, biliary ductular, mild, multifocal, random with scattered macrovesicular hepatocellular cytoplasmic vacuolation and rare trematodes
Slide 3:
Liver: As in slide 2.
Slide 4:
Liver: As in slide 2.

Sample 10181010C:
Slide 1:
1). Liver: Hyperplasia, biliary ductular, mild, multifocal, random
Slide 2:
1). Liver: As in slide 1, but with rare intraluminal protozoa like organisms
Slide 3:
1). Kidney: Glomerular tufts and tubules, presumptive protozoa, moderate, multifocal, random**
2). Skeletal muscle: Myocellular degeneration, mild, multifocal, acute
Slide 4:
1). Gills, primary lamellae: Branchitis, interstitial, mild, multifocal, granulomatous to fibrogranulomatous with numerous encapsulated intralesional trematodes
Slide 5:
1). Heart, myocardium and bulbous arteriosus: Myocarditis, mild, multifocal, random, granulomatous with scattered trematode parasites
Slide 6:
1). Kidney: As in slide 3.
There are no overt lesions within the spleen.
Slide 7:
1). Pyloric caeca: Enteritis, mild to moderate, multifocal, granulomatous, with glandular blunting, fusion and effacement, disruption of the stratum compactum, with numerous intraluminal cestode parasites
2). Coelomic cavity: Peritonitis, mild, multifocal, lymphohistiocytic, with mesothelia hypertrophy and occasional hyperplasia (reactive)
3). Pancreas: Islet hyperplasia, moderate to marked, multifocal
Sample 10181011C:
Slide 1:
1). Heart, myocardium and bulbous arteriosus: Myocarditis, mild, multifocal, random, granulomatous with scattered trematode parasites
Slide 2:
There are no overt lesions in the spleen.
Slide 3:
1). Skeletal muscle: Myocellular degeneration, mild, multifocal, acute
There are no overt lesions in the anterior kidney.
Slide 4:
1). Gills, primary lamellae: Branchitis, interstitial, mild, multifocal, granulomatous to fibrogranulomatous with numerous encapsulated intralesional trematodes
Sample 10181012C:
Slide 1
1). Adipose tissue: Peritonitis, mild, multifocal to coalescing, lymphohistiocytic with islet hyperplasia, periductular fibrosis, and scattered acinar cell degeneration
There are no overt lesions within the spleen.
Slide 2
1). Heart: Trematodiasis, marked, multifocal, random, with encapsulation and occasional degeneration and scattered reactive endocardia
Slide 3
1). Gills, primary lamellae: Branchitis, interstitial, mild, multifocal, granulomatous to fibrogranulomatous with numerous encapsulated intralesional trematodes
2). Heart, as in slide 2.
Slide 4
1). Kidney: Nephritis, interstitial, mild, multifocal, granulomatous, with numerous intralesional encapsulated trematodes
Slide 5
1). Pyloric caeca: Enteritis, moderate, multifocal, granulomatous, erosive and ulcerative with glandular blunting, fusion and effacement, multifocal calcification and scattered degenerate and mineralized presumptive parasite remnants
3). Coelomic cavity: Peritonitis, mild to moderate, multifocal, lymphohistiocytic, with mesothelia hypertrophy and occasional hyperplasia (reactive) and cytoplasmic condensation of adipocytes
Sample 10181013C:
Slide 1
1). Skeletal muscle: Myocellular degeneration, mild, multifocal, acute
2). Kidney: Trematodes, encapsulated remnant, mild, multifocal, random
3). Kidney: Fibrosis, periglomerular, mild, multifocal with occasional synechiae, and membranous glomerulopathy and obsolescence
Slide 2
1). Pyloric caeca: Enteritis, marked, diffuse, granulomatous, erosive and ulcerative with glandular blunting, fusion and effacement, disruption and convolution of the stratum compactum, numerous intraluminal cestode parasites and possibly atrophy of the muscularis
2). Coelomic cavity: Peritonitis, moderate, multifocal, lymphohistiocytic, with mesothelia hypertrophy and micropapillary hyperplasia, acute hemorrhage and fat atrophy
3). Pancreas: Pancreatitis, mild, multifocal, nonsuppurative with acinar cell degeneration and necrosis and scattered islet hyperplasia and islet amyloidosis
4). Spleen: Congestion, moderate, diffuse
Slide 3
1). Stomach, pylorus: Gastritis, moderate, granulomatous, multifocal to transmural, circumferential
2). Coelomic cavity: as in slide 2
3). Heart: Trematodiasis, moderate, multifocal, random, with encapsulation and occasional degeneration
Slide 4
1). Liver: Cholangiohepatitis, mild, multifocal, nonsuppurative
Slide 5
1). Gills: Branchitis, interstitial, mild to moderate, multifocal, random, lymphohistiocytic to fibrogranulomatous with scattered intralesional trematodes

2). Gills, distal limits secondary lamellae: Telangiectasia, moderate, multifocal to laminar, with occasional respiratory epithelial hypertrophy, hyperplasia and synechiae with scattered karyorrhectic debris
3). Heart and bulbous arteriosis: as in slide 3.

Sample 10181014C:

Slide 1:
1). Liver: Cholangiohepatitis, mild, multifocal, random, subacute with biliary ductular hyperplasia and scattered trematode
2). Liver: Cytoplasmic vacuolation, hepatocellular, macrovesicular, moderate, multifocally extensive
Slide 2:
1). Liver: As in slide 1, but moderate inflammation and more extensive cytoplasmic vacuolation, scattered pigment laden macrophages, and marked biliary ductular hyperplasia
Slide 3:
1). Kidney: Trematodes, encapsulated, mild, multifocal, random
2). Kidney: Fibrosis, periglomerular, mild, multifocal with occasional contracted tufts, synechiae, and membranous glomerulopathy
3). Kidney: Nephritis, interstitial, mild, multifocal, granulomatous with scattered tubuloproteinosis
4). Skeletal muscle: Myocellular degeneration, mild, multifocal, random with scattered endomysial and perivascular lymphohistiocytic infiltrates
Slide 4:
1). Coelomic cavity: Peritonitis, moderate, multifocal lymphohistiocytic, with mesothelial papillary hyperplasia and presumptive islet hyperplasia
2). Spleen: Possible lymphoid depletion, moderate, diffuse
Slide 5:
1). Gills: Branchitis, mild, multifocal, random, subacute with intralesional trematodes and scattered respiratory epithelial hyperplasia and hypertrophy
2). Heart: Myocarditis, mild, multifocal, random, lymphohistiocytic with scattered finely encapsulated trematode parasites and occasional platelet aggregates
Slide 6:
1). Heart: As in slide 5, but with multifocal to segmental attenuation of the outer compact layer (possibly cardiomyopathy) and scattered acute myocardial degeneration and necrosis
Slide 7:
1). Pyloric caeca: Enteritis, marked, diffuse, granulomatous and necroulcerative, multifocal to transmural with dissolution of the stratum compactum, depletion of the eosinophilic granular layer, peritonitis with reactive mesothelia and rare intralesional degeneration and necrotic helminths
2). Pancreas: Pancreatitis, moderate, multifocal, granulomatous with effacement and entrapment of acinar cells and moderate diffuse Islet and ductular hyperplasia
Slide 8:
1). Pyloric caeca: as in slide 7, but more severe and with florid intralesional helminths
2). Pancreas: As in slide 7

Sample 11011015C:

Slide 1
1). Heart: Myocarditis, moderate, multifocal, random, lymphohistiocytic with numerous intralesional trematode parasites
2). Kidney: Trematodiasis, mild, multifocal, random
There are no overt lesions within the skeletal musculature.
Slide 2
1). Pyloric caecae: Enteritis, mild, multifocal, granulomatous with occasional blunting and fusion of glandular elements and neovascularization of the muscularis
2). Coelomic cavity: Peritonitis, moderate, multifocal, subacute with scattered intralesional nematode parasites
3). Pancreas: Pancreatitis, moderate, multifocal, lymphohistiocytic with islet hyperplasia
There are no overt lesions within the spleen.
Slide 3
1). Gills: Branchitis, mild to moderate, multifocal, random, subacute with intralesional trematodes and scattered chondrodysplasia and respiratory epithelial hyperplasia and hypertrophy
2). Small intestine: Enteritis, moderate, multifocal, subacute with intraluminal cestodes
Slide 4
1). Heart: Myocarditis, moderate, multifocal, random, lymphohistiocytic with numerous intralesional trematode parasites
2). Heart: Possible malformation, moderate, segmental (ventricular hypertrophy)
Slide 5
1). Liver: Cytoplasmic vacuolation, hepatocellular, macrovesicular, moderate, diffuse with scattered biliary ductular hyperplasia and rare intravascular trematode
2). Liver: Hepatitis, mild, multifocal, random, subacute
Slide 6
1). Liver: As in slide 5, but with scattered presumptive regenerative nodules of hepatocytes

Sample 11011016C:

Slide 1:
There are no overt lesions within the posterior kidney, liver or swim bladder.
Slide 2:
1). Liver: Hyperplasia, biliary ductular, mild, multifocal, random with scattered macrovesicular hepatocellular cytoplasmic vacuolation
There are no overt lesions within the spleen.
Slide 3:
1). Heart: Myocarditis, moderate, multifocal, random, lymphohistiocytic with numerous intralesional trematode parasites
2). Heart: Possible malformation, moderate, segmental
Slide 4:
1). Gills: Branchitis, mild, multifocal, random, subacute with intralesional trematodes and scattered chondrodysplasia and respiratory epithelial hyperplasia and hypertrophy
Slide 5:
1). Skeletal musculature: Myocellular degeneration, mild, multifocal, acute
Slide 6:

1). Pyloric caeca: Enteritis, marked, diffuse, granulomatous, erosive and ulcerative with multifocal acute hemorrhage, glandular blunting, fusion and effacement, disruption and convolution of the stratum compactum, numerous intraluminal cestode parasites
2). Coelomic cavity: Peritonitis, moderate, multifocal, fibrogranulomatous with intralesional degenerate helminths
Slide 7:
1). Pyloric caecae: as in slide 6 with florid intralesional cestodes and coelomic cavity nematodes
2). Pancreas: Pancreatitis, moderate, multifocal to coalescing, lymphohistiocytic with islet hyperplasia
Sample 11011017C:
Slide 1
1). Liver: Hyperplasia, biliary ductules, moderate, multifocal with occasional intraluminal protozoal like organisms **
2). Small intestine: Enteritis, moderate, multifocal, subacute
Slide 2
1). Liver: As in slide 1 with multifocal mild subacute cholangiohepatitis
2). Heart: Myocarditis, moderate, multifocal, random, lymphohistiocytic with numerous intralesional trematode parasites
Slide 3
1). Skeletal muscle: Myocellular degeneration, mild to moderate, multifocal
2). Heart: As in slide 2
Slide 4
1). Skeletal muscle: Hemorrhage, moderate, focally extensive with acute myocellular degeneration and necrosis
There are no overt lesions within the spleen.
Slide 5
1). Gills: Branchitis, mild, multifocal, random, subacute with intralesional trematodes and scattered respiratory epithelial hyperplasia and hypertrophy
Slide 6
1). Pyloric caeca: Enteritis, marked, multifocal, granulomatous, with glandular blunting and fusion, scattered erosions and occasional intraluminal cestode and nematode parasites
2). Coelomic cavity: Peritonitis, moderate, multifocal, subacute with scattered intralesional nematode parasites
3). Pancreas: Pancreatitis, moderate, multifocal, lymphohistiocytic with islet hyperplasia
Slide 7
1). Pyloric caecae: As in slide 6.
Sample 11011018C:
Slide 1:
1). Heart: Myocarditis, mild, multifocal, random, nonsuppurative with occasional myocardial degeneration and numerous intralesional trematodes
There are no overt lesions within the spleen.
Slide 2:
1). Gills: Branchitis, mild, multifocal, random, subacute with intralesional trematodes and scattered respiratory epithelial hyperplasia, hypertrophy and telangiectasia
Slide 3:
1). Liver: Autolysis
Slide 4:
1). Liver: Autolysis
Slide 5:
1). Kidney, posterior: Trematodiasis, marked, multifocal to coalescing
2). Skeletal muscle: Hemorrhage, endomysial, mild, multifocal, acute
Slide 6:
1). Pyloric caeca: Enteritis, marked, diffuse, granulomatous, with glandular blunting and fusion, scattered erosions and occasional intraluminal cestode parasites
2). Coelomic cavity: Peritonitis, moderate, multifocal, subacute
3). Pancreas: Pancreatitis, moderate, multifocal, lymphohistiocytic with diffuse islet hyperplasia
Sample 11011019C:
Slide 1:
1). Pyloric caeca: Enteritis, marked, diffuse, granulomatous, with glandular blunting and fusion, scattered erosions, intermittent degeneration of the stratum compactum, and numerous intraluminal cestode parasites
2). Coelomic cavity: Peritonitis, moderate, multifocal, subacute with multifocal mesothelial micropapillary hyperplasia
3). Pancreas: Pancreatitis, moderate, multifocal, lymphohistiocytic with diffuse islet hyperplasia
Slide 2:
1). Small intestine and presumptive pyloric caecae: As in slide 1, but focal expansion of a lumina structure by multilobulated mineralized deposits
Slide 3:
1). Kidney: Trematodiasis, moderate, multifocal, random with a focal extracapsular nematode
2). Skeletal muscle: Myocellular degeneration, mild to moderate, multifocal
Slide 4:
1). Heart: Myocarditis, mild, multifocal, random, nonsuppurative with moderate numbers of intralesional trematodes
2). Spleen: Autolysis
Slide 5:
1). Gills: Branchitis, mild, multifocal, random, subacute with scattered intralesional trematodes, respiratory epithelial hyperplasia and hypertrophy
Slide 6:
1). Heart: As in slide 4, but with numerous trematodes throughout the spongy layer of the myocardium
Slide 7:
1). Liver: Autolysis
Slide 8:
1). Liver: Autolysis
Sample 11011020C:
Slide 1:
1). Liver: Hyperplasia, biliary ductules, mild, multifocal

Slide 2:
1). Liver: As in slide 1, but with scattered intralesional protozoal like organisms
There are no significant lesions within the spleen.
Slide 3:
1). Liver: As in slide 2, but with scattered nonsuppurative hepatitis
Slide 4:
1). Heart: Myocarditis, mild, multifocal, random, nonsuppurative with florid intralesional trematodes
Slide 5:
1). Gills, primary lamella and arch: Branchitis and myositis, mild, multifocal, random, subacute with scattered intralesional trematodes
2). Kidney: Autolysis
Slide 6:
Skeletal muscle: Myocellular degeneration, mild, multifocal, random, acute
Sample 1129102C*:**
Slide 1
1). Heart: Myocarditis, mild to moderate, multifocal, random, nonsuppurative with myocardial degeneration and necrosis and numerous intralesional trematodes
Slide 2
1). Kidney: Trematodes, encapsulated, moderate, multifocal, random with scattered tubuloproteinosis
2). Heart: As in slide 1
There are no overt lesions within the spleen.
Slide 3
1). Gills: Branchitis, moderate, focally extensive, necrotising with florid intralamellar and intravascular fungal hyphae morphologically consistent with Saprolegnia and florid extra and occasionally intracellular coccobacilli
2). Gills: Branchitis, mild, multifocal, random, subacute with intralesional trematodes and scattered respiratory epithelial hyperplasia and hypertrophy
Slide 4
1). Skeletal muscle: Hemorrhage, mild, multifocal, endomysial with scattered acute myocellular degeneration
Slide 5
1). Pyloric caecae: Enteritis, severe, multifocal to segmental, transmural, necroulcerative with florid intralesional bacilli, fibrinoserous exudate, dissolution of the stratum compactum, depletion of the eosinophilic granular layer and numerous intralesional syncytia*
Slide 6
1). Pyloric caecae: As in slide 5, but with more extensive mucosal necrosis*
Slide 7
1). Liver: Cytoplasmic vacuolation, macrovesicular, marked, diffuse with multifocal fat cyst formation, ductular cholestasis, biliary ductular hyperplasia**, and focal subcapsular hemorrhage
Slide 8
1). Liver: As in slide 7**
Sample 1129103C:
Slide 1
1). Heart: Trematodiasis, mild, multifocal, random, with focal intracellular protozoal pseudocyst morphologically consistent with Kudoa spp*
Aside from profound post mortem change, there are no overt lesions within the liver.
Slide 2
1). Heart: As in slide 1.
2). Liver: As in slide 1
Slide 3
Aside from pronounced post mortem change, there are no overt lesions within the small intestine or liver.
Slide 4
1). Small intestine, muscularis: Enteritis, marked, multifocal to coalescing, granulomatous with intralesional nematodes
Aside from pronounced post mortem change, there are no overt lesions within the pyloric caecae or pancreas.
Slide 5
1). Small intestine, as in slide 4.
Sample 11291010C:
Slide 1
1). Gills: Branchitis, interstitial, mild, multifocal, random, lymphohistiocytic with scattered intralesional trematodes
2). Gill, distal primary lamella: Branchitis, mild, segmental and laminar, necrotising and proliferative with florid intralesional fungal hyphae morphologically consistent with Zygomycetes and scattered polygonal cells with abundant amounts of refractile granular granules (possible eosinophilic granular cells)
3). Heart: Myocarditis, mild, multifocal, random, lymphohistiocytic with scattered finely encapsulated trematode parasites and occasional platelet aggregates
Slide 2
1).Heart: As in slide 1:
2). Pyloric caeca: Enteritis, moderate, diffuse, granulomatous and necroulcerative, multifocal to transmural with intralesional nematode parasites and rare cestodes
Slide 3
1). Heart: As in slide 1
2). Spleen: Congestion, moderate, multifocal, acute
3). Skeletal muscle: Myocellular degeneration, moderate, multifocal to coalescing
Slide 4
1). Pyloric caecae: As in slide 2, but milder involvement and occasional syncytia within the inflammatory infiltrate
2). Pancreas: Hyperplasia, islets, moderate, multifocal
Slide 5
1). Kidney: Trematodes, encapsulated remnant, moderate, multifocal, random
2). Small intestine: As in slide 2.

There are no overt lesions within the skeletal muscle.
Slide 6
1). Liver: Hyperplasia, bile ductules, mild, multifocal, random
Slide 7
1). Liver: As in slide 6.
Sample 11291011C:
Slide 1
1). Liver: Cholangiohepatitis, mild to moderate, multifocal, subacute with biliary ductular hyperplasia
2). Liver: Hepatitis, mild, multifocal, random, lymphohistiocytic, with intralesional trematodes
Slide 2
1). Liver: Hepatitis as in slide 1, but with scattered intralesional syncytia **
Slide 3
1). Pyloric caeca: Enteritis, marked, diffuse, granulomatous with blunting and fusion of glandular elements, intermittent dissolution of the stratum compactum, and intralesional cestodes
2). Pancreas: Islet hyperplasia, moderate, multifocal to confluent
3). Pancreas: Pancreatitis, mild, multifocal, nonsuppurative with acinar cell degeneration and necrosis, mesothelial hypertrophy and hyperplasia and scattered islet hyperplasia
Sample 11291012C:
Slide 1
1). Liver: Cholangiohepatitis, mild, multifocal, random, subacute with biliary ductular hyperplasia
2). Liver: Cytoplasmic vacuolation, hepatocellular, macrovesicular, mild, multifocal
Slide 2
Liver: As in slide 1.

APPENDIX V: Tabulated Summary of Histopathologic Findings from Sampled Fish

MORPHOLOGIC DIAGNOSES	mild	moderate	marked	severe	NSF
Bile duct hyperplasia	25	11	2	0	11
Bile duct protozoa	2	1	0	0	46
Liver flukes	4	0	0	0	45
Liver vacuolation	5	6	1	0	36
Cholangitis, cholangiohepatitis	26	4	0	0	19
Small intestine, enteritis	3	8	1, nematodes	0	36
Pyloric caeca, enteritis	1	6	13	1	27
Pyloric caeca, cestodes	3	3	11	0	31
Pyloric caeca, nematodes	1	1	0	0	46
Pyloric caeca, trematodes	1	0	0	0	51
Pyloric caeca, peritonitis	2	13	0	0	33
Adipose tissue, atrophy	0	2	0	0	46
Adipose tissue, steatitis	2	0	0	0	46
Gastritis	5	1	0	0	
Pancreatitis	5	6	0	0	38
Pancreatic Islet hyperplasia	1	7	2	0	29
Gill, branchitis	26	17	2	1	4
Gill, trematodes	22	10	9	4	5
Gill, protozoa	1	0	0	0	49
Gills, mycosis	1	0	1	0	48
Gills, telangiectasia	1	1	0	0	48
Glomerulonephritis	5	1	0	0	42
Glomerulopathy	2	2	0	0	44
Renal trematodes	6	3	2	0	39
Renal protozoa	1	0	0	0	47
Renal tubular vacuolation	0	2	0	0	46

MORPHOLOGIC DIAGNOSES	mild	moderate	marked	severe	NSF
Myocarditis	30	13	0	0	6
Myocardial trematodes	16	3	23	1	6
Myocardial protozoa	1	0	0	0	48
Myocardial malformation	0	4	0	0	45
Skeletal muscle degeneration	13	6	0	0	30
Skeletal muscle hemorrhage	5	1	0	0	43
Skeletal muscle, myositis	1	0	0	0	48
Skeletal muscle, trematodes	1	0	0	0	48
Spleen, congestion	20	0	0	0	28
Skin, erosions	2	10	0	0	15
Skin, trematodes	2	0	0	0	15