Health and Condition of Endangered Young-of-the-Year Lost River and Shortnose Suckers Relative to Water Quality in Upper Klamath Lake, Oregon, 2014–2015

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Conversion Factors

International System of Units to Inch/Pound

Multiply	By	To obtain
Length		
nanometer (nm)	3.93701×10^{-8}	inch (in.)
micrometer (μm)	0.000039	inch (in.)
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
meter (m)	1.094	yard (yd)
Area		
square kilometer (km^2)	2.471	acre
square kilometer (km^2)	0.3861	square mile (mi^2)
Volume		
microliter (μL)	3.381×10^{-5}	ounce, fluid (fl. oz)
milliliter (mL)	0.0338	ounce, fluid (fl. oz)
Mass		
gram (g)	0.03527	ounce, avoirdupois (oz)
milligram (mg)	3.5274×10^{-5}	ounce, avoirdupois (oz)
microgram (μg)	3.5274×10^{-8}	ounce, avoirdupois (oz)
nanogram (ng)	3.53×10^{-11}	ounce, avoirdupois (oz)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:
$$°F=(1.8 \times °C)+32.$$
Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L) or in micrograms per liter (µg/L).

Datum

Horizontal coordinate information is referenced to North American Datum of 1983 (NAD 83).

Abbreviations and Acronyms

Abbreviation or Acronym	Meaning
DNA	deoxyribonucleic acid
DO	dissolved oxygen
K	number of population clusters
MCLR	microcystin LR
MCMC	Markov chain Monte Carlo simulation
NH_3	ammonia
PAS	periodic acid-Schiff
ppb	Parts per billion
Prob [LRS]	probability of species being a Lost River sucker based
R^2	coefficient of determination
USGS	U.S. Geological Survey

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Executive Summary

Most mortality of endangered Lost River (*Deltistes luxatus*) and shortnose (*Chasmistes brevirostris*) suckers in Upper Klamath Lake, Oregon, occurs within the first year of life. Juvenile suckers in Clear Lake Reservoir, California, survive longer and may even recruit to the spawning populations. In a previous (2013–2014) study, the health and condition of juvenile suckers and the dynamics of water quality between Upper Klamath Lake and Clear Lake Reservoir were compared. That study found that apparent signs of stress or exposure to irritants, such as peribiliary cuffing in liver tissue and mild inflammation and necrosis in gill tissues, were present in suckers from both lakes and were unlikely to be clues to the cause of differential mortality between lakes. Seasonal trends in energy storage as glycogen and triglycerides were also similar between lakes, indicating prey limitation was not a likely factor in differential mortality. To better understand the relationship between juvenile sucker health and water quality, we examined suckers collected in 2014–2015 from Upper Klamath Lake, where water quality can be dynamic and, at times, extreme.

While there were notable differences in water quality and fish health between years, we were not able to identify any specific water-quality-related causes for differential fish condition. Water quality was generally better in 2014 than in 2015. When considered together afflictions and abnormalities generally indicated healthier suckers in 2014 than 2015. Low dissolved-oxygen events (< 4 milligrams per liter) were less frequent and occurred earlier; high pH events (≥ 9.5) were less frequent and shorter in duration; large diel fluctuations in pH (≥ 1.4) were less frequent; water temperatures were warmer, particularly in July and September; and concentrations of microcystin in both large and small fractions of samples were lower in 2014 than in 2015. Total and therefore also un-ionized ammonia were low in 2014–2015 relative to concentrations known to affect suckers. Petechial hemorrhages of the skin, attached *Lernaea* spp. and eosinophilic hyaline droplets in the kidney tubules were less prevalent in 2014 than in 2015; however, hyperplastic and hypertrophic gill tissue and trichodinids on the gills were observed more frequently in 2014. There were more suckers with normal liver color and texture in 2014 than in 2015. The prevalence of suckers with liver inflammation was greater in 2014 and only observed in suckers collected after August 5, whereas liver inflammation occurred intermittently in 2015. Liver glycogen among suckers decreased in late-August 2014 and increased from early August to mid-September 2015. Lost River suckers had greater whole-body triglyceride content but a larger proportion with an absence of visceral fat observed in 2014 than in 2015. In contrast, shortnose suckers were similar between years in regard to

both whole-body triglyceride and visceral fat. Black-spot-forming parasites (trematode metacercariae) were observed in a higher prevalence on shortnose suckers but not Lost River suckers in 2014 than in 2015. Opercular deformities were less prevalent in both species in 2014 than in 2015.

Neither gross nor histological examination revealed a high prevalence of abnormalities in suckers that clearly indicate a primary mechanism for juvenile mortality in Upper Klamath Lake. Histological abnormalities were almost always focal and minimal or mild except where associated with parasites. Mild to severe focal abnormalities associated with *Lernaea* sp. attachment sites and encysted digenean (trematode) metacercariae are unlikely to be associated with mortality. Severe and diffuse inflammation and hyperplasia of the gills associated with *Ichthyobodo* sp. on one Lost River sucker, may indicate a potential cause of mortality. High mortality may have primarily occurred outside our study period (for example, in spring or over winter), or was caused by a factor that could not be detected with our methods (for example, predation). Alternatively, abnormalities in a small percentage of passively captured suckers in Upper Klamath Lake may indicate health-related issues that were more prevalent in populations than in our samples. Temporary decreases in liver glycogen stores may also indicate periods of stress, which may eventually lead to mortality of young suckers.

Background

Lost River (*Deltistes luxatus*) and shortnose suckers (*Chasmistes brevirostris*) were once abundant throughout the Upper Klamath Basin, but populations of both species notably decreased by the 1960s and were listed as endangered in 1988 (U.S. Fish and Wildlife Service, 1988). These fish were once so numerous that they were an important food source for Native American tribes and supported a commercial cannery on the Lost River (National Research Council, 2004). The largest extant populations of Lost River suckers occur in Upper Klamath Lake, Oregon, whereas the largest extant population of shortnose suckers occurs in Clear Lake Reservoir, California. Although a ban on fishing for suckers in 1987 may have improved adult survival, the populations did not rebound (Markle and Cooperman, 2002; National Research Council, 2004). In fact, sucker populations in Upper Klamath Lake continue to decrease, and there probably are one-third as many suckers today as there were a decade ago (Hewitt and others, 2017).

Upper Klamath Lake sucker populations are decreasing because mortality is not being balanced by recruitment. Cohorts produced in the early 1990s now make up the largest proportion of adult sucker populations currently living in Upper Klamath Lake. About 5–10 percent of adult Lost River suckers die each year, but there has not been measurable recruitment since the cohorts produced in the early 1990s joined spawning aggregations (Hewitt and others, 2017). About 5–20 percent of adult shortnose suckers die each year. Mark-recapture analysis indicates that a small number of new recruits have joined the spawning aggregations of adult shortnose suckers since 2008. However, shortnose suckers first detected in the spawning aggregations since 2008 are not as small as new recruits detected in the late 1990s, making recent recruitment estimates for this species suspect (Hewitt and others, 2017).

Recruitment to spawning aggregations in Upper Klamath Lake likely is limited by mortality in the juvenile life stage. Both Lost River and shortnose suckers spawn in the Williamson and Sprague Rivers each spring. Lost River suckers also aggregate to spawn along the eastern shoreline of Upper Klamath Lake each year between March and June (Burdick, Hewitt and others, 2015). Larvae of both species were captured drifting downstream in the Williamson and Sprague Rivers each year from 2004 to 2010, indicating that spawning and hatching of eggs were both successful (Martin and others, 2013). Most larval suckers drifting in these rivers reach Upper Klamath Lake, where they can feed freely and food is abundant, within a day after they emerge from the gravel

(Cooperman and Markle, 2003). Age-0 juvenile suckers are then captured throughout Upper Klamath Lake until late August or early September, when catches decrease in most years (Burdick and Martin, 2017). Despite intensive sampling throughout Upper Klamath Lake and in the lower reaches of the lake's tributaries and at the outlet of the lake, age-1 endangered sucker captures are uncommon, and older juveniles rarely are encountered in Upper Klamath Lake (Burdick and Martin, 2017).

The difference in catch rates between age classes in Upper Klamath Lake is likely due primarily to mortality rather than emigration or a reduction in sampling efficiency. Seasonal decreases in age-0 catches and from age-0 to age-1 are unlikely due to emigration, because juvenile suckers have not been found to migrate out of Upper Klamath Lake or from near to offshore habitats in Upper Klamath Lake from June to August (Hendrixson and others, 2007). We presumed that sampling efficiency did not decrease to near zero for age-1 or older suckers in Upper Klamath Lake based on catches of age-1 and older suckers in Clear Lake Reservoir using the same gear type at the same time of year (Burdick, Elliott and others, 2015).

Juvenile suckers in Upper Klamath Lake appear mostly healthy until they disappear from catches. Juvenile suckers in Upper Klamath Lake grow rapidly throughout August and September each year, but growth slows in October (Bottcher and Burdick, 2010; Burdick and Brown, 2010; Burdick and Martin, 2017). Energy stored as triglycerides appears to remain constant or to increase throughout the summer for these suckers (Foott and others, 2012; Burdick, Elliott and others, 2015). Skeletal deformities usually occur in less than 10 percent of age-0 Lost River and shortnose suckers collected annually from Upper Klamath Lake but have been reported in as many as 27 percent in some years (Burdick, Anderson and VanderKooi, 2009). The most commonly reported deformity is shortened opercula, but scoliosis and fused vertebrae also are observed in less than 1 percent of individuals (Burdick and Hewitt, 2012; Burdick, Elliott and others, 2015). Wild-caught and mesocosm-held juvenile suckers from Upper Klamath Lake rarely are diseased, and histological abnormalities are uncommon and usually minor (Foott and others, 2012, 2014; Burdick, Elliott and others, 2015).

Although Upper Klamath Lake juvenile sucker health has been investigated (for example, Foott and others, 2012; Burdick, Elliott and others, 2015), annual variation in health in Upper Klamath Lake has not been well documented. If variation exists, it may be owing to differences in water quality.

In this report, we examined water-quality dynamics in 2014–2015 and co-occurring sucker health observations. Our goal was to identify covariation between water quality and sucker health that will assist in the development of new reasonable hypotheses about the effects of water quality on juvenile sucker health.

Description of Study Area

Major land-use changes in the Upper Klamath Lake watershed have shaped the landscape and its aquatic environments. Landscape changes occurred after the city of Klamath Falls, located at the southern terminus of the lake, was settled in 1867. Overgrazing by cattle was identified as an ecological concern in the Upper Klamath Basin as early as 1883 (National Research Council, 2004). Dominant primary production in Upper Klamath Lake switched from diatoms to cyanobacteria, with increased phosphorus loading in the early 20th century following reclamation of littoral wetlands for agricultural use (Bradbury and others, 2004). Diking and draining wetlands in the first half of the

20th century resulted in a loss of more than 20,000 acres of littoral wetlands surrounding Upper Klamath Lake, although about one-half of these wetlands have since been re-inundated (National Research Council, 2004). The loss of wetland habitat and overgrazing most likely contributed to nutrient loading and massive annual blooms of the blue-green cyanobacterium *Aphanizomenon flos-aquae* in Upper Klamath Lake (National Research Council, 2004). During prolonged periods each summer, the algal community in Upper Klamath Lake is a near monoculture of *A. flos-aquae* (Eldridge, Wood, and Echols, 2012). Shoreline habitat also was altered in 1909 when large boulders were used to stabilize the eastern shore of the lake for the construction of the Southern Pacific Railroad (National Research Council, 2004). Present-day land use in the watershed primarily is hay farming and cattle ranching, but the headwaters are in the U.S. Forest Service's Fremont-Winema National Forest, and a substantial amount of the lakeshore is managed by public and private entities for fish, wildlife, and water storage. Finally, Upper Klamath Lake is host to numerous non-native fishes that may compete with or prey upon suckers.

Upper Klamath Lake is uniformly shallow, with an average water depth of about 2.6 m and a surface area of about 305 km^2 at full pool (National Research Council, 2004). A 6.4–9.5-m-deep trench runs along the western shore of the lake. Most of the flow enters through the Williamson River on the eastern shore (fig. 1) and the smaller Wood River. A small but notable amount of water also upwells through the volcanic soils along the lakeshore. The bottom of the lake is covered with fine organic detritus composed primarily of decaying diatoms and cyanobacteria. Shoreline wetlands in the northern part of the lake are heavily vegetated with wocus (*Nuphar* spp.), tules (*Schoenoplectus acutus*), and willows (*Salix* spp.). Spring-fed creeks enter the lake in the area of these wetlands and are associated with relatively good summertime water quality when compared to the rest of the lake (Banish and others, 2009).

Water quality in Upper Klamath Lake is strongly correlated with the bloom dynamics of the cyanobacterium *A. flos-aquae* (Eldridge, Wood, and Echols, 2012). One or two massive blooms of *A. flos-aquae* occur every year in Upper Klamath Lake from May to October (Eldridge, Wood, and Echols, 2012). The first annual bloom starts with the increase in water temperature in May or June and ends with a period of *A. flos-aquae* senescence known as the "bloom crash" in July or early August in most years. The second bloom, if it occurs, frequently is less intense than the first bloom, and water quality does not reach the same extremes during the second bloom crash. Peak blooms are correlated with high pH (>9.5), and substantial swings in dissolved-oxygen (DO) concentrations. Although dissolved total nitrogen concentrations generally are lower during heavy bloom periods, the elevated pH increases the potential toxicity of ammonia in the lake because ammonia-nitrogen (NH_3-N) shifts toward the more toxic un-ionized form (NH_3) when pH is high and toward the less toxic ionized form (NH_4^+) when pH is low (Wedemeyer and others, 1976). During "bloom crash" periods, DO concentrations can be hypoxic (< 4 mg/L), pH stabilizes at around 7, and un-ionized ammonia can remain high (> 0.5 mg/L as NH_3; Eldridge, Caldwell Eldridge, and others, 2012). Microcystin, a powerful hepatotoxin produced by the cyanobacterium *Microcystis aeruginosa*, also increases during the bloom crash, with concentrations peaking at greater than 40 parts per billion (ppb) in 2014 (Eldridge, Wood, and Echols, 2012; Burdick, Elliott and others, 2015). Each of these water-quality variables may independently affect the survival of juvenile suckers, or combinations of multiple variables near the thresholds of sucker tolerance may cause direct mortality.
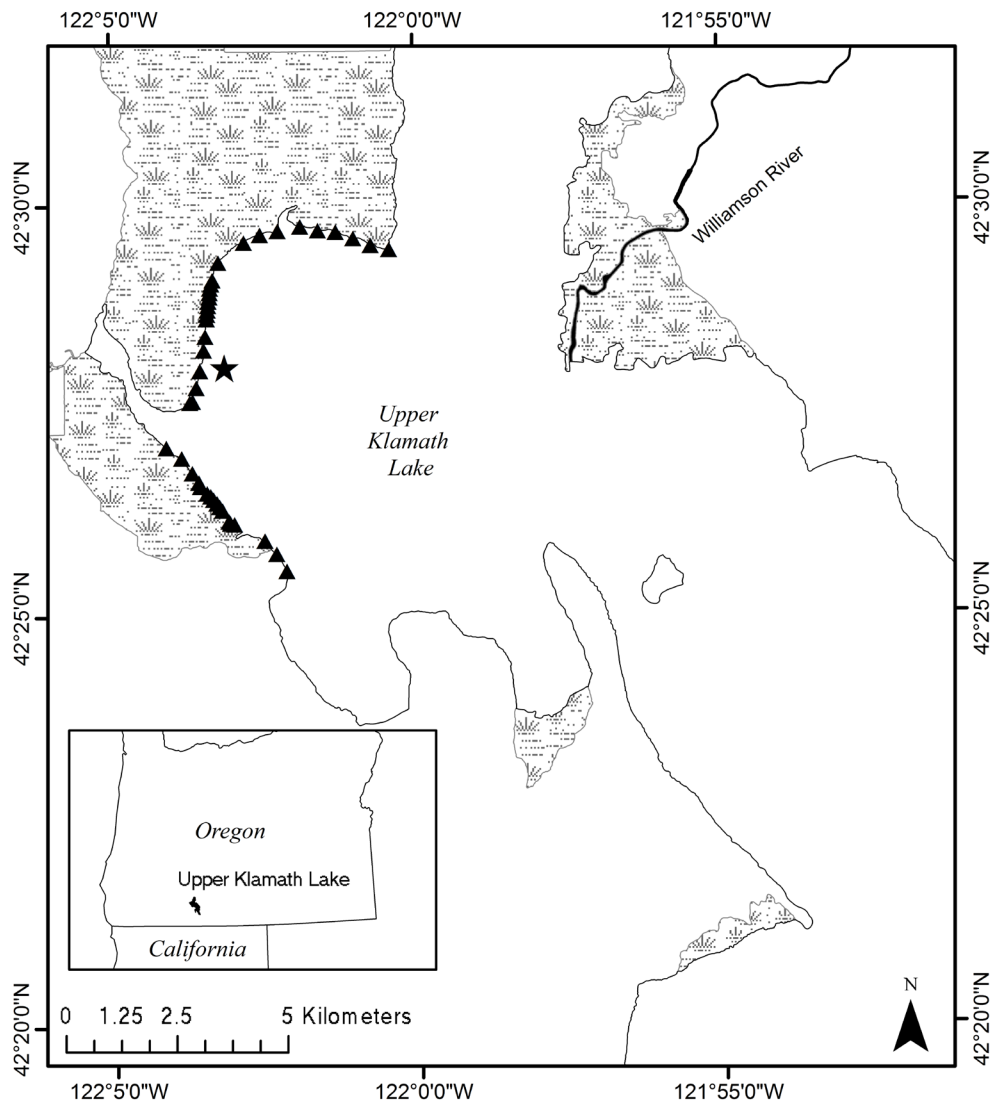
Figure 1. Map showing Upper Klamath Lake, Oregon. Fish were sampled along Fish Banks (star) on the northwestern lakeshore (triangles). Continuous water-quality measurements were collected at U.S. Geological Survey monitoring site No. 422820122032100 near Fish Banks (https://waterdata.usgs.gov/nwis/uv?site_no=422820122032100).

Review of Water-Quality Effects on Juvenile Sucker Health and Survival

Hypoxic conditions may directly or indirectly cause juvenile sucker mortality in Upper Klamath Lake. During bloom crash periods, DO concentrations within 1 m of the substrate in Upper Klamath Lake commonly decrease to less than 2 mg/L for several hours to as long as a day (http://nwis.waterdata.usgs.gov/nwis/qwdata). On occasion, DO concentrations near the lake bottom decrease to less than 1 mg/L for short periods of time ranging from 1 to 16 h (http://nwis.waterdata.usgs.gov/nwis/qwdata). Mean of median 24–96-h lethal DO concentrations (LC_{50}) range from 1.14 to 1.34 mg/L for juvenile shortnose suckers, and from 1.58 to 1.62 mg/L for juvenile Lost River suckers (Saiki and others, 1999). In 14-day trials, the mortality of Lost River suckers increased as DO concentrations decreased from 2.1 to 1.4 mg/L (Meyer and Hansen, 2002). DO concentrations are rarely less than 2 mg/L for more than 24 h, but shortnose suckers cannot tolerate DO concentrations of less than 0.7 mg/L for even 1 minute (Castleberry and Cech, 1993). This finding was corroborated by Stone and others (2017) who observed complete mortality of captive suckers held near the lake bottom when DO concentrations were measured at less than or equal to 1 mg/L for less than 2 h. Although there are no observable adverse physiological effects to Lost River suckers exposed to DO concentrations at 2.10 mg/L or greater for 14 days (Meyer and Hansen, 2002), suckers may exhibit a behavioral response to low DO. Although other fishes exposed to very low DO concentration are known to cease feeding and become lethargic (Svobodova and others, 1993), juvenile suckers have been observed to leave their normally occupied benthic habitats and swim near the surface (Saiki and others, 1999). When allowed access to the surface, Lost River suckers were able to survive several hours in completely anoxic water by gulping air (Foott and others, 2007). This behavior may make suckers temporarily more susceptible to predation by surface-feeding birds.

High pH in Upper Klamath Lake that commonly exceeds 9.75 and occasionally reaches 10.50 also has potential to affect juvenile sucker mortality. In laboratory trials, juvenile Lost River suckers did not begin to express signs of pH-related stress until pH was greater than or equal to 10.00 (Meyer and Hansen, 2002). When pH was greater than 10.00, juvenile suckers convulsed, swam erratically, produced excessive mucus, and exhibited hemorrhaging from the gills and eyes (Saiki and others, 1999). The 96-h median lethal pH for larval juvenile Lost River and shortnose suckers is estimated to be at least 10.30 (Saiki and others, 1999). Falter and Cech (1991) reported that hatchery-reared juvenile shortnose suckers exposed to rapidly increasing pH had a mean acute tolerance of only 9.55, with death occurring within 1 minute of exposure. A critical difference between pH experiments may be water temperature. Saiki and others (1999) conducted their experiments at a constant 20 °C, whereas Falter and Cech (1991) conducted their experiments at water temperatures ranging from 14 to 16 °C. Stone and others (2017) found mortality of juvenile Lost River suckers held in cages for 7 days in Upper Klamath Lake was greatest when pH and water temperatures were both relatively high (pH ≥ 10 and temperature ≥ 20.1 °C).

Un-ionized ammonia concentrations in Upper Klamath Lake commonly are high enough to affect suckers and, in some years, exceed the median lethal thresholds for shortnose suckers. Gill lamellar thickness increases at concentrations of un-ionized ammonia of 0.20 mg/L and higher (Lease and others, 2003). Hyaline droplets in the proximal convoluted tubules of the kidneys (indicative of excess protein from the filtrate) were associated with experimental exposure of Lost River suckers to high un-ionized ammonia concentrations (0.44 mg/L; Foott and others, 2000), although the pathological significance of this finding is unknown (Ferguson, 1989; Wolf and others, 2015). Foott and others (2014) speculated that increased urine output, stimulated by elevated ammonia, may result in the kidney tubule changes. Liver, pancreatic, and intestinal damage have not been associated with high concentrations of un-ionized ammonia (Foott and others, 2000). The 48-h

median LC$_{50}$ for un-ionized ammonia was 0.48 mg/L for shortnose suckers and 0.92 mg/L for Lost River suckers in laboratory experiments (Saiki and others, 1999). Un-ionized ammonia concentrations exceeded 0.48 mg/L in only 2 percent or fewer samples from May to September each year from 1990 to 1995 and from 2004 to 2010. In comparison, un-ionized ammonia exceeded 0.48 mg/L in 7–35 percent of samples each year from 1996 to 2003 (Klamath Tribes, unpub. data). Stone and others (2017) found that un-ionized ammonia at concentrations that varied from 0.016 to greater than 0.3 mg/L were uncorrelated with mortality in captive suckers in Upper Klamath Lake.

Microcystin in Upper Klamath Lake could cause severe damage to the liver and other tissues of juvenile suckers and may result in mortality. In Upper Klamath Lake, the extremely toxic microcystin-LR congener primarily is produced by *Microcystis aeruginosa* during the period when *A. flos-aquae* senesces (Carmichael and others, 2000; Eldridge, Wood, and Echols, 2012). The primary route of exposure to microcystins for fish is through their food and, to a lesser extent, the toxins also may be taken up in gills when there are high concentrations of dissolved microcystins in the water column (Carbis and others, 1996). Tolerance of microcystins varies among fishes and has not been established specifically for Lost River or shortnose suckers (Fischer and Dietrich, 2000). The most relevant study on lethal concentrations may be that of Fischer and Dietrich (2000), who determined that a cyprinid, common carp (*Cyprinus carpio*), died within 10 h when gavaged with 6.6 µg/g body weight and within 24–48 h when gavaged with 1.7 µg/g body weight microcystin-LR.

Because juvenile suckers consume benthic material during feeding (Markle and Clauson, 2006), they may be exposed to *Microcystis* cells deposited in the sediment. Alternatively, they may be exposed to the toxin through the food chain. Peak concentrations can be greater than 10 µg/L in the water column, but vary substantially among years. The highest microcystin concentrations generally occur in colonies of cells (> 63 µm), rather than in single independent cells (1.5–63 µm) or the dissolved (extra-cellular) part of water samples from Upper Klamath Lake, although concentrations also may be elevated in the dissolved fraction. Microcystin concentrations in the sediment commonly are as high as 38.2 µg/g dry weight (Eldridge and others, 2013). The hypothesis that suckers are exposed to microcystin through the food chain is supported by positive immunohistochemical staining for microcystin toxin (MCLR) in digesta and intestinal epithelium of juvenile sentinel suckers held in cages or net pens in Upper Klamath Lake during the summers of 2011, 2012, and 2013 (Foott and others, 2014). Apparent suppression of protein phosphatase 2A (PP2A), an enzyme responsive to MCLR, observed in a small sample of age-0 Lost River suckers caged in Upper Klamath Lake during the summer 2013, also is consistent with microcystin exposure (Foott and others, 2014). However, liver damage observed in caged fish has been minor, and positive immunohistochemical staining was not associated with focal necrosis in hepatocytes of sentinel age-0 Lost River suckers (Foott and others, 2013). Therefore, microcystin concentrations may not be high enough, last long enough, be metabolized fast enough, or be composed of large enough portions of the most toxic variants to be a major factor in juvenile sucker mortality.

Juvenile suckers in Upper Klamath Lake are host to a number of parasites, but it is unclear if any of these parasites substantially contribute to juvenile sucker mortality. Because of low prevalence (for example, *Epistylus* sp. and *Diplostomum* sp.) or lack of pathological response related to parasite infestation (for example, *Trichodina ciliatesis*, *Myxobolus* sp*., Parvicapsula* sp.), most of the identified parasites are likely to be benign (Foott and Stone, 2005; Simon and others, 2013; Kent and others, 2014). Notably, *Parvicapsula minibicornis* is known to have deleterious effects on the survival of juvenile salmonids (Kent and others, 1997), but no pathological effects were associated with the presence of a *Parvicapsula* species in suckers from Upper Klamath Lake (Foott and Stone, 2005; Kent and others, 2014). A digenean (trematode) metacercaria identified by Kent and others (2014) as *Bolbophorus* sp. can be prevalent in Upper Klamath Lake suckers and when present is

associated with focal, mild to severe inflammation (Burdick, Elliott and others, 2015). A trichodinid gill parasite was observed on 25 percent of age-0 suckers sampled from Upper Klamath Lake in 2014, but was not associated with gill tissue inflammation, necrosis, hyperplasia, or hypertrophy (Burdick, Elliott and others, 2015). The gill parasite *Ichthyobodo* sp. is occasionally reported on suckers collected from Upper Klamath Lake (Banner and Stocking, 2007). *Ichthyobodo* sp. heavily infested the gills of 14 moribund age-1 suckers held in a mesocosm in Upper Klamath Lake in 2014 and were thought to be associated with the imminent mortality of these fish (Hereford and others, 2016). The nematode *Contracaecum* sp. infected the heart of 6.4 percent of 110 age-0 suckers inspected in 2015 (Kent and others, 2017). When present, *Contracaecum* sp. is associated with granulomatous inflammation and coagulative necrosis in the heart (Burdick, Elliott and others, 2015). More importantly, a single larval *Contracaecum* sp. can fill the heart of a sucker and therefore infections by this parasite are considered to be lethal (Kent and others, 2017).

Numerous facultative bacterial pathogens have been isolated from asymptomatic juvenile suckers (Foott and others, 2013). Among the bacterial genera detected by molecular methods in juvenile sucker mucus were genera such as *Aeromonas*, *Pseudomonas*, *Vibrio*, and *Flavobacterium*, which include fish pathogenic species (Burdick, Ottinger and others, 2009). The myxosporean parasite *Myxobolus* sp. also was identified in the kidneys of about 15 percent of juvenile suckers held as sentinels in Upper Klamath Lake, but was not associated with inflammation or other signs of disease or distress (Foott and others, 2012).

Methods

Water-Quality Monitoring

Water quality was monitored at U.S. Geological Survey (USGS) Fish Banks West site No. 422820122032100. This site was located at the approximate mid-point of locations where suckers were captured, along a vegetated shoreline in the northern part of Upper Klamath Lake (fig. 1). Dissolved-oxygen concentrations, pH, and water temperature were recorded hourly using YSI model 600XLM data Sondes positioned horizontally. In 2014, one water-quality meter was placed 0.3 m above the substrate (Lower) and one was placed 0.3 m below the water surface (Upper). In 2015, a single water-quality meter was placed at mid-water column depth (Middle). The water-quality meters recorded data hourly from July 14 to September 9, 2014, and from July 9 to September 20, 2015. The performance of water-quality monitors was checked during weekly site visits through comparison with freshly calibrated reference monitors. During weekly visits, water-quality monitors were either cleaned or replaced with clean and calibrated monitors. Monitors were replaced with freshly calibrated monitors at least every 3 weeks.

Integrated water column samples were collected for microcystin analysis by lowering two 2-L vented HDPE bottles at a constant rate from the surface to 0.5 m from the bottom. Each sample was mixed in a churn splitter and then divided into a primary and a quality-control sample. These samples were transferred to 500 mL amber glass bottles and stored on ice. To ensure that sampling equipment was free from microcystin prior to sampling, blank samples were collected on 3 days each year. Blank samples were the first samples collected on a given day, and were collected by pouring inorganic blank water into the 2-L vented sample bottles, transferring it to the churn splitter, and then transferring the sample to sample bottles identical to bottles used for environmental samples. To assess the variability owing to mixing and analysis, the churn mixed sample was split into two samples for identical analysis every other week on each lake. To assess environmental variability in sample dates and locations, replicate samples were collected by repeating the entire sampling process on alternate weeks from split samples in each lake.

Water samples were filtered within 24 h through a 63-µm-mesh sieve to separate the large particulates from the rest of the sample. Samples were then frozen until they could be analyzed. The fraction of the sample retained on the sieve was considered the particulate fraction, and the filtrate from the sieving process was considered the dissolved fraction. The particulates were concentrated and re-suspended in tap water after filtering. The total volume of the field sample and the volume of tap water used to concentrate and re-suspend the particulates were recorded and later used to calculate the concentration per sample volume. Both particulate and dissolved fractions were processed through three freeze-thaw cycles prior to analysis to lyse whole *M. aeruginosa* cells and release toxin into water.

Before analysis, the dissolved and large particulate fractions of samples were filtered through either a 0.45-µm or a 0.30-µm filter. We used 0.30-µm filters in 2014, but we were unable to obtain the same filters in 2015 and switched to 0.45-µm filters. To confirm that the filter size was not biasing results, we ran three samples from August 6, August 20, and September 15 2014 with both filter types. Samples filtered through 0.45-µm-mesh size were filtered using a 0.45-µm UniPrep™ syringeless glass microfiber filter (Whatman, Inc., Clifton, New Jersey). Samples filtered through 0.3-µm mesh size were filtered using 25-mm 0.3-µm pre-fired glass filters (Advantec®) and a glass filter holder, glass filter flask, and vacuum hand pump. The filtrate was analyzed and the fraction remaining on the filter was discarded.

Particulate and dissolved fractions were diluted, when necessary, to bring the sample within the detection range of the analysis. Particulate and dissolved fractions were analyzed for unbound microcystin using enzyme-linked immunosorbent assays (ELISA, kit PN 520011, Abraxis LLC, Warminster, Pennsylvania). Sample absorbances were measured at 450 nm, and calibration standards were analyzed with the samples for regression analysis of the mean absorbance of the standards. Microcystin concentrations were calculated using the regression from the calibration standards. Concentrated particulate results were multiplied by the concentration factor (volume of re-suspended particulates ÷ volume of field sample) to determine the final microcystin concentration for the particulate samples.

Water samples for ammonia were collected at the mid-water column depth using a peristaltic pump and hose with a 0.45-µm capsule filter. Filtered samples were directly deposited into 125-mL HDPE bottles and stored on ice. To ensure that sampling equipment was free from ammonia contamination, blank samples were collected prior to each environmental sample. To assess variability in the analytical procedure, two sequential samples were collected without changing the pump filter at each site every other week (split samples). To assess environmental and procedural variability within sites and sample dates, replicate samples using new filters were collected on alternating weeks from split samples. Samples were refrigerated at 4 °C for as many as 2 days prior to being transferred on ice to the Sprague River Water Quality Laboratory for analysis. Upon arrival, samples were refrigerated at 4 °C for as many as 10 days before being analyzed for ammonia as nitrogen (N) according to U.S. Environmental Protection Agency (1979a) protocols. The pH and temperature data recorded during sample collection were used to calculate the amount of un-ionized ammonia (NH_3) in each sample (U.S. Environmental Protection Agency, 1979b). Total and un-ionized ammonia in samples were examined relative to the lowest level in the literature shown to cause a physiological response (0.20 mg/L; Lease and others, 2003).

Fish Capture and Processing

Fish were captured using trap nets from the northwestern shore of Upper Klamath Lake (fig. 1). Sampling occurred from July 14 to October 22, 2014, and July 14 to September 17, 2015. Eight to twelve sample locations were randomly selected each week from 30 sites that were predetermined to be accessible under most water levels. Fish were collected in trap nets with rectangular mouth dimensions of 0.609 × 0.914 m, a 10-m lead, and three internal fykes. These nets were green with 6.4-mm mesh nylon netting. Nets were set between approximately 0800 h and 1300 h each day and pulled the next day between approximately 0800 h and 1700 h for a target fishing time of 20 h.

Juvenile suckers were sacrificed with an overdose of tricaine methanesulfonate (MS222; Western Chemical, Ferndale, California) to determine whole-body triglyceride content and to look for histological evidence of disease and pathogens. One of every three sacrificed suckers were wrapped in dry paper, placed in clear plastic bags, and immediately placed on dry ice. These suckers were transferred to a -80 °C freezer upon returning to the laboratory and were later analyzed for triglyceride content. One of three sacrificed suckers in 2014 and two of three sacrificed suckers in 2015 were slit open ventrally, examined for signs of physical health as described below (Field Necropsies), and placed in 10 percent phosphate buffered formalin fixative for histological examination. Digestive tracts were snipped in several places prior to preservation to facilitate fixative penetration. Suckers were transferred from formalin to 70 percent ethanol after 72–96 h. All suckers were weighed to the nearest 0.1 g after returning to the laboratory.

To separate out any suckers that were not young-of-the-year, complete left-leading pectoral fin rays were removed from all captured juvenile suckers by cutting as close to the fin base as possible prior to fixation or freezing. Fin rays were dried and mounted in epoxy. Sections were taken from the proximal end of the fin ray and read under transmitted light at 5–10 times magnification. Two technicians with a minimum of 3 years of experience each counted the number of annuli on each sectioned structure. Technicians first examined structures independently with no knowledge of the other's age estimate so that aging bias among readers could be examined. When there was a discrepancy in the number of annuli, technicians examined fin ray sections together and came to a consensus about the estimated age of each fish. Structures from fish that were estimated to be older or younger than seemed reasonable based on their length were examined an additional time by both readers. Only data for suckers that had no annuli (age-0 suckers) were examined for this report.

Field Necropsies

Field necropsies were performed to rapidly assess health and condition. External examinations were conducted for all suckers, and internal organ examination was conducted on one of every three suckers collected in 2014 and two of every three suckers collected in 2015. Internal examinations were not conducted on suckers collected for triglyceride analysis. We classified organ condition following a modified version of methods described by Adams and others (1993). Eyes were examined for exophthalmia, hemorrhages, and blindness. Gills were examined for frayed ends, clubbed ends, discoloration on all or part of the gill filaments and lesions. The presence and location of external hemorrhages were documented. External parasites visible without magnification were counted, and the location on the body of the fish was noted. Opercula shortening or malformation was noted as occurring on both sides, left side, right side, or neither side. Other deformities also were noted. After external examination was complete, the somatic cavity was carefully opened along the ventral line for internal examination. The amount of visceral fat was qualitatively assessed as high, low, or absent. Liver color was classified as normal (dark red) or off-color (pale, pink, or tan), and

liver texture was noted as smooth, grainy, or lumpy. Livers described as normal were dark red or pink and had a similar appearance to those observed for age-1 hatchery-reared Lost River suckers.

We used logistic regression to compare the frequency of occurrence of external abnormalities and conditions observed during field necropsies among taxa and between years. In these models, the response variable was the presence or absence of a particular affliction or condition and the explanatory variables were categorically assigned taxa and years. We applied single variable models, additive year and taxa models and interactive year and taxa models to each affliction or condition examined. We applied Akaike information criterion (AICc), which ranks models based on parsimony and accounts for sample size, to each model set. Models having the lowest AICc were considered the best explanation of the data (Burnham and Anderson, 2002). The most parsimonious model for each affliction analysis was examined to determine significant differences at the $\alpha = 0.05$ level. Power analysis indicated that sample sizes of dissected fish restricted power to less than about 0.3 for an $\alpha = 0.05$, given moderate differences in proportions among groups. Therefore, the prevalence of conditions observed during dissection were descriptively rather than quantitatively compared.

Species Identification

To identify juveniles as shortnose suckers or Lost River suckers and apparent shortnose-Lost River hybrids, we applied genetic identification methods described by Hoy and Ostberg (2015). Another sucker species in the study area, Klamath largescale suckers (*Catostomus snyderi*), are indistinguishable from shortnose suckers based on non-lethal methods including genetics. Therefore, the suckers we call shortnose could be either Klamath largescale or shortnose suckers. Caudal fin tissue was collected and dried. Deoxyribonucleic acid (DNA) was extracted from the caudal tissues using DNeasy kits (Qiagen, Inc.). A total of 18 nuclear DNA TaqMan® assays that differentiate the species based on single nucleotide polymorphisms (SNPs) was used (Hoy and Ostberg, 2015). A mitochondrial DNA (mtDNA) TaqMan® assay also was applied to identify the maternal lineage (Lost River or shortnose sucker) for each individual (Hoy and Ostberg, 2015).

We used the program STRUCTURE, version 2.3 (Pritchard and others, 2000; Evanno and others, 2005), to probabilistically assign individual multilocus genotypes to the Lost River sucker and shortnose sucker based on the posterior distribution of the program output. STRUCTURE uses a Markov chain Monte Carlo (MCMC) simulation approach to identify the posterior probability (q) for the proportion of an individual genotype derived from each of K population clusters. We applied the admixture model with independent allele frequencies, given the high differentiation between Lost River and shortnose suckers. A total of 10 repetitions were run in STRUCTURE, and the model parameters were as follows: (1) markers assumed to be unlinked; (2) 361 individuals; (3) 18 nuclear loci; (4) 2 populations assumed; and (5) 50,000 burn-in steps, followed by 100,000 MCMC iterations. We followed the procedure of Evanno and others (2005) to estimate the most probable number of K population clusters. The most probable number of population clusters was $K = 2$ (that is, Lost River and shortnose suckers). Therefore, admixture proportions between Lost River and shortnose suckers were estimated for each individual using the mean posterior probability over the 10 repetitions. When an assignment to a sucker taxa was required for describing histology or whole body triglyceride content, we call suckers with a probability of assignment as a Lost River sucker less than or equal to 0.05 a shortnose sucker and with a probability of assignment as a Lost River sucker greater than or equal to 0.95 a Lost River sucker. Suckers with intermediate probabilities of species assignment were called "Intermediate Prob[LRS]". To simplify the description of these groupings, we refer to these as separate taxa.

Triglyceride Analysis and Condition Assessment

Age-0 suckers captured from Upper Klamath Lake from Fish Banks in 2014 and 2015 were assayed for whole body triglyceride content at the California-Nevada Fish Health Center in Anderson, California, using a modification of methods described by Weber and others (2003) (table 1). Water was added to fish tissue at a ratio of 1 mL per gram of fish to enable homogenization. Isopropanol was added to an aliquot of homogenate in the ratio of 4 mL per gram of fish. Samples were processed for 20 minutes at room temperature in a revolving sample mixer before being centrifuged at 3,220 times gravity for 5 minutes. Three replicate 10-µL samples of 10 times diluted supernatant were added to microplate wells and reacted with a triglyceride reagent (triglyceride GPO kit, Pointe Scientific, Inc.™, Canton, Michigan). The triglyceride reagent produces red quinoneimine dye in concentrations directly proportional to triglyceride concentrations. The optical density was measured in a colorimetric spectrometer and the milligrams of triglycerides per gram of fish were calculated.

We calculated Fulton's K as another way to compare overall condition of suckers among sucker taxa and years (table 2). Condition was calculated for frozen fish only as Fulton's K ([weight/standard length^3]*100,000; Neumann and others, 2012). We first compared condition factor to whole-body triglyceride content to determine if the two metrics were measuring the same or different aspects of condition. We compared whole body triglyceride to Fulton's K using linear regression in program R version 2.15.2 (R Core Team, 2012). Fulton's K was considered the dependent variable, and whole-body triglyceride content was the independent variable. For this analysis, we checked for normality in the distribution of Fulton's K and whole body triglyceride content. We then used a log transformation to better meet the criteria of having a normal distribution. We considered relationships significant at the $\alpha = 0.05$ level.

Table 1. Number of age-0 suckers listed by taxa and month collected that were analyzed for triglyceride content in Upper Klamath Lake, Oregon, 2014–2015.

	2014			2015		
	Lost River	**Intermediate Prob[LRS]**	**Shortnose**	**Lost River**	**Intermediate Prob [LRS]**	**Shortnose**
July	20	19	6	19	6	5
August	11	13	21	3	8	29
September	1	2	4	3	3	10

Table 2. Number of frozen age-0 suckers identified as Lost River or shortnose suckers for which condition factor (Fulton's K) was calculated, Upper Klamath Lake, Oregon, 2014–2015.

	2014			2015		
	Lost River	**Intermediate Prob[LRS]**	**Shortnose**	**Lost River**	**Intermediate Prob[LRS]**	**Shortnose**
July	28	30	11	19	6	5
August	22	25	29	3	8	29
September	1	2	5	3	3	10

To examine differences in whole-body triglyceride content and in Fulton's *K* among taxonomic groups and between years, we fit a series of five ANOVA models in program R using the glm function. In this analysis, whole-body triglyceride content was the response variable, and year, taxa, or both were categorically defined explanatory variables. One model included an interaction between year and taxa. We also fit a no-effect (dot) model to test the null hypothesis. We log transformed whole-body triglyceride data to help meet the assumption of normality required for this analysis. We assumed homogeneity of variance in whole-body triglycerides and Fulton's *K* among taxa and years. To test this assumption, we conducted Bartlett tests in the base package of program R using the bartlett.tests function (R Core Team, 2012). Models were compared using AICc. We conducted post-hoc comparisons among groups to determine where significant differences were using Tukey's honestly significant differences test applied using function TukeyHSD in program R base package.

To determine if differences in whole-body triglyceride content of Lost River suckers between years was primarily an artifact of the timing of sample collection we conducted a post-hoc analysis. In this analysis we compared whole-body triglyceride content of Lost River suckers collected in July between years with a t test. Prior to conducting a *t* test, we first used a power analysis to determine the probability of detecting differences among years when data were restricted to July, August, or September.

Histopathology

Tissue Preparation and Staining for Histopathology

Fish sacrificed for necropsy and histological examination were preserved in Carson's modified Millonig phosphate-buffered formalin (table 3; Carson and others, 1973). Tissues sampled included the first and second gill arches, heart, anterior kidney, posterior kidney, liver, spleen, pancreatic tissue (including interpancreatic connective tissue), intestinal tract, skin, and skeletal muscle. Observations and photographs of external abnormalities and parasites were made at the time of collection, and these areas were also sampled for histological examination. Additional tissues included the snout, mouth, head, eyes, opercles, fins, body, and peduncle. All tissues were dehydrated through a graded ethanol series and xylene substitute, followed by infiltration with paraffin. Tissues were sectioned at 5 µm and stained with hematoxylin and eosin (H&E) to examine the degree of histological response. Sections of areas where abnormalities or parasites occurred were also stained with May-Grünwald giemsa (M-GG) to detect bacteria.

Table 3. Numbers of suckers sacrificed for necropsy and histological examination, Upper Klamath Lake, Oregon, 2014–2015.

Sucker taxa	2014	2015
Lost River sucker	12	35
Shortnose sucker	5	32
Sucker with intermediate Prob [LRS]	13	20
Total	30	87

As a possible indicator of nutritional status of the fish, liver energy storage was investigated by examination of the presence and appearance of vacuoles and staining characteristics of hepatocyte cytoplasm. Liver tissue sections were stained with periodic acid-Schiff (PAS) and PAS-diastase (Carson, 1997) to determine the presence of glycogen stored in the cytoplasm of hepatocytes. Levels of glycogen were scored as none, focal (low levels), or diffuse (high levels). The presence of cytoplasmic vacuoles morphologically consistent with lipid storage in hepatocytes was also rated as "none," containing occasional presumptive lipid vacuoles and flocculent cytoplasm (low levels) or consisting almost entirely of round, well-defined vacuoles (high levels).

Tissues were examined by light microscopy with a Zeiss Axiophot photomicroscope, and the degree of tissue response including inflammation, fibrosis, and necrosis per field at 200× magnification was recorded and scored using a four-point scale. The severity of histological changes was scored as none to minimal, mild, moderate, or severe; distribution was scored as none, focal, multifocal, or diffuse. The location and identification of parasites and the degree of host response to parasites were also recorded. We summarize data in 1-week time periods in order to most effectively relate histological observations with data collected on water quality.

Results

Continuous Water-Quality Monitoring Data

Periods of moderately low DO concentrations (< 4 mg/L) occurred later in the sampling season and were more frequent in the Middle water column in 2015, than in the Lower water column in 2014 (fig. 2). The duration of moderately low DO events and the frequency of more extreme low DO events (< 2 mg/L) were similar between 2014 and 2015. Periods of low DO concentrations also were more frequent and lasted longer in the Lower water column than in the upper water column in 2014. Dissolved oxygen was less than 4 mg/L on 8 different occasions in the Lower water column in 2014 and on 34 occasions in the Middle water column in 2015. Periods of DO concentrations less than 4 mg/L lasting 10 or more hours occurred 4 times in the Lower water column in 2014 and 5 times at the Middle water column in 2015. Periods of low DO concentrations lasting at least 10 hours occurred from July 13 to August 13 in 2014 and from September 1 to September 20 in 2015. There were four periods in 2014 at the Lower water column where DO concentrations were measured at less than 2 mg/L, lasting from 1 to 9 hours each. During these periods, DO concentrations were more than 4 mg/L greater in the Upper water column than in the Lower water column for most of each episode. There were seven events in 2015 at the Middle water-column where DO concentrations were measured at less than 2 mg/L, lasting from 1 to 10 hours. Three of the longest periods of time in 2015 when DO concentrations less than 2 mg/L occurred in the Middle water column from September 11 to September 13 and lasted 10, 7, and 6 hours each. DO concentrations decrease to less than or equal to 1.0 mg/L on July 15–16 and August 11, 2014, and September 12–13, 2015.

Figure 2. Graphs showing dissolved-oxygen concentrations at the Fish Banks West site in Upper Klamath Lake, Oregon (U.S. Geological Survey site No. 422820122032100) (https://waterdata.usgs.gov/nwis/uv?site_no=422820122032100). In 2014, water-quality measurements were collected 0.3 meter below the water surface (Upper water column) and 0.3 meter from the substrate (Lower water column). In 2015, water-quality measurements were collected in the middle of the water column (Middle water column). Site locations are shown in figure 1. The dates that suckers were sampled for histology (preserved in formalin; dots), condition analysis (preserved by freezing; triangle), or both are shown along the x axis. Each dot indicates a day on which samples were collected.

pH was higher and high for longer periods in the Middle water column in 2015 than at either depth in 2014 (fig. 3). Maximum pH recorded in both Lower and Upper water columns in 2014 was 9.7 and it reached 9.9 in the Middle water column in 2015. The percentage of hours in which the recorded pH was greater than or equal to 9.5 was 2.5 percent in the Lower water column in 2014, 5 percent in the Upper water column in 2014 and 31 percent in the Middle water column in 2015. The maximum number of consecutive hours that pH was greater than or equal to 9.5 was 7 h in the Lower water column in 2014, 15 h in the Upper water column in 2014 and 126 h (August 11–August 16) in the Middle water column in 2015. There were three periods in 2014 when pH reached greater than or equal to 9.5 on several consecutive days; July 10–14, August 28–31, and September 1–5. In 2015, pH ≥ 9.5 was recorded for no fewer than 2 h a day from July 11 to July 31, and from August 5 to August 16, but pH never exceeded 9.5 after August 26, 2015. The diel variation in pH exceeded 1.4 pH units frequently after September 5, 2015; whereas, diel variation this extreme only occurred on one day in 2014 (August 20) in the Upper water column. During high pH events, water temperatures ranged from 16.5 to 23.19 in 2014 and 18.7 to 22.3 in 2015.

Water temperature averaged about 1 °C warmer in 2014 than in 2015, with greater differences between years occurring in July and September (fig. 4). Mean (± SD) July water temperature was warmest in the Upper water column in 2014 (24.22 ± 2.35 °C), intermediate in the Lower water column in 2014 (23.54 ± 2.06 °C), and coolest at the Middle water column in 2015 (22.16 ± 1.34 °C). Mean September water temperatures were similar between Upper and Lower water columns in 2014 (18.46 ± 1.75 °C). Mean September water temperatures were cooler in the Middle water column in 2015 (16.67 ± 2.61 °C) than either Lower or Upper water column in 2014.

The most extreme values for each of the water-quality parameters did not always coincide with extreme values for other parameters. When DO concentrations were low (<4 mg/L) mean (± SD) pH was moderate (7.75 ± 0.65). When pH was relatively high (≥ 9.5) mean (± SD) DO concentrations were also relatively high (11.53 ± 2.25 mg/L). There was a wide range of water temperatures during periods of low DO concentrations (11.6–25.3 °C) and periods of relatively high pH (16.5–23.3 °C). The most extreme combination of high pH (≥9.5) and warm water temperatures (≥28 °C) occurred from July 10 to July 14, 2014 only in the Upper water column.
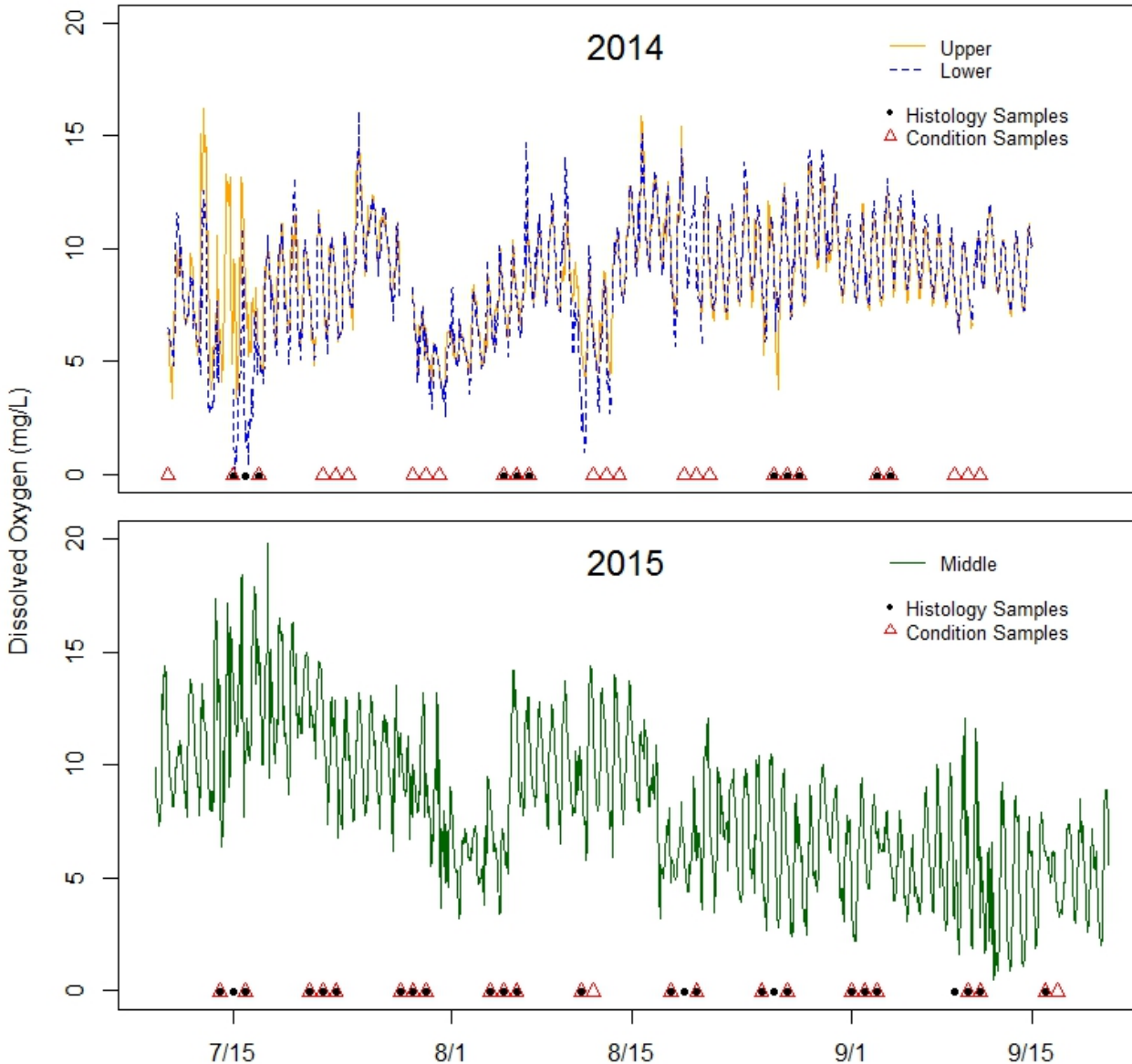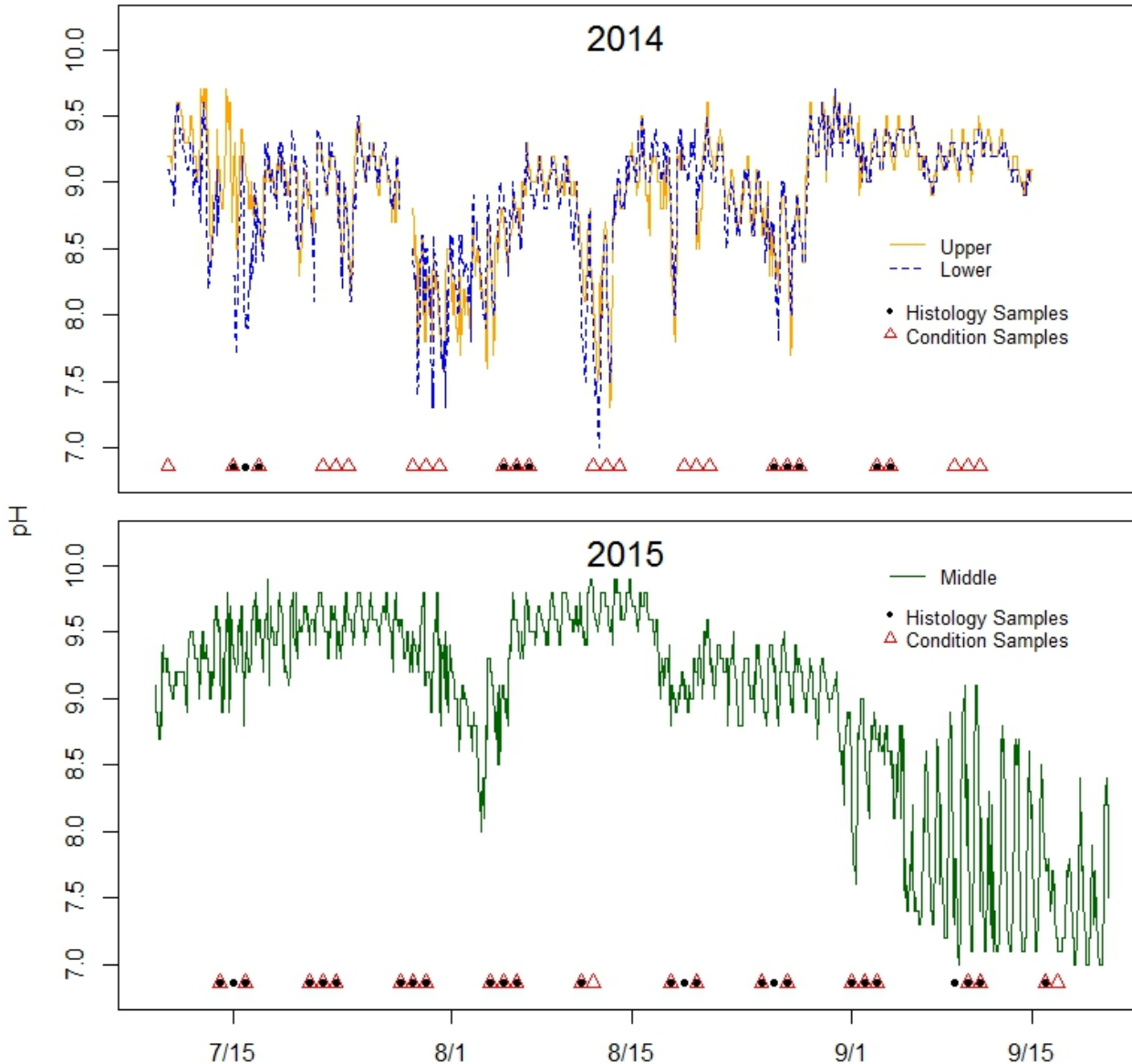
Figure 3. Graphs showing pH at the Fish Banks West site in Upper Klamath Lake, Oregon (U.S. Geological Survey site No. 422820122032100) (https://waterdata.usgs.gov/nwis/uv?site_no=422820122032100). In 2014, water-quality measurements were collected 0.3 meter below the water surface (Upper water column) and 0.3 meter from the substrate (Lower water column). In 2015, water-quality measurements were collected in the middle of the water column (Middle water column). Site locations are shown in figure 1. The dates that suckers were sampled for histology (preserved in formalin; dots), condition analysis (preserved by freezing; triangles), or both are shown along the x axis.

Figure 4. Graphs showing water temperature at the Fish Banks West site in Upper Klamath Lake, Oregon (U.S. Geological Survey site No. 422820122032100) (https://waterdata.usgs.gov/nwis/uv?site_no=422820122032100). In 2014, water-quality measurements were collected 0.3 meter below the water surface (Upper water column) and 0.3 meter from the substrate (Lower water column). In 2015, water-quality measurements were collected in the middle of the water column (Middle water column). Site locations are shown in figure 1. The dates that suckers were sampled for histology (preserved in formalin; dots), condition analysis (preserved by freezing; triangles), or both are shown along the x axis.

Ammonia

Quality-control samples indicated that total ammonia measurements were precise but occasionally biased slightly high. Total ammonia concentrations varied between split and replicate samples by 0.016 mg/L or less in 2014 and 0.012 mg/L or less in 2015, indicating that variation in the data owing to sampling or analytical procedures was minor. In 2014, total ammonia exceeded the minimum reporting limit by no more than 0.002 mg/L in 52 percent of blank samples and was lower in all other blank samples. The capsule filters were determined to be the source of this minor contamination, and the problem was corrected in the last week of sampling in 2014 by flushing the filters for a few additional minutes prior to sample collection. Total ammonia exceeded the minimum reporting limit by 0.001 mg/L in a blank sample collected July 29, 2015, for which there is no explanation. Finally, total ammonia concentration in a blank sample collected September 16, 2015, exceeded the minimum reporting limit by 0.005 mg/L when thick forest fire smoke was present. In all cases, the potential error introduced from ammonia contamination of samples was very low and unlikely to bias the measured concentrations by an amount that would have changed our conclusions about the effects to suckers.

Total and un-ionized ammonia concentrations measured at Fish Banks in Upper Klamath Lake in 2014 and 2015 were low relative to the minimum threshold for un-ionized ammonia (0.20 mg/L) as established based on the literature for eliciting a physiological response in suckers (Lease and others, 2003). Total ammonia concentrations only exceeded the minimum reporting limit (0.012 mg/L) in weekly samples collected from July 16 to July 30 and on September 10, 2014. The highest total ammonia concentration detected in 2014 was 0.11 mg/L in a sample collected on July 16. Given the concurrently measured pH (8.87) and water temperature (23.38 °C), 27.3 percent of the total ammonia in this sample was in the more toxic un-ionized form. In 2015, concentrations of total ammonia as N were no more than 0.015 mg/L in samples collected from July 13 to August 12. From August 19 to September 19, total ammonia as N ranged from 0.24 mg/L on August 19 to 0.027 mg/L on September 10. The corresponding concentrations of un-ionized ammonia (NH_3-N), given the pH and water temperature measured during sample collection, were less than or equal to 0.094 mg/L.

Microcystin Concentrations

Quality-control samples indicated that microcystin measurements were unbiased by contamination and mixing procedures, and localized heterogeneity in concentrations was mostly minor (fig. 5). Microcystin concentrations were less than the minimum reporting limit of 0.10 ppb in all blank samples from both years. Microcystin concentrations varied between split samples in the small sample fraction by no more than 0.63 ppb in either year. The variation in concentrations in the large particulate fraction among split samples was as much as 0.79 ppb in 2014 and 5.9 ppb in 2015, due to varying degrees of cell-clustering among samples. Concentrations in large particulate fraction varied among environmental replicates by no more than 3.9 ppb in 2014 and no more than 2.18 in all but one sample pair in 2015. The greatest difference between concentrations in the particulate fraction between two replicates was a 16.5 percent difference when measured concentrations were relatively high on September 10, 2015.
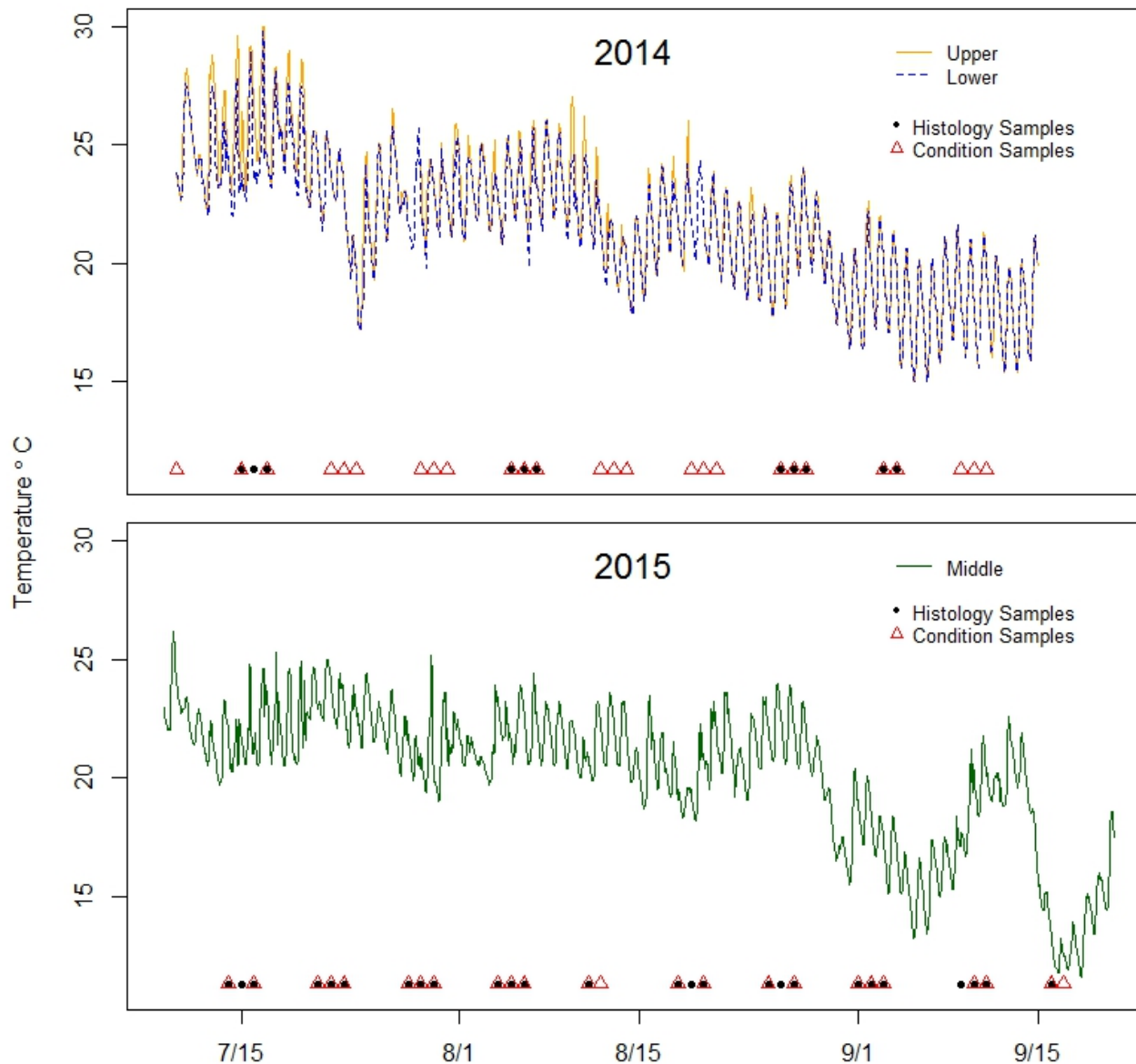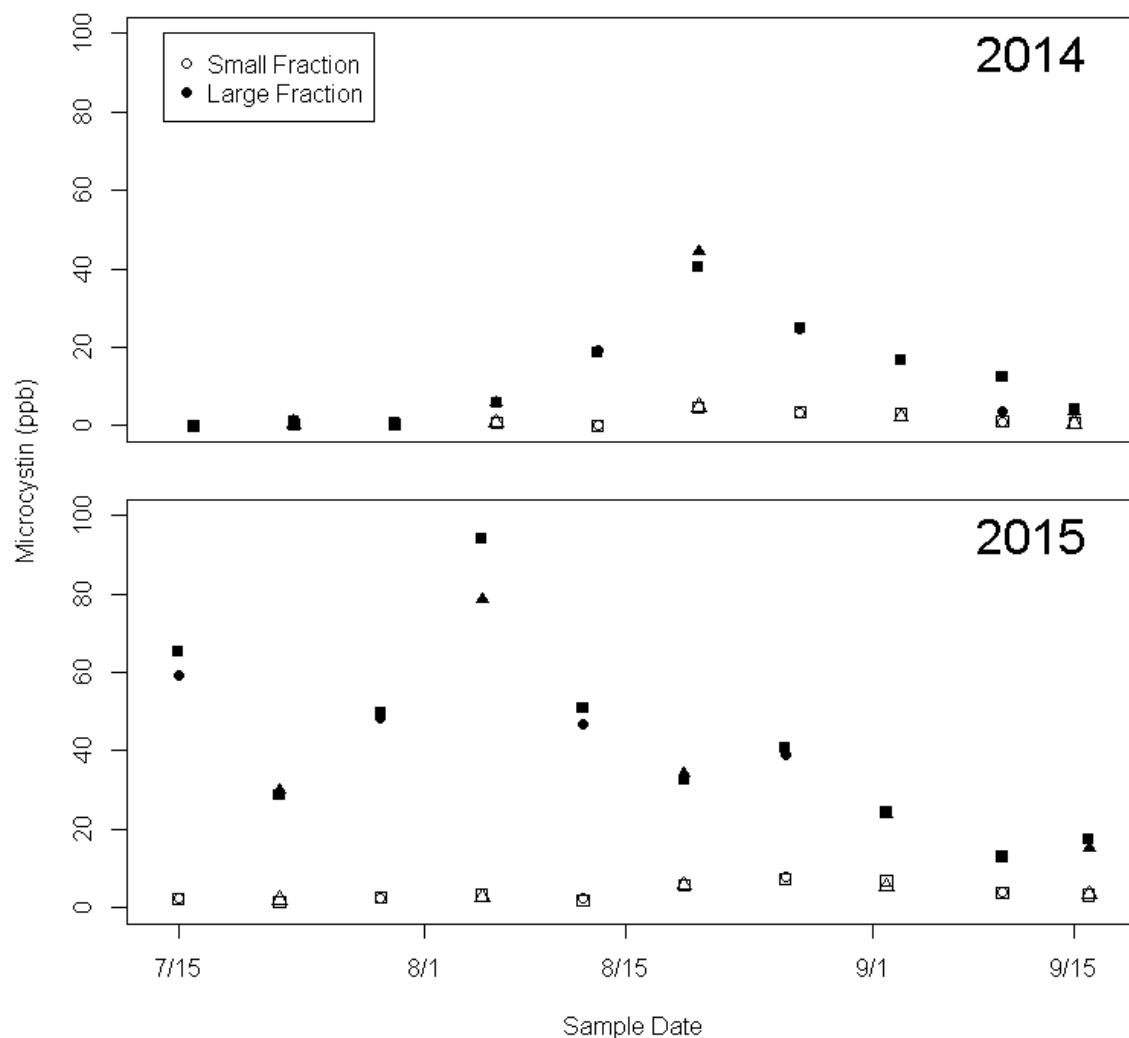
Figure 5. Graphs showing concentrations of microcystin at the Fish Banks (U.S. Geological Survey site No. 422820122032100) (https://waterdata.usgs.gov/nwis/uv?site_no=422820122032100) site in Upper Klamath Lake, Oregon, 2015. Large (≥ 63 micrometers) and small fractions (< 63 micrometers) of the same water sample are shown. Squares indicate the primary sample concentration. Circles indicate that quality-assurance samples were conducted as splits and triangles indicate environmental replicates.

Mircocystin concentrations in the large particulate fraction of water samples were lower and peaked later in 2014 than in 2015 (fig. 5). Microcystin concentrations in the large particulate fraction were less than 1 ppb from July 16 to July 30, 2014, and increased to 8.5 or less on August 6. Microcystin concentrations in the large particulate fraction then increased to at least 16.62 ppb from August 13 to September 3 and peaked at 44.3 ppb in the sample collected on August 20, 2014. Mircocystin concentrations in the large particulate fraction were at least 12.8 ppb on all sample dates in 2015. The highest concentrations of microcystin in the large particulate fraction in 2015 were measured on July 15 (65.3 ppb) and August 5 (78.6 ppb). Microcystin concentrations were much lower in the small fraction of water samples, peaking at 4.55 ppb on August 20, 2014 and at 8.03 ppb on August 26, 2015.

Field Examinations

Totals of 113 age-0 suckers in 2014 and 248 age-0 suckers in 2015 were examined for external condition (tables 4 and 5). Most suckers examined in 2014 (51 percent) and 2015 (76 percent) were identifiable to taxa based on our criteria. There were four main afflictions noted during external field examinations of suckers (tables 4 and 5). Petechial skin hemorrhages ($p < 0.001$) were more prevalent in 2015 than in 2014 in all sucker taxa. When petechial skin hemorrhages occurred, they most commonly covered the whole body, but were restricted to the head or caudal peduncle in about 15 percent of suckers each year. Thirty percent of skin hemorrhages observed in 2014 occurred during the week of August 5. In contrast, the hemorrhages occurred consistently throughout the season in 2015. Deformed opercula were less common in shortnose suckers in 2014 than in 2015 and more common in Lost River suckers and suckers with intermediate Prob [LRS] in 2014 than in 2015 ($p < 0.001$; tables 4 and 5). Deformed opercula were bilateral in 74 percent of affected suckers in 2014 and 22 percent in 2015. Black-spot-forming parasites (presumably the metacercarial stage of the digenean *Bolbophorus* sp.) were more prevalent in shortnose suckers and suckers with intermediate Prob [LRS] in 2014 than in 2015 ($p < 0.001$). A difference in prevalence of black-spot-forming parasites in Lost River suckers between years was not detected ($p = 0.07$). *Lernaea* sp. were less prevalent on suckers of all three taxa in 2014 than in 2015 ($p < 0.001$). As many as four *Lernaea* sp. per sucker were observed in 2014, and as many as 9 per sucker were seen in 2015.

Table 4. Percentages of suckers with afflictions by taxa, Upper Klamath Lake, Oregon, 2014.

Afflictions	Lost River sucker	Shortnose sucker	Suckers with intermediate Prob [LRS]	Total
Number examined	36	28	49	113
Petechial skin hemorrhages	28%	36%	43%	36%
Opercula deformity	19%	4%	14%	13%
Black-spot-forming parasite	11%	71%	43%	40%
Lernaea sp.	39%	4%	20%	22%

Table 5. Percentages of suckers with afflictions by taxa, Upper Klamath Lake, Oregon, 2015.

Afflictions	Lost River sucker	Shortnose sucker	Suckers with intermediate Prob [LRS]	Total
Number examined	102	91	55	248
Petechial skin hemorrhages	75%	74%	82%	76%
Opercula deformity	11%	18%	9%	13%
Black-spot-forming parasite	5%	16%	11%	10%
Lernaea sp.	81%	20%	49%	52%

Field necropsies indicated that suckers were in similar condition in both years, except for Lost River suckers, which had lower amounts of visceral fat in 2014. Pale gills were reported on two suckers from 2014, but these are suspected to be the result of bleeding during a dissection performed prior to examining gills. Gill color and condition was normal in all suckers in 2015. The amount of observable visceral fat ranged from absent to high in both years and all three taxa. Of the three taxa, shortnose suckers had the highest proportion of individuals with no visible visceral fat (table 6). There were more Lost River suckers with an absence of visceral fat in 2014 (22 percent) than in 2015 (3 percent) (table 6). The proportion of suckers lacking visceral fat was similar between years for the other two sucker taxa. Liver color was light pink to dark red in 95 percent of suckers examined in 2014 and 87 percent in 2015. Tan livers were observed in four (10 percent) suckers with intermediate prob [LRS] examined in 2014, two (6 percent) Lost River suckers examined in 2015, five (16 percent) shortnose suckers examined in 2015, and four (20 percent) suckers with intermediate prob [LRS] examined in 2015. Tan livers were observed throughout the entire sampling season in 2015. Liver texture was smooth and normal on all suckers examined in 2014, and all but two suckers examined in 2015. Two suckers with intermediate prob [LRS] had grainy pink livers. Gall bladders varied from empty to full and were pale yellow to bright green, indicating suckers were in various stages of digestion. Other conditions, each noted on less than 3 percent of suckers in both years, included deformed or damaged fins, hemorrhages in the eyes, exophthalmos of the eyes, missing eyes, scale loss, scarring on the body, deformed caudal peduncles, open wounds associated with lamprey bites, and apparent bruising on the caudal peduncle.

Table 6. Numbers and percentages of suckers in each of three taxa that had no visible visceral fat observable during dissection, Upper Klamath Lake, Oregon.

[Total numbers of suckers examined by taxa are shown in tables 4 and 5]

Year	Lost River suckers	Shortnose suckers	Suckers with intermediate Prob [LRS]
2014	6 (22%)	3 (30 %)	4 (10 %)
2015	1 (3%)	9 (26%)	2 (10%)

Triglyceride Content and Condition

There was a weak but significant relationship between Fulton's K and whole-body triglyceride content (p = 0.04, df = 181, and coefficient of determination [R^2] = 0.017), indicating that the two metrics were measuring aspects of sucker condition that were only slightly correlated. For the data used in this comparison (n = 486), Fulton's K was normally distributed around a mean (± SD) of 1.59 ± 0.26, and the log of whole-body triglyceride content was relatively normally distributed with a mean (± SD) of 2.03 ± 0.45. Triglyceride content increased on average (± SD) 0.011 ± 0.004 mg triglyceride per g of body weight for every one unit of increase in Fulton's K.

The whole-body triglyceride content in Lost River suckers was lower in 2015 than in 2014 and was lower than either shortnose or intermediate Prob[LRS] suckers in either year (Tukey's HSD, p less than or equal to 0.002). No other differences in whole-body triglyceride content were detected among taxa or years (Tukey's HSD p ≥ 0.83). The most parsimonious model fit to the whole-body triglyceride data contained a taxa-by-year interaction and had a model weight of 0.835, indicating that there was taxa-specific annual variation in age-0 sucker triglyceride content. Lost River suckers collected in 2015 had a mean (± SD) of 5.4 ± 2.5 mg of triglyceride per gram of body weight. All other suckers analyzed combined had a mean whole-body triglyceride content of 8.9 ± 3.8 mg of triglyceride per gram of body weight (fig. 6).

When data were restricted to July captured Lost River suckers, mean ± SD whole body-triglyceride content was significantly greater (p < 0.0001, df=30, n=39) in 2014 (8.3 ± 2.1) than in 2015 (5.1 ± 2.6). This indicates that the difference in whole-body triglyceride content between years detected for Lost River suckers in the previous analysis was unlikely to be solely an artifact of differential temporal distribution in sample collection dates. Power analysis indicated that the probability of detecting even large annual effect sizes (≥ 0.8) was less than 0.5 for all other same month comparisons between years and therefore other comparisons were not made.
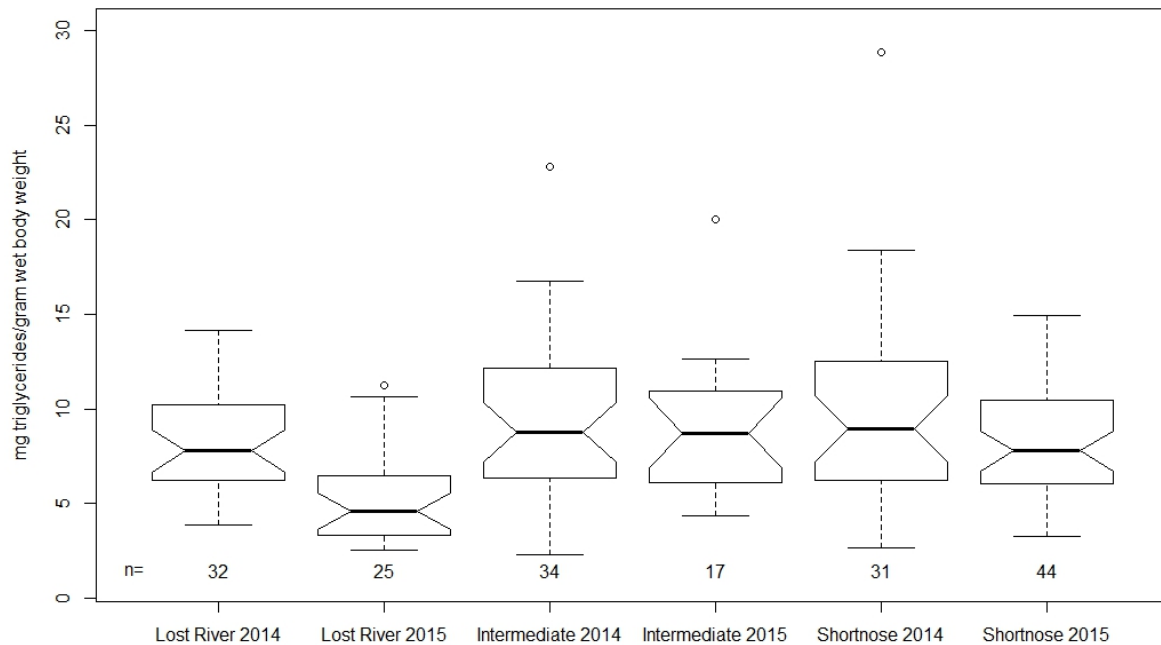
Figure 6. Graph showing whole-body triglyceride content of age-0 suckers collected from Upper Klamath Lake near Fish Banks, Oregon, 2014–2015. Suckers were defined as Lost River suckers, shortnose suckers, or suckers with a genetic probability of assignment to species intermediate of these two species. Dots indicate outliers, whiskers indicate 5th and 95th percentiles, boxes indicate the 25th and 75th percentiles, and the bold black bars indicate medians. The sample sizes (n) used to create each box are given on the x-axis. Non-overlapping notches indicate significant differences (Chambers and others, 1983).

Fulton's condition factor for age-0 suckers sampled from Upper Klamath Lake near Fish Banks varied among taxa but not years. The most parsimonious model contained an additive effect of taxa and year on Fulton's K (table 7). The second-best model, which accounted for a year effect, had a delta AIC of 0.75 indicating that this model was nearly equally parsimonious to the first model. Examination of the additive Taxa and Year model indicated that Year had an insignificant effect on Fulton's K. Tukey's HSD indicated that there were significant differences in Fulton's K between all three taxa (p less than or equal to 0.005). Fulton's K was lowest for Lost River suckers (1.41 ± 0.24), moderate for suckers with intermediate prob [LRS] (1.60 ± 0.19), and highest for shortnose suckers (1.71 ± 0.22).

Table 7. AIC model selection table for models fit to describe variation in Fulton's *K* data for age-0 Lost River and shortnose suckers captured from Upper Klamath Lake near Fish Banks, Oregon, 2014–2015.

Model	df	AICc	ΔAICc	Weight
Taxa + Year	5	-43.2	0	0.53
Taxa	4	-42.4	0.75	0.37
Taxa * Year	7	-39.9	3.26	0.10
Year	3	18.4	61.56	0.00
Dot	2	20.2	63.37	0.00

Histopathology

In tissues where histological abnormalities were observed, most changes were focal and minimal or mild except those associated with parasites. Inflammation was characterized by the presence of a variety of cell types including lymphocytes, macrophages, polymorphonuclear cells, and eosinophilic granular cells. In 2014, the presence of rodlet cells in areas of inflammation also was noted. Fibrosis and necrosis were usually focal, minimal to mild, and characterized by the presence of fibrocytes and individual cell (apoptotic) necrosis or necrotic debris, respectively. Except where associated with parasites, minimal histological changes were considered to be normal according to the criteria of Foott and others (2000).

Parasites affecting the skin and skeletal muscle of suckers were observed throughout sampling seasons in both years. Focal, mild to severe inflammation associated with *Lernaea* sp. attachment sites was observed in the skin and skeletal muscle of 7 percent (2 Lost River suckers) of fish sampled in 2014 and 32 percent of fish sampled in 2015 (table 8). Moderate to severe focal inflammation, fibrosis, and necrosis associated with an apparent *Lernaea* attachment organ were observed in the pancreas/interpancreatic tissue and adjacent spleen of a Lost River sucker sampled in 2015. Melanization surrounding encysted digenean (trematode) metacercariae was found in the skeletal muscle of two shortnose suckers collected in 2014 and 7 percent of fish (two Lost River suckers and four shortnose suckers) collected in 2015. Host response to this trematode, identified by Kent and others (2017) as *Bolbophorus* sp., including inflammation and fibrosis, was focal and mild. Examination of skin and skeletal muscle found no hemorrhaging or other lesions in fish where petechial hemorrhaging or other abnormalities were observed macroscopically. No bacteria were observed in areas associated with parasites, petechial hemorrhaging, or other abnormalities. Mild, focal granulomatous inflammation and coagulative necrosis surrounded a metacercaria located in the ventriculobulbar valve of the heart of one fish sampled in August 2014 (data not shown).

Histological changes in the gills were observed in 13 percent of suckers examined in 2014 and included inflammation, hyperplasia, and hypertrophy in interlamellar tissue. Hyperplastic and hypertrophic gill tissue was observed in 40 percent of suckers collected in late August 2014 during periods of decreasing microcystin concentrations, low un-ionized ammonia concentrations, moderate pH, and relatively high DO concentrations (table 9). In 2015, histological changes in the gill tissue were limited to hyperplasia in 3 percent of fish except for severe, diffuse inflammation, hyperplasia, and hypertrophy associated with *Ichthobodo* sp. parasite infestation in one Lost River sucker collected on August 11, 2015 (table 9).

Trichodinid parasites occurred on the gills of 40 percent of fish examined in 2014 and 20 percent of fish examined in 2015. The presence of trichodinid parasites was not associated with histological abnormalities in either year. Trichodinid parasites were observed on suckers sampled from August 5 to September 11, 2014, and during most sampling periods in 2015 (table 9). The prevalence of fish with trichodinids was greatest (38 percent) in late July through early August 2015, with a mean of 4 trichodinids observed per field at 200× magnification. In 2014, trichodinids were attached to the gills of 42 percent of Lost River suckers and 80 percent of shortnose suckers. Trichodinids were attached to the gills of 29 percent of Lost River suckers and 16 percent of shortnose suckers in 2015.

Inflammatory cell infiltration around liver blood vessels and bile ducts (perivascular and peribiliary cuffing) or foci of inflammatory cells in the hepatocellular tissue (liver parenchyma) was observed in 21 percent of suckers sampled in 2014 and 8 percent of suckers sampled in 2015 (table 10). Cuffing was observed in 17 percent of suckers sampled in 2014 and 8 percent in 2015. In fish identified to species, liver inflammation occurred in 60 percent of shortnose suckers and 9 percent of Lost River suckers in 2014 and 6 percent of shortnose suckers and 11 percent of Lost River suckers in 2015.

No hepatocyte vacuolation was observed in 48 percent of fish and 33 percent of fish sampled in 2014 and 2015, respectively (table 10). No vacuolation occurred in 55 percent of Lost River suckers and 40 percent of shortnose suckers in 2014, and 63 percent of Lost River suckers and 13 percent of shortnose suckers in 2015. Low hepatocyte vacuolation (with flocculent cytoplasm) was observed in 52 percent of fish sampled in 2014; 45 percent of Lost River suckers and 60 percent of shortnose suckers. Low hepatocyte vacuolation was also observed in 66 percent of fish sampled in 2015; 34 percent of Lost River suckers and 88 percent of shortnose suckers. Only one fish in 2015 (a Lost River sucker sampled August 5) had hepatocyte vacuolation consistent with lipid storage. All other hepatocyte vacuoles appeared to contain mostly glycogen. There were apparent changes in hepatocellular vacuolation during the sampling season during both years. In 2014, hepatocellular vacuolation was classified as absent or low in approximately equal numbers of fish during all sample periods from July 15 through September 11 with the exception of the August 26–28 sample, in which hepatocellular vacuolation was absent in one fish and low in four of the five fish sampled. In comparison, hepatocellular vacuolation was classified as absent in 45 percent, low in 53 percent, and high in less than 2 percent of suckers sampled from July 14, 2015, to August 20, 2015 (n = 64). All suckers sampled from August 25 to September 15, 2015 (n = 23) had low levels of hepatocellular vacuolation.

Overall percentages of low to high hepatocyte cytoplasmic glycogen were similar, but the timing of apparent changes in glycogen storage differed between years. Three percent of suckers sampled each year had no hepatocyte cytoplasmic glycogen. Levels of hepatocellular glycogen were low in 34 percent of suckers sampled in 2014 and 26 percent sampled in 2015. Levels of hepatocellular glycogen were high in about 62 percent of suckers sampled in 2014 and in about 70 percent of suckers sampled in 2015 (table 10). Among fish identified to species, low glycogen levels occurred in 9 percent of Lost River suckers and 80 percent of shortnose suckers in 2014, and in 43 percent of Lost River suckers and 16 percent of shortnose suckers in 2015. High glycogen levels were seen in 82 percent of Lost River suckers and 20 percent of shortnose suckers in 2014 and 49 percent of Lost River suckers and 84 percent of shortnose suckers in 2015. Glycogen levels were high in 89 percent of fish sampled in 2014 from mid-July to early August and low in 80 percent of suckers sampled from mid-August to mid-September 2014 (table 10). There appeared to be a temporary decrease in liver glycogen storage in late July to early August, 2015, followed by an increasing trend in storage as the sampling season progressed. All the fish collected during the July

14–20 period had high glycogen storage. The only sampling period in which some (30 percent) of the fish examined exhibited no hepatocellular glycogen was July 21–27, and the only period in which low hepatocellular glycogen levels were observed in the majority (75 percent) of fish examined was July 28–August 3. Samples collected during the August 4–10 sampling period were nearly equally split between low and high glycogen storage. In the samples collected between August 11 and September 15, 71–100 percent of fish had high hepatocellular glycogen levels.

Eosinophilic hyaline droplets in kidney tubule epithelial cells were observed in only one fish sampled in 2014 (a shortnose sucker sampled August 5) and in 15 percent of fish sampled in 2015 (data not shown). Hyaline deposits were present in 14 percent of Lost River suckers and 16 percent of shortnose suckers. The tissues of the heart, hematopoietic kidney, and intestinal tract of all examined fish were unremarkable.

Table 8. Histological response and host response to parasites observed microscopically in suckers sampled from Upper Klamath Lake, Oregon, 2014–2015.

[Number of fish in which a host response to parasites was observed and the parasite identification are shown in parentheses for each histological response category. n, total number of fish examined. LN, *Lernaea* sp.; BSMT, black spot metacercaria]

Sample dates	n	Pancreas/ Interpancreatic tissue	Skin and skeletal muscle		
		Inflammation	Inflammation	Fibrosis	Necrosis
2014					
July 15–17	10	0	1 (BSMT)	0	0
Aug. 5–7	10	0	3	0	0
Aug. 26–28	5	0	1 (LN)	1 (LN)	1 (LN)
Sept. 3–11	5	0	3 (1, LN)	1 (LN)	1 (LN)
2015					
July 14–20	7	0	0	0	0
July 21–27	10	0	5 (5, LN; 1, BSMT)	4 (4, LN; 1, BSMT)	0
July 28–Aug. 3	12	0	5 (5, LN)	4 (4, LN)	2 (2, LN)
Aug. 4–10	14	0	6 (6, LN)	5 (5, LN)	0
Aug. 11–17	14	[1]1 (1, LN)	5 (5, LN; 1, BSMT)	3 (3, LN; 1, BSMT)	0
Aug. 18–24	7	0	2 (2, LN)	2 (2, LN)	0
Aug. 25–31	13	0	4 (2, LN; 2, BSMT)	1 (1, LN)	0
Sept. 1–7	7	0	3 (3, LN; 1, BSMT)	2 (2, LN; 1, BSMT)	0
Sept. 8–15	3	0	0	0	0

[1]Host response to the apparent *Lernaea* attachment organ included fibrosis and necrosis that extended into the spleen.

Table 9. Number of fish positive by histopathological examination of gill samples for inflammation, hyperplasia, hypertrophy and parasites among suckers sampled from Upper Klamath Lake, Oregon, 2014–2015.

[n, total number of fish examined; <, less than]

Sample dates	n	Inflammation	Hyperplasia	Hypertrophy	Trichodinids	Trichodinid intensity (number per 200× field)
2014						
July 15–17	10	0	0	0	0	0
Aug. 5–7	10	0	0	0	5	4
Aug. 26–28	5	0	2	2	3	4
Sept. 3–11	5	[1]1	2	2	4	1
2015						
July 14–20	7	0	1	0	1	< 1
July 21–27	10	0	0	0	1	1
July 28–Aug. 3	12	0	0	0	4	6
Aug. 4–10	14	0	0	0	6	2
Aug. 11–17	14	[2]1	[2]1	[2]1	3	2
Aug. 18–24	7	0	1	0	0	0
Aug. 25–31	13	0	0	0	1	2
Sept. 1–7	7	0	0	0	0	0
Sept. 8–15	3	0	0	0	1	3

[1]Mild, focal apoptotic necrosis was also observed.

[2]Host response to numerous *Ichthyobodo* sp. parasites attached to gill filaments and lamellae.

Table 10. Number of fish positive by histopathological examination for liver inflammation, hepatocyte vacuolation, and glycogen storage among suckers sampled from Upper Klamath Lake, Oregon, 2014–2015.

[n, number of fish examined]

Sample dates	n	Inflammation		Vacuolation			Glycogen		
		Perivascular/ peribiliary	Parenchyma	None	Low	High	None	Low	High
2014									
July 15–17	10	0	0	5	5	0	0	1	9
Aug. 5–7	[1]9	2	0	5	4	0	0	1	8
Aug. 26–28	5	1	1	1	4	0	0	4	1
Sept. 3–11	5	2	2	3	2	0	1	4	0
2015									
July 14–20	7	0	0	2	5	0	0	0	7
July 21–27	10	2	0	6	4	0	3	1	6
July 28–Aug. 3	12	1	0	8	4	0	0	9	3
Aug. 4–10	14	0	0	6	7	1	0	6	8
Aug. 11–17	14	1	1	6	8	0	0	3	11
Aug. 18–24	7	0	0	1	6	0	0	2	5
Aug. 25–31	13	1	0	0	13	0	0	2	11
Sept. 1–7	7	0	0	0	7	0	0	0	7
Sept. 8–15	3	2	2	0	3	0	0	0	3

[1]One liver not sectioned.

Discussion

Suckers collected from Upper Klamath Lake did not show signs of widespread or severe disease or parasite problems despite results of previous studies that report annual lake-wide decreases in catches of age-0 suckers to near-0 by about September each year (Burdick and Martin, 2017). The lack of severely diseased fish could be explained, at least in part, by the passive gear used in our study that may be biased toward healthy fish that are active enough to swim into our nets. Although other gear types have been tried, it is difficult to obtain reasonable sample sizes of suckers using active gears. The conditions we observed in a small proportion of fish may in fact be present in a larger proportion of the population, especially if these conditions reduce the swimming activity of suckers. However, other studies that examined the health of captive suckers held in Upper Klamath Lake for 7 days (Stone and others, 2017) or several months (Hereford and others, 2016) found a low prevalence of abnormalities in livers and gills except when associated with parasites. Another explanation for the lack of diseased fish in our catches may be that the conditions that cause the highest annual mortality do not occur during our sampling period or are owing to predation. Alternatively, diseased fish could experience higher rates of predation. Despite the lack of moribund fish in our catches, we did observe a number of abnormalities in suckers by macroscopic examination, histopathology, or both, and some of these abnormalities may provide clues to causes of apparent mortality in young suckers.

Water quality measured in this study was in most cases less extreme than the water quality that suckers were exposed to in previous laboratory trials. For example, pH never reached 10.0, a level that Saiki and others (1999) showed to cause effects on sucker behavior. Low DO concentrations (less than or equal to 2 mg/L) measured in this study never lasted more than 10 h. In laboratory trials, 50 percent of juvenile shortnose suckers died after exposure to 1.14 and 1.34 mg/L of DO after 24 or 96 h, respectively, and 50 percent of juvenile Lost River suckers died after exposure to 1.58 and 1.62 mg/L of DO after 24 or 96 h, respectively (Saiki and others, 1999). DO concentrations less than 1 mg/L for less than 2 h are lethal for Lost River suckers (Stone and others, 2017) and did occur in both years for short periods of time. Although there is no literature to help guide our expectation of sucker condition after exposure to long periods (up to 126 h) of moderately high pH ($\geq$ 9.5) or moderately high pH at high water temperatures (16–23 °C), we did not observe abnormalities in suckers during these incidents at a higher rate than at other times. Abnormalities in suckers observed on the few occasions when water-quality reached extreme levels (DO $\leq$ 2 mg/L, un-ionized or total ammonia $\geq$ 0.2 mg/L) were no more frequent (if observed at all) than when water quality was less extreme.

Petechial hemorrhaging of the skin was less frequent in 2014 than in 2015. This affliction can have various causes, including infectious agents or abrasion (Ferguson, 1989). Although there was not a strong temporal pattern in the occurrence of this condition in 2014, it was somewhat more common in early August, when un-ionized ammonia concentrations were low, pH was decreasing, and microcystins in the small fraction of water samples were not detected, but water temperatures were relatively high. It was also noted throughout the sampling season in 2015, during a variety of water conditions. This affliction was rarely observed on older juvenile suckers captured in Clear Lake Reservoir using similar sampling methods, which may indicate that the causative factor for this affliction is not present, or rarely present, in Clear Lake or that older suckers are less susceptible to this condition (Burdick, Elliott and others, 2015).

Opercular deformities, which were less prevalent in shortnose suckers collected in 2014 than in 2015, are considered non-lethal for hatchery-reared fish (Beraldo and others, 2003). However, Barkstedt and others (2015) found that that the prevalence of opercular deformities on three species of wild-caught catostomids decreased with age and concluded that mortality related to the deformity was the likely cause. This deformity has not been reported for older juvenile suckers sampled in Clear Lake Reservoir, indicating that the deformity is more common on younger juvenile suckers or due to a factor not present in Clear Lake. Determining the prevalence of this deformity in suckers throughout their first year of life could provide information about the life stage at which this deformity first appears. Such information could be useful in determining if deformed opercles are associated with mortality.

Opercular deformities may lower resistance to oxygen stress and predispose fish to infections by bacteria, parasites, and fungi (Galeotti and others, 2000; Beraldo and others, 2003), or reduce predator avoidance. These deformities have numerous potential causes including low pH, inbreeding, hybridization (Winemiller and Taylor, 1982; Tringali and others, 2001), nutritional deficiency (Chávez de Martinez, 1990; Lall, 2002), heavy metals, pesticides, high egg incubation temperatures (Boglione and others, 2013) and parasites (Quist and others, 2007). The pH was neutral to high rather than low in Upper Klamath Lake during the 2 years of our study, indicating that was not the cause. Nutrition related to skeletal deformities in fish includes too much or too little dietary bioavailable phosphorus (P) relative to calcium uptake and deficiencies in vitamins C and D, phospholipids, unsaturated fatty acids, or magnesium (Lall, 2002; Cahu and others, 2003). Deformed opercula also have been noted at low rates in hatchery-reared Lost River suckers, which may indicate that the causes are genetic, temperature related, or nutritional (U.S. Geological Survey, oral commun. 2017). Additionally, opercular deformities are observed in redband trout (*Oncorhynchus mykiss newberrii*) and the federally listed threatened bull trout (*Salvelinus confluentus)* in tributaries to Upper Klamath Lake including the upper Sprague River drainage, Wood River drainage, and Threemile Creek (N. Banish, U.S. Fish and Wildlife Service, oral commun. 2015; W. Tinniswood, Oregon Fish and Wildlife Service, oral commun. 2015). However, it is not clear that the same factors that cause the deformity in these trout cause deformity in suckers.

Several parasites identified on the skin and skeletal muscle tissue of suckers in this study could potentially harm the fish. The ectoparasitic copepod *Lernaea* sp., which was more prevalent in 2014 than in 2015, can cause severe inflammatory lesions and ulceration at the attachment site, which can in turn provide portals of entry for opportunistic bacterial pathogens (Berry and others, 1991). However, inflammation caused by these parasites, although severe, was limited to the area directly around the attachment site. The host response was focal and mild to the encysted metacercariae of the black-spot-forming trematode *of Bolbophorus* sp., which was more prevalent in external macroscopic examinations of shortnose suckers and suckers with an intermediate prob [LRS] in 2014 than in 2015. The mild host response suggested that this parasite was unlikely a major cause of immediate mortality for suckers in either year, although it is unknown whether heavy infections may make fish more vulnerable to predation (Barber and others, 2000).

One internal parasite may be of concern given that it was associated with significant inflammation. It is unknown whether the presence of the single metacercaria encysted in a heart valve of a single sucker collected in 2014 could affect blood flow and cardiac function, but heavy infections of digenean metacercariae encysted in the bulbus arteriosus of some fish species have been associated with blockage of blood flow, decreased cardiac function and swimming performance, and decreased ability to survive under conditions of reduced DO concentrations (Coleman, 1993; Coleman and Travis, 1998; Hicks and Steele, 2013). Our use of histological samples for detection of these parasites was not ideal, as histopathology is not as sensitive as examination of wet tissues for

quantification of parasites (Ferguson and others, 2011). Nevertheless, histopathology is valuable for evaluating host response to parasites, and for detecting pathogens or other abnormalities that cannot be observed by gross examination (Kent and others, 2013).

Minimal to mild changes including hyperplasia, hypertrophy, inflammation, and necrosis in the gills of suckers collected in August and September 2014 could have resulted from exposure to a broad range of irritants including both infectious and noninfectious agents (Lafayette, 1975; Ferguson, 1989). However, microcystins were unlikely the cause of gill changes. For example, gill necrosis in common carp was documented after they were exposed to 1,700 µg/mL of dissolved MCLR (Carbis and others, 1996). In our study, microcystin concentrations in the small fraction of water samples that peaked at only 4.5 µg/L in 2014, were not likely high enough to cause gill tissue necrosis observed in that year. Microcystin concentrations in the small fraction of water samples were less than or equal to 2.55 ppb the day after the one fish with gill abnormalities was collected in 2015. Given the presence of *Ichthyobodo* sp. on the gills and the low ammonia we presume the former was the more likely cause of gill abnormalities.

A higher prevalence of trichodinid parasites co-occurred with a higher prevalence of gill tissue abnormalities in 2014 than in 2015, but no hyperplasia, hypertrophy, inflammation, or necrosis was observed in direct association with trichodinids. The number of trichodinids per field may have been biased slightly high because only one gill arch, instead of two, was sectioned. Trichodinids are ciliated protozoans that primarily are ectocommensals when present in low numbers and use fish as a substrate for attachment while feeding on waterborne particles and bacteria, as well as detritus and particles from the fish surface (Lom and Dyková, 1992; Bruno and others, 2006).

Elevated total or un-ionized ammonia concentrations do not explain observed gill abnormalities in suckers collected in August and September, 2014 and in one fish collected August 11, 2015. Mean gill lamellar thickness has been reported to increase in Lost River suckers exposed to un-ionized ammonia concentrations greater than or equal to 0.20 mg/L (Lease and others, 2003). Un-ionized ammonia concentrations only exceeded 0.20 mg/L on two sample dates in mid-July 2014 when hyperplasia and hypertrophy were not observed. Other histological signs of ammonia toxicity were not observed, such as increased mucosal cells and infiltration of white blood cells into lymphatic spaces (Lease and others, 2003). Even at the most extreme values measured in this study, un-ionized ammonia concentrations were not in the ranges that are known to cause most structural changes in gill tissues (Lease and others, 2003), and were much less than the 24- to 96-h mean LC_{50} for juvenile Lost River ($\geq$ 0.78 mg/L) or shortnose ($\geq$ 0.53 mg/L) suckers (Saiki and others, 1999). The total ammonia concentration on the 1 day in 2015 when a sucker with gill abnormalities was captured was less than the reporting limit.

Because of the documented effects of microcystin on fish liver tissue (Malbrouck and Kestemont, 2006), this tissue was one of the primary targets of histopathological analysis in our study. Perivascular cuffing in the liver similar to that observed in fish in our study is one of the clinical signs noted in net-pen disease of Atlantic salmon (*Salmo salar*), which is thought to be associated with microcystin exposure (Kent and others, 1996). Therefore, perivascular/peribiliary cuffing or parenchymal foci of inflammatory cells in livers, observed in 3 of 10 suckers collected in 2014 after mid-August, could be interpreted as responses to microcystin, except that this same condition was also observed in 50 to 100 percent of sucker livers sampled weekly from Clear Lake Reservoir in 2014 where no microcystin was detected (Burdick, Elliott and others, 2015). Furthermore, a lower percentage of suckers sampled from Upper Klamath Lake had perivascular/peribiliary cuffing in the liver in 2015 than 2014, when microcytin concentrations were higher. Perivascular/peribiliary cuffing represents an inflammatory/immune response that can have various causes, and generally is not considered an indicator of a specific etiology (Lang and others,

2006). Cuffing has been associated with infectious agents (Kent and Myers, 2000; Iwanowicz and others, 2006; Grésoviac and others, 2007) as well as exposure to certain toxicants (Boorman and others, 1997). Additionally, certain other signs of microcystin toxicity in liver tissue including hepatocyte shrinkage and increased nuclear/cytoplasmic ratio (Carbis and others, 1996) were not observed.

The methods we used to examine energy storage and condition resulted in only slightly correlated data, indicating they each measured different aspects of sucker condition. For example, Fulton's *K* and whole-body triglyceride content were only slightly correlated in the same fish. Fulton's *K* measures body shape rather than energy storage (Neumann and others, 2012). Lost River suckers had greater whole-body triglyceride content and a higher prevalence of fish without visceral fat in 2014 than in 2015, suggesting opposite conclusions. This may have been a result of the two types of data collected on different fish, or it may indicate that most whole-body triglycerides are not stored visceral fat. Although there were apparent temporal trends in the amount of liver glycogen storage in both years, no seasonal trends were detected in whole-body triglycerides.

When energy intake exceeds the needs for metabolism, growth, swimming exertion, reproduction, and other activity, a fish tends to store a part of the excess energy as glycogen and (or) lipid in the cytoplasm of hepatocytes (Wolf and others, 2015). Therefore, high glycogen levels in fish sampled from Upper Klamath Lake in mid-July and early August 2014 and mid-August to mid-September 2015 indicated periods of increased feeding and storage of excess glycogen. Conversely, lower glycogen levels in late August and early September 2014 and mid-July 2015 seemed to indicate a period of either decreased feeding or increased energy demands. Measured water-quality parameters were variable during periods when lower hepatocellular glycogen levels were observed in fish. For example, relatively high vacuolation and lower glycogen storage during the August 26–28, 2014 sample period were correlated with decreasing DO concentrations, decreasing pH, and decreasing particulate microcystin concentrations. In the following sampling period (September 3–11, 2014), lower glycogen storage, and more frequent (four of five fish) liver inflammation was associated with DO concentrations around 7–10 mg/L, pH greater than 9.0, and large fraction microcystin concentrations less than 20 ppb. Low glycogen storage from July 21 to August 3, 2015 was associated with decreasing DO concentrations, pH from 8.0 to 9.8, and particulate microcystin concentrations at 29 ppb. The 2015 sampling period with the lowest glycogen levels (July 21–27) also coincided with high mortality of mesocosm held suckers in an independent study in Upper Klamath Lake (Stone and others, 2017). Alternatively, the type and amount of hepatic energy stores also can vary by species, age, sex, reproductive phase, season, or effects of inflammatory or toxic processes (Wolf and others, 2015) and may not necessarily represent nutritional status or energy expenditure. The amount of hepatocellular glycogen stored in the crucian carp (*Carassius carassius*) has been shown to vary greatly by season, with lowest glycogen content observed in summer and the highest glycogen content measured in late autumn and winter when fish are preparing to survive in hypoxic conditions under ice (Hyvärinen and others, 1985). When deprived of food, many fishes metabolize glycogen prior to triglycerides (Garvey and Chipps, 2012), but several migratory species and at least one cyprinid (*Cyprinus carpio*) metabolize triglycerides prior to glycogen (Enes and others, 2009).

Hypoxia-tolerant fishes such as Lost River and shortnose suckers tend to have high levels of hepatic glycogen storage (Dhillon and others, 2013). Reduction or depletion of liver glycogen that occurs during prolonged hypoxia or anoxia in such fishes indicates that it is an important glucose energy source during periods of oxygen depletion (Zhou and others, 2000). For example, the crucian carp uses enormous stores of liver glycogen to survive extended periods of anoxia in winter. During anoxia, glucose is the only available cellular fuel, and the crucian carp uses glycolytic metabolism,

with production of ethanol that is released into the water, to avoid lactate self-poisoning (Nilsson and Renshaw, 2004). The temporal decrease in hepatic glycogen in Upper Klamath Lake in 2014 could be interpreted as a result of prolonged hypoxia, except that glycogen levels generally increased in 2015 when low DO events were more frequent. Liver glycogen depletion also was observed in Clear Lake Reservoir in 2014 where DO concentrations were greater than or equal to 5.7 mg/L (Burdick, Elliott and others, 2015). We did not detect a seasonal decrease or increase in triglycerides in either year, which may be partly due to small weekly sample sizes. As with our findings, hatchery-reared Lost River suckers held in a mesocosm in Upper Klamath Lake in 2014 also maintained fairly constant levels of whole-body triglycerides (Hereford and others, 2016). In contrast to these findings, Foott and others (2012) noted that triglyceride content seemed to decrease between August and October for sentinel Lost River suckers held in Upper Klamath Lake in 2011. The difference among study results may indicate annual variability in seasonal energy storage or may be a spurious result due to small sample sizes in Foott and others (2012).

Triglyceride densities measured in this study were variable and within the range of values reported for Lost River and shortnose suckers and other species (Foott and others, 2005; Bennett and others, 2007). Triglyceride content in our study was lower than for older juvenile shortnose suckers collected from Clear Lake Reservoir in 2014 (Burdick, Elliott and others, 2015) and similar to whole-body triglyceride content of wild-caught suckers from Upper Klamath Lake in 2004 (3.1–16.1 mg/g) (Foott and Stone, 2005). The difference in whole-body triglyceride between Upper Klamath Lake and Clear Lake Reservoir in 2014 may be a result of differences in age classes, individuals, or environments (Post and Parkinson, 2001; Bennett and others, 2007). Lower whole-body triglyceride content for Lost River suckers in 2015 than in 2014 co-occurred with slower Lost River sucker growth and cooler July and September water temperatures (Burdick and Martin, 2017). In contrast, shortnose suckers had similar triglyceride concentrations and similar growth in 2015 and 2014 (Burdick and Martin, 2017).

Hyaline droplets observed in kidney tubular epithelial cells of juvenile suckers, which were less prevalent in suckers collected in 2014 than in 2015, have previously been described in juvenile and adult suckers from Upper Klamath Lake and Clear Lake Reservoir, but may not indicate a specific etiology (Foott and others, 2013; Burdick, Elliott and others, 2015). The droplets can be a sign of hyaline degeneration, which is due to lipoprotein accumulation in the epithelial cell phagolysosomes (Foott and others, 2013). Lost River suckers form hyaline droplets in the proximal tubules in the kidneys when exposed to un-ionized ammonia concentrations at 0.22 mg/L or greater for 62 h (Foott and others, 2000), but fish with hyaline droplets in our study were collected when un-ionized and total ammonia concentrations were low. Hyaline droplets also can form as an initial response to hypoxia (Tervonen and others, 2006). The only sucker in 2014, and three of seven of the suckers in 2015 with this kidney abnormality were collected when DO concentrations decreased to about 4 mg/L during the day. However, all other suckers with hyaline droplets in the kidney tubular epithelium were collected when DO concentrations fluctuated from about 7 to 12 mg/L. Finally, hyaline droplets can occur in the epithelium of kidney tubules of fish that have had no exposure to toxins or pathogens and otherwise appear healthy (Ferguson, 1989; Wolf and others, 2015). Suckers captured in Clear Lake in 2014, where DO concentrations never decreased to less than 6 mg/L, had similar hyaline droplets, indicating this condition may be normal for suckers.

Conclusions

Although there were differences in water quality between years, differences in the health of the fish we examined were mostly minor and could not be directly attributed to water quality. Neither gross nor histological examination of suckers revealed obvious etiologies of high mortality. Our failure to detect correlations between water quality and fish health may be partially due to having only 2 years of consistently collected samples. Another possibility is that our sample collection method was biased toward healthy fish. It also is possible that annual differences in water quality in Upper Klamath Lake are not extreme enough to create annual differences in sucker health. The main causes of recruitment failure in Upper Klamath Lake may be caused by factors not examined in our study.

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