Technical Memorandum

# Analysis of Microcystin in Fish in Copco and Iron Gate Reservoirs in 2009

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# Executive Summary

Since 2005, Copco and Iron Gate reservoirs, located on the Klamath River, have experienced elevated levels of cyanobacteria including *Microcystis aeruginosa* (MSAE) and the toxin microcystin. Yellow perch (*Perca flavescens*) from Copco and Iron Gate reservoirs were sampled during summer 2009 for possible microcystin accumulation as part of the 2009 Agreement in Principle (AIP) Interim Measure 12 Monitoring Plan. Tissue and liver samples from 43 yellow perch were collected during two sampling events (August and September). Samples were sent to the California Department of Fish and Game Laboratory (Laboratory) in Rancho Cordova CA for analysis of microcystin using the liquid chromatography mass spectrometry method (LC-MS/MS). All of the 2009 tissue and liver samples results were non-detect for microcystin.

Two previous studies have reported on microcystin analysis in yellow perch in Copco and Iron Gate reservoirs. In September 2007, tissue and liver samples from 19 yellow perch were collected from each reservoirfor a total of 38 total tissue samples and six liver samples (composited from three specimens each). Microcystin were detected in 31 of the 38 tissue samples and in five of the six liver composites. In 2008, tissue samples from 81 and 85 yellow perch were collected from Copco and Iron Gate reservoirs, respectively, over four occasions between May and November. All the 2008 samples were non-detect for microcystin.

These varying study results (that is, detection in some samples in 2007, and non-detection in all samples from 2008 and 2009) illustrate that the presence of MSAE and the toxin microcystin within waters of the reservoirs does not correlate to microcystin concentrations in fish tissue. Reasons for this lack of correlation may include, but are not limited to, the patchy distribution of algal blooms within waters of the reservoirs, the moblity of fish to move in and out of cyanobacteria bloom areas where microcystin is likely most prevalent, and the fact that uptake of toxins into fish tissue is through the food chain and not directly from the water.

# 1.0 Introduction

On November 13, 2008, the United States, the states of California and Oregon, and PacifiCorp executed an Agreement in Principle (AIP) describing the framework for an approach to study the water quality conditions of the Klamath River pursuant to the possible removal of four of PacifiCorp's dams on the Klamath River. Interim Measure 12 of the AIP provides for a water quality monitoring program, including on-going monitoring of blue-green algae (cyanobacteria) and associated toxins. Interim Measure 12 of the AIP further stipulates that PacifiCorp will provide funding of $500,000 per year for this measure, and that monitoring will be performed by an entity or entities agreed upon by the parties to the AIP and in consultation with the appropriate water quality agencies.

The North Coast Regional Water Quality Control Board (Regional Water Board), Oregon Department of Environmental Quality (DEQ), the Environmental Protection Agency (EPA), PacifiCorp, and the Karuk and Yurok Tribes cooperatively developed and implemented the AIP Interim Measure 12 Monitoring Plan (referred to as the 2009 Plan in the rest of this document)(AIP 2009). The 2009 Plan addressed public health monitoring of cyanobacteria and associated toxins, and comprehensive baseline water quality monitoring in the Klamath River.

Since 2004, Klamath River monitoring has documented elevated levels of cyanobacteria including *Microcystis aeruginosa* (MSAE) and the toxin microcystin. Microcystin is produced by some strains of cyanobacteria including MSAE, and are released into waters when cyanobacterial cells die or cell membranes degrade. MSAE cell counts and microcystin concentrations found in Klamath River waters within PacifiCorp's Copco and Iron Gate reservoirs have exceeded action levels defined by the California State Water Resources Control Board Blue Green Algae Work Group (SWRCB 2007). The action levels are threshold MSAE/microsystin levels which, when exceeded, trigger postings at recreational access points to inform the public of potential health risks associated with microcystin.

Human health effects from microcystins are reported as occurring primarily from direct ingestion or dermal contact with water containing elevated levels of microcystins (WHO 2003). Very little information is available on the accumulation and transfer of microcystin from the food chain to humans. Studies have shown that the toxin can accumulate in the liver and viscera of fish, and has been detected in muscle tissue (Magalhaes et al. 2001, Xie et al 2005). Yellow perch (*Perca flavescens*) provide a popular sport fishery in the reservoirs and mussels from the Klamath River are part of the traditional diet of tribal people. The 2009 Plan's public health monitoring included sampling of yellow perch from Copco and Iron Gate reservoirs, and mussel sampling from locations on the Klamath River below Iron Gate dam for possible microcystin accumulation.

PacifiCorp was the entity responsible for collecting fish from Copco and Iron Gate reservoirs for tissue analysis and the California Department of Game and Fish Laboratory (Laboratory) in Rancho Cordova, California analyzed the tissue samples for microcystin. This document reports the 2009 fish tissue sampling and results that were part of the 2009 Plan.

# 2.0 Methods

The 2009 sampling for yellow perch was performed in both Copco and Iron Gate reservoirs during the cyanobacterial bloom season that typically occurs from July to October. According to the 2009 Plan, two sampling events were conducted to capture the bloom period. One sampling event occurred after the bloom season started (August) and the other in the middle of the bloom season (September). As specified in the 2009 Plan, a minimum of 5 fish and a maximum of 15 fish were targeted for capture from each reservoir for each sampling event. The field and laboratory techniques used for this sampling are described below.

## 2.1 Field Procedure

The field sampling targeted yellow perch in the reservoirs because yellow perch is the species most commonly captured and consumed recreationally. Fish specimens were collected by hook and line sampling ("angling") in areas that are frequented by anglers (e.g., "the Narrows" in Iron Gate reservoir and Mallard Cove in Copco reservoir). Upon capture, individual specimens were put in sealed plastic bags and placed in coolers packed with ice. At the end of the sampling day, fish were taken from the plastic bags and measured (fork length), weighed (grams), and assessed for any abnormal external conditions (e.g., lesions, parasites). Fish were then individually labeled and double wrapped in aluminum foil, and frozen until shipped by overnight delivery to the Laboratory. The procedures followed the Standard Operating Procedures (SOP) contained in the 2009 Plan and additional directions received from the Laboratory (David Crane, CDFG, pers. comm.).

## 2.2 Laboratory Analysis

At the Laboratory, fish tissue (muscle) samples were taken from fillets of the individual fish specimens. Fish liver samples were prepared from a composite of livers from five fish, and followed the "75 percent rule" (i.e., the length of the smallest fish collected in a sampling event for a given location should be at least 75 percent of the length of the largest fish sampled).

The prepared tissue and liver samples were analyzed for microcystin using the liquid chromatography mass spectrometry method (LC-MS/MS). Although research indicates that microcystins have more than 70 structural variants only a relatively limited number are available as accurate and reliable analytical standards (Mekebri 2009). The LC-MS/MS analysis allows quantification and confirmation of six microcystin congeners (MC-LA, LR, LW, LY, RR, and YR), and the determination of two microcystin metabolites (Desmethyl-LR and RR), with a higher degree of specificity and sensitivity than other analytical approaches (Mekebri 2009).

Samples were kept frozen in the Laboratory until the time of analysis. Prior to the liquid chromatography analysis, tissue samples were homogenized, sonified, and extracted. Samples were analyzed in sets, with each set including a procedural (method) blank, laboratory control sample (LCS), matrix spike and duplicate and field sample duplicate. Identification and quantification of microcystins were performed by liquid chromatography-tandem mass spectrometry in multiple-reaction monitoring (MRM) mode. All quantification was performed using certified standards, except the demethylated (dm) congeners which are quantified as the parent non-methylated analog since no certified standard is commercially available. Nodularin was used for the internal standards. The reporting limit of all microcystins is 1 ug/L (ppb). For a detailed description of the LC-MS/MS method, see Mekebri (2009).

## 2.3 Water Analysis

While water quality sampling was not done specific to the fish tissue sampling, water quality data collection occurred throughout the summer in the reservoirs as part of the 2009 Plan. Water quality data included bimonthly sampling for analysis of algae (including speciation and enumeration) and microcystin in each reservoir. Water quality samples were obtained at three or more sites in each reservoir, including at least two public access sites and one open water site. The algae and microcystin samples were analyzed on a "rushed" schedule and biweekly memos of results were circulated to members of the Klamath Blue Green Algae Work Group (KBGAWP) and regulatory agencies such as the Regional Water Board to keep apprised of potential public health concerns. These memos were also posted on PacifiCorp's Klamath Project website (http://www.pacificorp.com/es/hydro/hl/kr.html).

# 3.0 Results

## 3.1 Specimens Collected

Sampling events occurred on August 3 and September 9, 2009. A total of 43 yellow perch were collected over the two sampling events, including 22 and 21 yellow perch caught in Iron Gate and Copco reservoirs, respectively (Table 1). Two black crappie (*Pomoxis nigromaculatus*) also were caught in Iron Gate reservoir but were not included as specimens for the Laboratory analysis (Table 1).

Table 1. Number of the fish collected in Copco and Iron Gate reservoirs during 2009.

| Species | Location | Number of Samples |
|---|---|---|
| Yellow perch | Copco Reservoir | 21 |
| | Iron Gate Reservoir | 22 |
| Black crappie | Iron Gate Reservoir | 2 |

The sizes of the yellow perch caught in the two reservoirs were similar. The sizes of the yellow perch caught in Iron Gate reservoir ranged from 175 to 230 mm (average 202 mm) in length and from 58 to 162 grams (average 108 grams) in weight (Table 2). The sizes of yellow perch caught in Copco reservoir ranged from 185 to 250 mm (average 201 mm) in length and from 46 to 229 grams (average 107 grams) in weight (Table 2).

## 3.2 Laboratory Results

No detectable levels of microcystin were found in either the individual tissue samples or the composite liver samples. Copies of the laboratory results are posted on PacifiCorp's Klamath Project website (http://www.pacificorp.com/es/hydro/hl/kr.html).

## 3.3 Water Quality

The presence of MSAE was first detected in both Iron Gate and Copco reservoirs during the water quality sampling of July 6, 2009. Cell count levels were above the California health advisory guidelines.[1] Microcystin levels exceeded the California guideline of 8 µg/l in both

[1] The California State Water Resources Control Board (SWRCB 2007) and Oregon Department of Health Services (ODHS 2005) provide guidelines for posting advisories in recreation waters. These guidelines were developed using information provided in WHO (2003). Both SWRCB (2007) and ODHS (2005) recommend posting advisories in recreation waters under three circumstances: (1) if "scum is present associated with toxigenic species"; (2) if scum is not present, but the density of *Microcystis* or *Planktothrix* is 40,000 cells/ml or greater; and (3) if scum is not present, but the density of all potentially toxigenic BGA is 100,000

reservoirs in July, August and September. These data were reported in biweekly memos posted on PacifiCorp's Klamath website and communicated to the Klamath Blue-Green Algae Work Group. Water quality data including the phytoplankton results are reported in a separate document covering the entire 2009 sampling program (Raymond 2010) and is available on PacifiCorp's Klamath website (www.pacificorp.com/es/hydro/hl/kr.html#).

Table 2. Month, Sample ID, Length and weight of the yellow perch collected for microcystin analysis from Iron Gate and Copco reservoirs in 2009.

| Iron Gate Reservoir | | | |
|---|---|---|---|
| Month | Sample ID | Fork Length (mm) | Weight (g) |
| Aug | IG09-1-YP-01 | 175 | 59 |
| Aug | IG09-1-YP-02 | 185 | 84 |
| Aug | IG09-1-YP-03 | 210 | 129 |
| Aug | IG09-1-YP-04 | 210 | 119 |
| Aug | IG09-1-YP-05 | 230 | 162 |
| Aug | IG09-1-YP-06 | 185 | 80 |
| Aug | IG09-1-YP-07 | 195 | 102 |
| Aug | IG09-1-YP-08 | 215 | 129 |
| Aug | IG09-1-YP-09 | 170 | 58 |
| Aug | IG09-1-YP-10 | 210 | 129 |
| Aug | IG09-1-YP-11 | 190 | 91 |
| Aug | IG09-1-YP-12 | 210 | 117 |
| Sept | IG09-2-YP-01 | 220 | 140 |
| Sept | IG09-2-YP-02 | 230 | 172 |
| Sept | IG09-2-YP-03 | 200 | 96 |
| Sept | IG09-2-YP-04 | 220 | 134 |
| Sept | IG09-2-YP-05 | 190 | 70 |
| Sept | IG09-2-YP-06 | 220 | 137 |
| Sept | IG09-2-YP-07 | 190 | 88 |
| Sept | IG09-2-YP-08 | 185 | 85 |
| Sept | IG09-2-YP-09 | 195 | 91 |
| Sept | IG09-2-YP-10 | 200 | 100 |
| | Average | 202 | 108 |

| Copco Reservoir | | | |
|---|---|---|---|
| Month | Sample ID | Fork Length (mm) | Weight (g) |
| Aug | CO09-1-YP-01 | 190 | 88 |
| Aug | CO09-1-YP-02 | 190 | 87 |
| Aug | CO09-1-YP-03 | 195 | 83 |
| Aug | CO09-1-YP-04 | 200 | 102 |
| Aug | CO09-1-YP-05 | 205 | 108 |
| Aug | CO09-1-YP-06 | 205 | 135 |
| Aug | CO09-1-YP-07 | 210 | 106 |
| Aug | CO09-1-YP-08 | 185 | 76 |
| Aug | CO09-1-YP-09 | 230 | 162 |
| Aug | CO09-1-YP-10 | 185 | 76 |
| Aug | CO09-1-YP-11 | 185 | 67 |
| Sept | CO09-2-YP-01 | 195 | 90 |
| Sept | CO09-2-YP-02 | 235 | 172 |
| Sept | CO09-2-YP-03 | 155 | 46 |
| Sept | CO09-2-YP-04 | 190 | 84 |
| Sept | CO09-2-YP-05 | 180 | 82 |
| Sept | CO09-2-YP-06 | 250 | 229 |
| Sept | CO09-2-YP-07 | 200 | 98 |
| Sept | CO09-2-YP-08 | 195 | 81 |
| Sept | CO09-2-YP-09 | 215 | 130 |
| Sept | CO09-2-YP-10 | 220 | 135 |
| | | | |
| | Average | 201 | 106 |

cells/ml or greater. Based on WHO (2003) information, SWRCB (2007) and ODHS (2005) indicate that cell counts of 40,000 and 100,000 cells/ml equate to microcystin toxin concentrations of 8 μg/L and 20 μg/L, respectively.

# 4.0 Discussion

The yellow perch collected in Copco and Iron Gate reservoirs in 2009 had no detectable levels of microcystin present in either the fish tissue or liver samples even though microcystin was detected in water samples from both reservoirs (Figure 1). Possible factors contributing to the non-detection of microcystin could include: (1) the patchy distribution of algal blooms within waters of the reservoirs; (2) the moblity of fish to move in and out of cyanobacteria bloom areas where microcystin is likely most prevalent; and (3) and the fact that uptake of toxins into fish tissue is through the food chain and not directly from the water.

With regard to the first possible factor, MSAE and microcystin are both highly variable in time and space, and nearshore habitats usually have higher concentrations than open water habitats (Raymond 2009; Figure 1). Microcystin levels can also vary by depth with surface samples typically having the highest concentrations (Figure 2).

With regard to the second possible factor, yellow perch are a schooling fish that prefer deeper, open waters (Becker 1983; Wydoski and Whitney 1979) and do not typically inhabit the shallow nearshore areas or the water's surface where water samples are collected for public health monitoring. In a study evaluating the human health threat associated with microcystin in yellow perch in Lake Erie, Wilson et al. (2008) used integrated samples to estimate toxin concentrations throughout the water column since yellow perch are rarely found near the lake surface. Since fish are mobile organisms, correlating their potential exposure to toxins is difficult.

With regard to the third possible factor, feeding is the exposure route for aquatic biota to microcystin since microcystins tend to be water soluable and polar, and thus do not readily pass membranes such as gills (Ibelings and Haven 2008). As juveniles, yellow perch prey on zooplankton and move to larger aquatic insects and fish as they mature (Wydoski and Whitney 2008). Zooplankton can accumulate microcystins and may act as vectors of the toxins in the aquatic food web; however, this transfer has not yet been quantified (Smith and Haney 2006). There were no data collected on the stomach contents nor on the zooplankton community for this study.

The risk of being exposed to toxins via the food web is amplified if biomagnification is occurring. However, Ibelings and Haven (2008) found little support for biomagnification and suggested that biodilution seems to occur in the food web, whereby toxins are subject to degradation and excretion at each trophic level. In their review of the available literature on cyanobacterial toxin concentrations in biota, Ibelings and Haven (2008) determined that bioaccumulation has seldom been analyzed correctly.

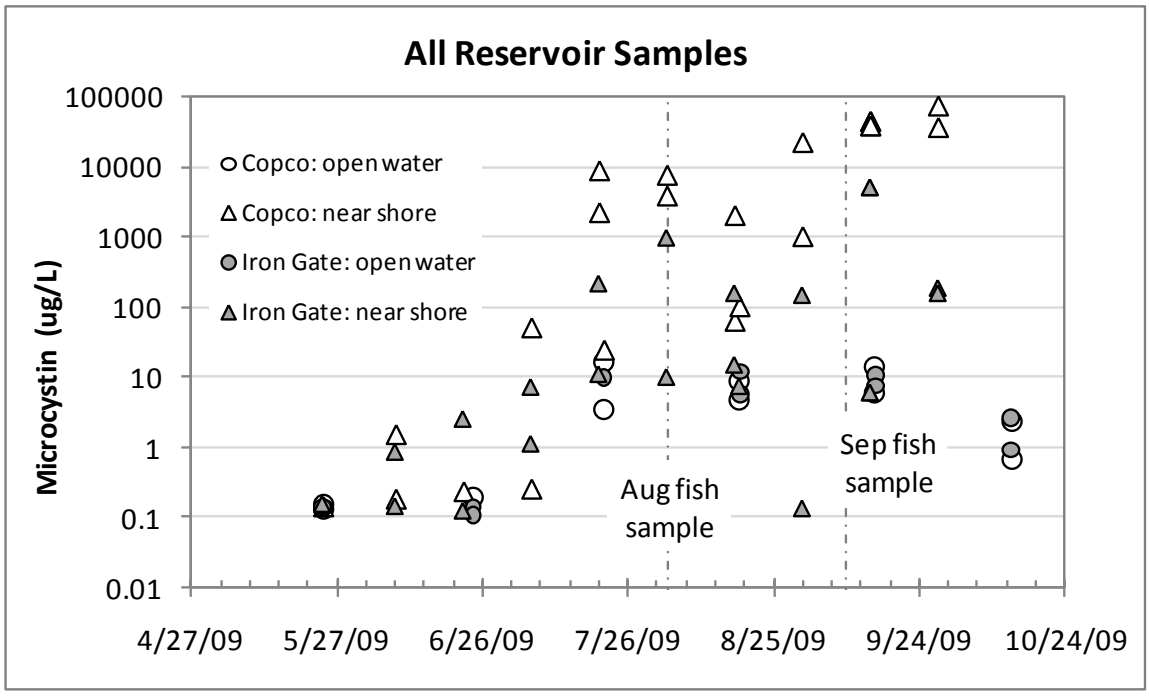


Figure 1. Microcystin levels in the near shore and open water habitats in Copco and Iron Gate reservoirs, 2009.

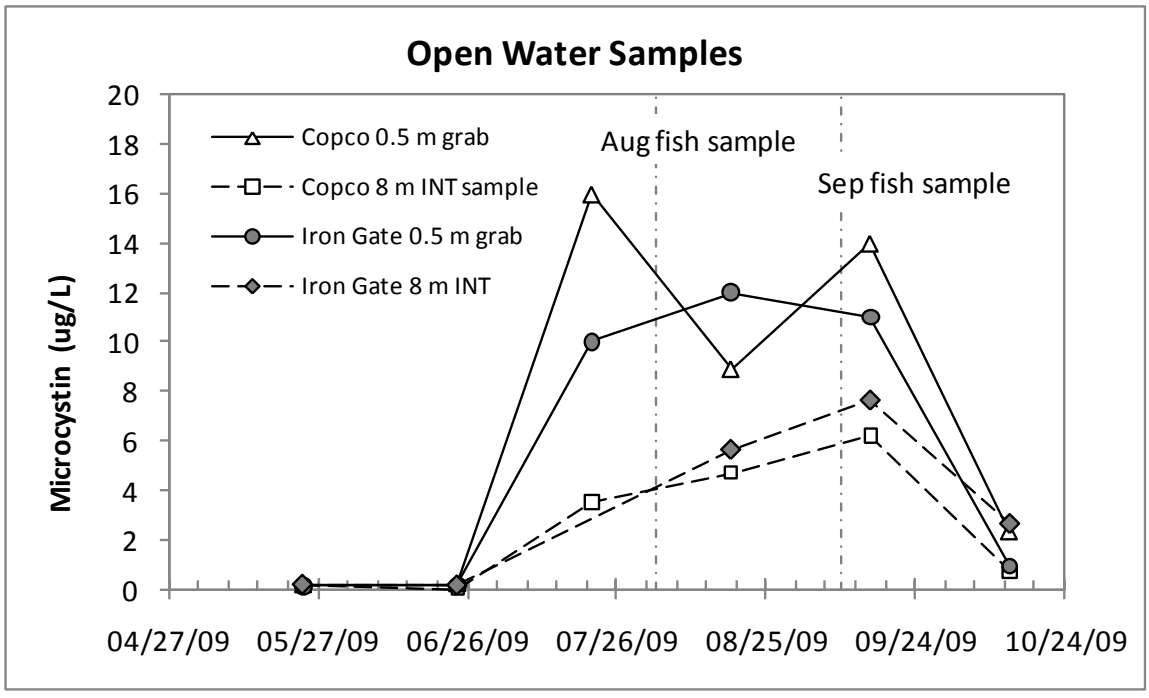


Figure 2. Microcystin levels for surface an integrated water samples collected in Copco and Iron reservoirs, 2009.

Two previous studies have reported on microcystin analysis in yellow perch in Copco and Iron Gate reservoirs (CH2M Hill 2009; Kann 2008; SWRCB 2008). In September 2007, tissue and liver samples from 19 yellow perch were collected from each reservoir, for a total of 38 tissue samples and six liver samples (composited from three specimens each). Microcystin were detected in 31 of the 38 tissue samples and in five of the six liver composites. In 2008, tissue samples from 81 and 85 yellow perch were collected from Copco and Iron Gate reservoirs, respectively, over four occasions between May and November. All the 2008 samples were non-detect for microcystin. CH2M Hill (2009) speculated that the difference between the 2007 and 2008 samples may be due to microcystin variablity between the years and/or the variation in analytical methods. This 2009 study used the same analyticial methods as the 2007 study but had different results. Possible explainations for these different results are speculative but may include variations in microcystin concentrations, prey abundance, and sampling locations within the reservoirs.

For all three years where fish tissue sampling was done in Copco and Iron Gate reservoirs, MSAE cell counts were at levels that exceeded public health guidelines and both reservoirs were posted with health advisory signs during the times when fish sampling was occurring. Even though there was a variation in the analytical method between the 2007 and 2009 samples, and the 2008 samples, both methods used high performance liquid chromatography with mass spectral detection. None of these studies looked at stomach contents or zooplankton distribution in the reservoirs which could affect toxin accumulation. These varying study results illustrate the difficulty in attempting to correlate microcystin concentrations in fish tissue with MSAE blooms.

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