THE FISH AND FISHERIES OF THE BOUNDARY WATERS CANOE AREA WILDERNESS AND VOYAGEURS NATIONAL PARK, AND THEIR VULNERABILITY TO COPPER SULFIDE MINING

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SUMMARY

This report was prepared for the 90-day public comment period on the U.S. Forest Service's proposed withdrawal of federal minerals in the Rainy River watershed. It describes the fish and fisheries of the Boundary Waters Canoe Area Wilderness (BWCAW) and Voyageurs National Park (VNP), and their vulnerability to copper sulfide mining.

The BWCAW and VNP region is home to almost half of Minnesota's native fish species, including two of the state's species of special concern (lake sturgeon, *Acipenser fulvescens*; northern brook lamprey, *Ichthyomyzon fossor*) (**Section I**). Only one non-native fish species, rainbow smelt (*Osmerus mordax*) is known to occur in the BWCAW and VNP.

The BWCAW and VNP support genetic strains and cold-water fish assemblages that are rare in other parts of the state. Approximately 50 lakes in the BWCAW and VNP have also been identified as refuges for cisco (*Coregonus artedi*) and other cold-water species such as lake whitefish (*Coregonus clupeaformis*), and lake trout (*Salvelinus namaycush*). A decline in water quality would jeopardize the ability of these lakes to serve as refuges.

Fishes in the BWCAW and VNP are an integral component of a larger ecological system, and provide a diversity of ecosystem services essential for ecosystem function and resilience. For example, fish regulate nutrients and food webs, provide important links to terrestrial ecosystems (e.g., as food for birds and mammals), and several species in the BWCAW and VNP are essential to the life cycle of state-listed freshwater mussels. Fish in the BWCAW and VNP also benefit humans directly by serving as ecological indicators and records of information, and as a source of food and recreation.

The BWCAW and VNP support important recreational and subsistence fisheries. The region is a popular tourist destination, and a comparison of fish catches inside and outside of the

BWCAW and VNP suggests that anglers target the BWCAW and VNP when fishing for lake trout (and cold-water species) and smallmouth bass (*Micropterus dolomieu*). State survey data suggest that some game fish are larger in the BWCAW and VNP (probably due to relatively large lakes and pristine habitat, and low fishing pressure). The BWCAW and VNP also support subsistence fisheries in Minnesota, the rights to which will be jeopardized by pollution. Pollution from mining would exacerbate the mercury problem in the region, and threaten Canadian waters that themselves support significant commercial, recreational, and/or subsistence fisheries.

The diversity and value of fish in the BWCAW and VNP are a direct result of past efforts to maintain the pristine nature and ecological integrity of the region. The scientific literature is replete with studies that indicate that acid mine drainage (AMD) causes long-term declines in fish abundance, species number, and genetic diversity, and may facilitate the establishment of invasive species (**Section II**).

Acid mine drainage lowers pH and exposes fish and other aquatic organisms to toxic metals (e.g., copper, cadmium, lead, mercury, nickel, and zinc). The downstream extent of AMD impacts on an aquatic ecosystem depends on initial concentrations; the duration and rate of release relative to the volume of the receiving waters; physical, chemical, and biological processes and interactions; species; and demographic factors such as sex, size, and age. Most fish species in the BWCAW and VNP will be lost between pH ~5 and ~6; well above the typical pH found in some AMD. Toxic metals also play a role by causing mass die-offs or accumulating in all major organs. The latter impairs systems function (e.g., feeding, digestion, respiration), causes deformities and behavioral issues, results in reproductive failure and mortality, and may render fish unfit for human consumption.

The magnitude, location, and timing of AMD impacts on fish are difficult to predict because of complex physical, chemical, and biological reactions and interactions that are context-dependent and vary over time. For example, AMD leaches toxic metals from the environment, which is a particular problem in the BWCAW and VNP because of metal-containing bedrock that is poor at neutralizing acid. The complex nature of AMD is perhaps best demonstrated by a review that found that the environmental impact assessments of hard rock mines failed to predict water quality exceedences almost 60% of the time.

Evidence from previous hard rock mines demonstrates overwhelmingly that a copper-sulfide mine in the Rainy River watershed will impact fish, and that such damage is likely to extend far from the mine site and persist for centuries causing damage to a relatively pristine region of the state, and the diversity of popular and rare fishes that it supports.

I. THE FISH AND FISHERIES OF THE BOUNDARY WATERS CANOE AREA WILDERNESS AND VOYAGEURS NATIONAL PARK

A. Diverse, rare, and mostly native species. The BWCAW and VNP are home to nearly 40% of Minnesota's ~150 native fish species (Table I-1), and nearly 55% of Minnesota's ~25 native fish families. This diversity likely stems from the fact that the region is relatively pristine (MPCA, 2016), and comprises an abundance and diversity of habitats (Ojakangas and Matsch, 2001). The region also represents the *de facto* northern range limit of some species (e.g., brassy minnow, *Hybognathus hankinsoni*; and hornyhead chub, *Nocomis biguttatus*) and the *de facto* southern range limit of others (e.g., lake whitefish, *Coregonus clupeaformis*; and lake chub, *Couesius plumbeus*).

Two fishes that occur in the BWCAW/VNP region are considered by the state to be species of special concern: lake sturgeon (*Acipenser fulvescens*) and northern brook lamprey (*Ichthyomyzon fossor*) (MNDNR, 2013). Another 10 species in the BWCAW/VNP are rare in Minnesota (Table I-1). The lake sturgeon was nearly extirpated from the state, in part because of pollution. Sturgeon are recovering because of the Clean Water Act of 1977, as well as conservation efforts such as stocking and habitat restoration; however, recovery rates are low because female lake sturgeon take ~25 years to mature and only spawn every ~5 years (Kallok, 2008). The northern brook lamprey is a Minnesota species of special concern because of pollution, other forms of habitat degradation, and efforts to control sea lamprey (*Petromyzon marinus*) in Great Lakes tributaries. Little is known about the northern brook lamprey in Minnesota, and the Minnesota Department of Natural Resources currently does not have a conservation plan in place (Berendzen and Schmidt, 2016).

The BWCAW/VNP region is effectively free of invasive fishes (Table I-1), although some lakes contain invasive invertebrates that are known to affect native fishes: rusty crayfish (*Orconectes rusticus*) (e.g., Wilson et al., 2004) and spiny water flea (*Bythotrephes cederstroemi*) (e.g., Rennie et al., 2009). There are ~15 non-native fishes in Minnesota, but only one species, the rainbow smelt (*Osmerus mordax*), is found in the BWCA/VNP region. A review by Rooney and Paterson (2009) suggests that rainbow smelt can benefit native game fishes by serving as an energy-rich (albeit unreliable) food source. However, rainbow smelt can also compete with or even prey upon young game fishes or their natural prey (especially in small lakes), and impact water quality. In addition to rainbow smelt, popular native game fishes such as brook trout (*Salvelinus fontinalis*) and both smallmouth (*Micropterus dolomieu*) and largemouth bass (*M. salmoides*) have been introduced into lakes and rivers in which they did not previously occur.

B. Unique species assemblages and genetic strains. The BWCAW, VNP, and surrounding region support cold-water fish assemblages that are rare in other parts of the state. Cold-water assemblages typically include game fishes such as cisco (*Coregonus artedi*), lake whitefish, lake trout (*Salvelinus namaycush*) (Table I-1) and other, non-game species that require mean surface water temperatures of $<15^{o}C$ during the ice-free period or, if temperatures exceed this threshold, large, deep lakes (Wehrly et al., 2012). Cold-water assemblages are also sensitive to low levels of dissolved oxygen and nutrient loading (Jacobson et al., 2008). Climate

Table I-1. Fish species known or suspected to occur in the Boundary Waters Canoe Area Wilderness or Voyageurs National Park according to VNP (2016) and Minnesota Pollution Control Agency collection data (Chad Anderson, personal communication, October 2016).

Scientific name	Common name	Scientific name cont'd	Common name cont'd
Acipenser fulvescens	Lake sturgeon[a]	*M. salmoides*	Largemouth bass
Ambloplites rupestris	Rock bass	*Moxostoma anisurum*	Silver redhorse
Ameiurus melas	Black bullhead	*M. macrolepidotum*	Shorthead redhorse
A. nebulosus	Brown bullhead	*Nocomis biguttatus*	Hornyhead chub
Catostomus catostomus	Longnose sucker	*Notemigonus crysoleucas*	Golden shiner
C. commersonii	White sucker	*Notropis atherinoides*	Emerald shiner
Chrosomus eos	Northern redbelly dace	*N. dorsalis*	Bigmouth shiner
C. neogaeus	Finescale dace	*N. heterodon*	Blackchin shiner[b]
Coregonus artedi	Cisco[b,c]	*N. heterolepis*	Blacknose shiner
C. clupeaformis	Lake whitefish[b,c]	*N. hudsonius*	Spottail shiner
Cottus bairdii	Mottled sculpin[b]	*N. volecullus*	Mimic shiner
C. cognatus	Slimy sculpin	*Noturus gyrinus*	Tadpole madtom[b]
Couesius plumbeus	Lake chub[b]	*Osmerus mordax*	Rainbow smelt[d]
Culaea inconstans	Brook stickleback	*Perca flavescens*	Yellow perch
Esox lucius	Northern pike	*Percina caprodes*	Log-perch
E. masquinongy	Muskellunge	*P. maculate*	Blackside darter
Etheostoma exile	Iowa darter	*P. shumardi*	River darter[b]
E. nigrum	Johnny darter	*Percopsis omiscomaycus*	Trout-perch
Hiodon tergisus	Mooneye	*Pimephales notatus*	Bluntnose minnow
Hybognathus hankinsoni	Brassy minnow	*P. promelas*	Fathead minnow
Ichthyomyzon fossor	Northern brook lamprey[a,b]	*Pomoxis nigromaculatus*	Black crappie
I. unicuspis	Silver lamprey[b]	*Pungitius pungitius*	Ninespine stickleback
Lepomis cyanellus	Green sunfish	*Rhinichthys atratulus*	Blacknose dace
L. gibbosus	Pumpkinseed	*R. cataractae*	Longnose dace
L. macrochirus	Bluegill	*Salvelinus fontinalis*	Brook trout
L. megalotis	Longear sunfish	*S. namaycush*	Lake trout[b,c]
Lota Iota	Burbot	*Sander Canadensis*	Sauger
Luxilus cornutus	Common shiner	*S. vitreus*	Walleye
Margariscus margarita	Pearl dace	*Semotilus atromaculatus*	Creek chub
Micropterus dolomieu	Smallmouth bass	*Umbra limi*	Central mudminnow

[a]Species of special concern (MNDNR, 2013)
[b]Rare in Minnesota
[c]Cold-water game fish
[d]Non-native

warming appears to be contributing to a decline in cisco in northeastern Minnesota (Jacobson et al., 2012). Importantly, ~50 lakes in the BWCAW and VNP are predicted to serve as refuges for cisco and other cold-water fishes under future climate scenarios (Fang et al., 2012; Jiang et al., 2012). Maintaining water quality in these lakes is a management priority because they represent ~80% of the 62 refuge lakes in the region, and ~35% of the 160 refuge lakes state-wide.

The BWCAW and VNP also contain unique genetic strains of fish. Genetic strains are groups within species that can be genetically differentiated from other groups or the species in general. This genetic variation results from reproductive isolation, random mutations, and local adaptation, and is not unique to the BWCAW and VNP. However, strains contribute to the genetic diversity of a species and, as a result of local adaptation, are usually best-suited to local conditions. For example, muskellunge (*Esox masquinongy*) from Shoepack Lake in VNP are relatively smaller than other strains and unlikely to persist when stocked in other Minnesota lakes (Miller et al., 2009; 2012). Protecting genetic diversity is a key principle of conservation biology (Groom et al., 2006), in part because the absence or loss of a genetic strain can complicate efforts to rehabilitate or reintroduce a species (e.g., Burnham-Curtis et al., 1995; He et al., 2016). Genetic strains should also be protected for their utility. Examples include stocking programs (Miller et al., 2009; 2012), research (Coe et al., 2009), and aquaculture (Brown et al., 2007).

C. Ecologically important. Fishes in the BWCAW and VNP are an integral component of a larger ecological system, and provide a diversity of ecosystem services (Table I-2). Detailed reviews by Holmlund and Hammer (1999) and Lynch et al. (2016) describe fish as ecological engineers that regulate nutrients and food webs, and contribute to the resiliency of aquatic systems to disturbance. These qualities are perhaps most famously demonstrated by trophic cascades, the process whereby changes in fish abundance cascade through a food chain to affect water quality (Carpenter and Kitchell, 1996). Fish also provide important links to terrestrial ecosystems (e.g., Nakano et al., 1999; Reimchen et al., 2003), and several species in the BWCAW and VNP are essential to the life cycle of state-listed freshwater mussels that also occur in the region (e.g., the threatened flutedshell, *Lasmigona costata*). Fish in the BWCAW and VNP also perform important ecosystem services (Table I-2) by serving as ecological indicators and records of information, and providing food and recreational opportunities (Homlund and Hammer, 1999; Lynch et al., 2016).

D. Recreationally and culturally important. The BWCAW and VNP are popular tourist destinations (Dvorak et al., 2012), and home to Minnesota's most popular game fishes (Pauly, 2005). According to a federal survey (US 2011), anglers in Minnesota prefer to fish for walleye and sauger (*Sander* spp.), northern pike and muskellunge (Esox spp.), smallmouth and largemouth bass, and panfish (*Lepomis* and *Pomoxis* spp.). To gain insight into angler species preferences in northeastern Minnesota, we gathered anonymized data in partnership with Fishidy; a popular smartphone application (app) that anglers use to log their catches by species, photos, location, method, weather, and date. Catches both inside of and adjacent to the BWCAW and VNP are largely consistent with the state-wide, 2011 survey in that they

Table I-2. Major fundamental and demand-derived ecosystem services generated by marine and freshwater fish populations (modified from Holmlund and Hammer, 1999).

Fundamental ecosystem services (essential for ecosystem function and resilience)	
Regulating services	**Linking services**
Regulation of food web dynamics	Linkage within aquatic ecosystems
Recycling of nutrients	Linkage between aquatic and terrestrial ecosystems
Regulation of ecosystem resilience	Transport of nutrient, carbon, and minerals
Redistribution of bottom substrates	Transport of energy
Regulation of carbon fluxes from water to air	Acting as ecological memory
Maintenance of sediment process	
Maintenance of genetic, species, ecosystem biodiversity	

Demand-driven ecosystem services (reflect human values/demands; not fundamental)	
Cultural services	**Informational services**
Production of food	Assessment of ecosystem stress
Aquaculture production	Assessment of ecosystem resilience
Production of medicine	Revealing evolutionary tracks
Control of hazardous diseases	Provision of historical information
Control of algae and macrophytes	Provision of scientific and educational
Reduction of waste	Information
Supply of aesthetic value	
Supply of recreational activities	

Table I-3. A summary of Fishidy catch data by species for inside of the BWCAW and VNP (40 fishing trips by 21 users to 8 lakes between 9/22/2011 and 9/25/2015), and for a region within 30 km of the BWCAW and VNP boundaries (152 trips, 89 users, and 33 catches between 6/25/2009 and 12/22/2015). We combined all species that contributed <3% of the total catch within an area (inside or outside of BWCAW and VNP) to the 'Other' category.

Species	Percent of catch inside	Percent of catch outside
Walleye	47	38
Smallmouth bass	26	16
Lake trout	10	4
Northern pike	9	21
Muskellunge	-	9
Largemouth bass	-	4
Other	8	8

seem to prefer walleye (*Sander vitreus*), smallmouth and largemouth bass, and northern pike and muskellunge (Table I-3). However, the proportion of lake trout in the catch is two and a half times higher inside of the BWCAW and VNP than it is outside. Assuming that app users and use is representative (Jiorle et al., 2016), these data suggest that cold-water assemblages are important to recreational fisheries in the BWCAW and VNP.

On average, lakes inside of the BWCAW and VNP contain more large fish than lakes outside of these areas (Figure I-1). This pattern was evident in species-specific lake survey data from the Minnesota Department of Natural Resources. They show that memorable brook trout (defined as fish that are within 60% of the world-record length for that species; Gabelhouse, 1984) are four times more common. Largemouth bass and northern pike were twice as common. The higher proportion of large fish in the BWCAW and VNP probably stems from a relatively high proportion of larger lakes and pristine habitat, and relatively low fishing pressure.

The waters that flow through and from the BWCAW and VNP also support subsistence fisheries in Minnesota and recreational fisheries in Canada. Subsistence fishing by the Bois Forte, Fond du Lac, and Grand Portage Chippewa bands of Lake Superior (~10,000 enrolled members) was formally recognized by treaties in 1854 and 1866. Courts typically consider pollution to be a threat to these rights (Perron, 2000; Blumm and Steadman, 2009). The fish and waters in the BWCAW and VNP are also a shared resource with Canada (Boundary Waters Treaty, 1909). The entire ~700 km border between Minnesota and Canada east of Lake of the Woods bisects lakes and rivers. Article IV of the 1909 Boundary Waters Treaty states that boundary waters “shall not be polluted on either side to the injury of health or property on the other” (but see DeWitt, 1994). Canada’s Quetico Provincial Park, Rainy Lake, Lake-of-the Woods, and Lake Winnipeg are all downstream of the BWCAW and VNP, and support significant commercial, recreational, and/or subsistence fisheries that would be impacted by mine contaminants. The region is already stressed by the atmospheric deposition of mercury (Wiener et al., 2006), and for many lakes the consumption advice is just one fish per week (MDH, 2017).

II. THE VULNERABILITY OF FISHES IN THE BOUNDARY WATERS CANOE AREA WILDERNESS AND VOYAGEURS NATIONAL PARK TO COPPER SULFIDE MINING

Mining-caused contamination of water resources is a global problem (Myers, 2016). Acid mine drainage occurs when sulfide ores are exposed to the atmosphere and subsequently oxidized (Jennings et al., 2008). The result is an acid mixture that contains high concentrations of sulfite and heavy metals (Jennings et al., 2008) and undergoes complex chemical, biological, and physical reactions in the natural environment (Lindsay et al., 2015) to affect algal production, aquatic macroinvertebrates, and fish (Hughes et al., 2016).

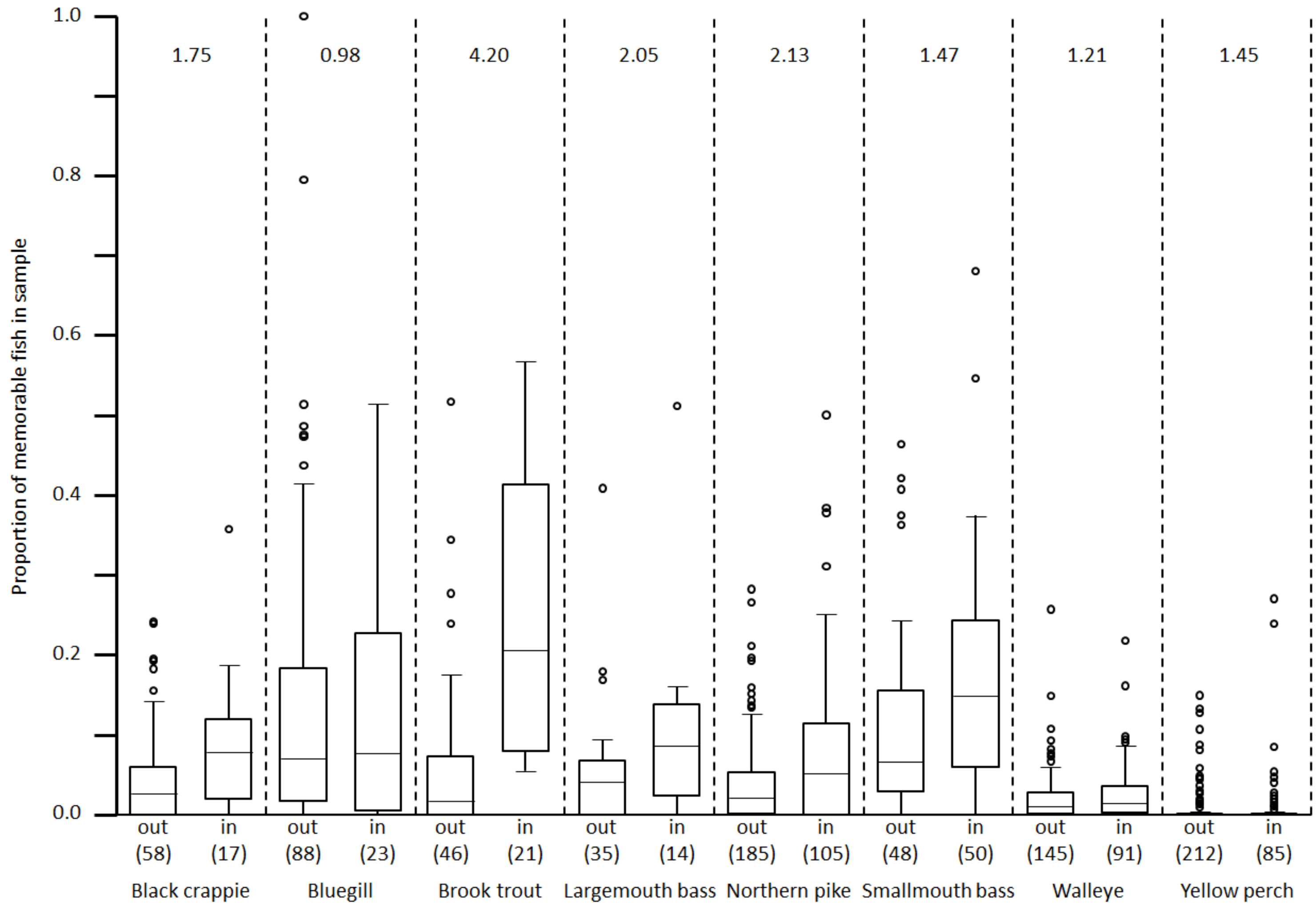

Figure I-1. The mean proportion of memorable-sized game fish sampled by the Minnesota Department of Natural Resources inside of the BWCAW and VNP (in) and for a region within 30 km of the BWCAW and VNP boundaries (out). Values in parenthesis are sample sizes (number of lakes), and at the top of the plot are means ratios (in/out). A lake was excluded from the analysis if species-specific sample size was <20 individuals, and species were excluded from the plot if inside or outside lake number was <10.

Myers (2016) developed a watershed-scale, ground-water fate and transport model for the Rainy Headwaters. This model showed that mining in the BWCAW watershed could contaminate surface and subsurface waters to the north and northwest in the BWCAW. Myers (2016) concluded that "Even relatively short-term leaks on the surface could cause substantial loads to reach the rivers and valuable downstream resources." Consistent with modeling studies (e.g., Coulthard and Macklin, 2003) and the direct observation (e.g., Peplow, 2000) of other mines, water contamination was predicted to extend far from the mine site, and persist for centuries to millennia.

A. Direct effects of low pH on fish. The acidity of sulfide mine tailings may far exceed the lethal limit for even the most tolerant fish species (Lindsay et al., 2015). Acidity interferes with the ability of fishes to exchange oxygen and carbon dioxide, and maintain an internal balance of water and salts (Alabaster and Lloyd, 2013). The extent to which pH impacts fishes downstream of an AMD input depends on initial pH, rate and duration of release relative to the receiving waters, the buffering capacity of the receiving waters, distance downstream, species, and life stage (e.g., egg vs. adult).

Reviews by Lind et al. (1978), Baker and Christensen (1991), Cook and Jager (1991), and Alabaster and Lloyd (2013) identify approximate thresholds at which acidification will lead to extirpation as a result of mortality, reproductive failure, avoidance, or some combination thereof. Most species in the BWCAW and VNP are likely to persist down to pH ~6 (Figure II-1). Exceptions are cisco, trout-perch (*Percopsis omiscomaycus*), and some species of dace, darter, and minnow. Conversely, the only species that are likely to persist at pH 5 are brook trout, northern pike (*Esox lucius*), largemouth bass, pumpkinseed (*Lepomis gibbosus*), yellow perch (*Perca flavescens*) and a handful of non-game species. These results are corroborated by laboratory studies showing that many fish species exhibit avoidance behaviors to pH in the range 5.0 to 5.5, and field observations of fish moving downstream ahead of an acid pulse (Alabaster and Lloyd, 2013). Few natural fish populations are found in waters below pH ~4.5 (Lind et al., 1978; Alabaster and Lloyd, 2013).

B. Direct effects of toxic metals on fish. AMD contains high concentrations of toxic metals such as copper, cadmium, lead, mercury, nickel, and zinc (Jennings et al., 2008). The bioavailability and toxicity of each of these metals depends on a diversity of interacting factors such as the physical and chemical characteristics of the water, exposure time, the species of fish, and demographic factors (e.g., sex, age, and size) (Lind et al., 1978; Spry and Wiener, 1991; Walczak and Reichert 2016).

In general, most toxic metals bioaccumulate in all major organs including muscles and gills. Damage to these organs results in impaired physiology and behavior, reproductive failure, and mortality. A detailed survey of fishes in heavily-polluted region of Russia identified diverse effects of toxic metals on the kidneys, liver, and skeleton (Moiseenko and Kudryavtseva, 2001). Incidence rates approached 90%.

Lind et al. (1978) found copper to be particularly toxic to fish; it reduced fish weight and/or growth at 1-10 μg/L, bioaccumulated at concentrations as low as 10-100 μg/L, impacted

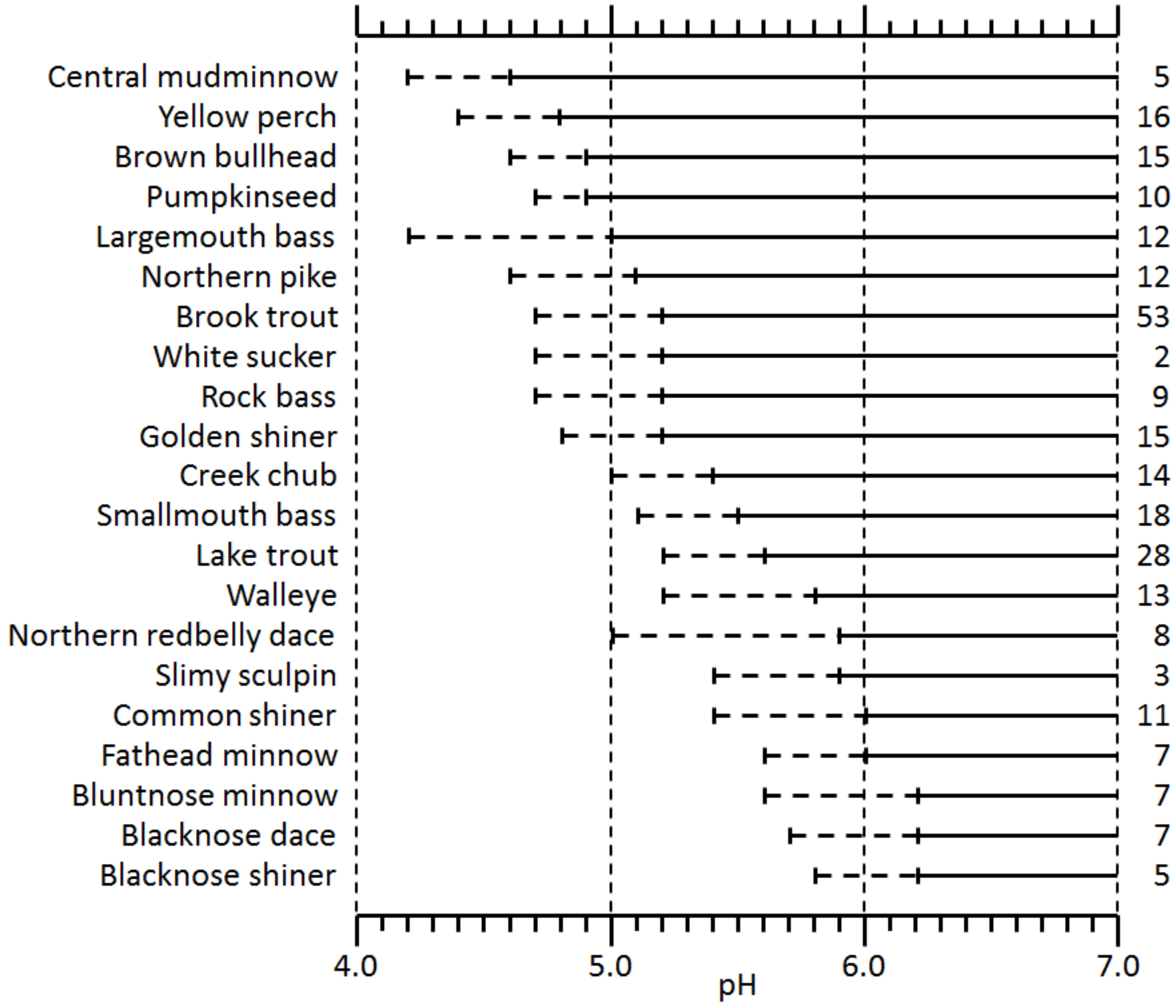

Figure II-1. Estimated "critical" pH values for effects on fish species that are known to occur in the BWCAW and VNP based on a qualitative literature review by Baker and Christensen 1991). Results do not incorporate interactive effects with aluminum (see Section II.C). Horizontal dashed lines show the approximate range of uncertainty in the estimate, and values to the right are the number of observations (n) used to derive the estimated "critical" pH.

reproductive success at 10-100 µg/L, and was lethal to half of all study fish that were held at 100-1000 µg/L for 4-10 days. Even short-term (e.g., 1-hour) exposure to sub-lethal copper concentrations can impact fish vision (Bodammer, 1985), smell (Sandahl et al, 2006), hearing (Linbo et al., 2006), and taste (Sutterlin and Sutterlin, 1970).

Although acute exposure to toxic metals can be lethal, most population-level effects result from avoidance behaviors or chronic exposure to sub-lethal concentrations. A review by Lind et al. (1978) suggests that fish can detect sub-lethal concentrations. These concentrations are avoided if the gradient is steep, but can be attractive if the gradient is gentle. Chronically-exposed fish are less likely to capture prey, avoid predators, fight off infections, and successfully reproduce (e.g., Zelikoff, 1993; Kime, 1995; Weis and Candelmo, 2012). The cumulative effect of these stressors is reduced abundance or complete extirpation (Bervoets et al., 2005).

C. Impacts of AMD on fish are complex and difficult to predict. Kuipers et al. (2006) compared the observed and predicted impact of 25 hard rock mines on water quality. Seventy-six percent of these mines exceeded water quality thresholds, but only 42% of these observed exceedences were predicted in environmental impact statements. Failures were attributed to insufficient or inaccurate characterization of hydrology (e.g., discharge, dilution, and storms) and geochemistry (e.g., as a result of inadequate sampling). It is also possible that these environmental impact statements placed too much emphasis on laboratory tests involving fish. A review by Lind et al. (1978) found that laboratory tests tend to grossly underestimate effects in the wild.

Effects of AMD on wild fish are difficult to predict because AMD undergoes complex physical, chemical, and biological reactions in the surrounding environment (Lindsay et al., 2015). For example, although laboratory studies suggest that many fish species can tolerate prolonged exposure to pH 5, these species show poor survival at pH ~6 in the wild (McDonald et al., 1989). This paradox is explained by two phenomena: the leaching of aluminum (a toxic metal) from the environment at any pH <7, and increased susceptibility of fish to aluminum and other toxic metals at pH <7. Leaching is a particular problem in the BWCAW and VNP because of large areas of exposed bedrock that contain toxic metals but provide little buffering capacity (Baker and Christensen, 1991).

Effects of AMD on fish are also difficult to predict because physical, chemical, and biological reactions vary over time. For example, seasonal or stochastic rainfall events can either dilute AMD (e.g., Davies et al., 2011) or flush it from mine areas (e.g., Suchanek et al., 2008). Reaction rates are also temperature-dependent. Although colder temperatures reduce metabolism and therefore toxic metal accumulation in fish (e.g., MacLeod and Pessah, 1973), metal toxicity is often higher at lower temperatures (Lind et al., 1978). Colder temperatures also reduce the rate at which toxic metals are 'neutralized' by bacteria (e.g., Neculita et al., 2007). Toxic metal concentrations in streams in Montana and Idaho also varied ~4-fold over a 24-hour period as a result of redox reactions, photoreduction, precipitation, dissolution, absorption, and other geochemical processes related to pH and temperature cycles (Nimick et al., 2003). The dynamics of toxic metals within fish are another complicating factor in that relatively benign bioaccumulations in fat tissue are suddenly released into the blood stream when fat is metabolized (e.g., during periods of spawning, migration, or starvation).

Food web effects can also made it difficult to predict AMD effects on fish. For example, lake trout in an experimentally acidified lake succumbed, not to pH, but to starvation due to a lack of forage fish (Mills et al., 1987). AMD can also reduce or eliminate organisms that are lower in the fish food chain, such as algae and plants (Niyogi et al., 2002) and aquatic macroinvertebrates (Lind et al., 1978). The Dunka taconite mine (no longer operational) near the BWCAW is an example of the latter. Sulfate waste rock leachate from this mine flowed into a creek for over 37 years, and has significantly reduced the abundance and diversity of macroinvertebrates that are both indicators of water quality and important food for fish (Johnson and Johnson, 2015).

D. Documented effects of AMD on fish. The scientific literature is replete with studies showing that AMD eliminates all fish species from an area (reviewed by Lind et al., 1978) or causes long-term declines in diversity (e.g., Cooper and Wagner, 1973; Lind et al., 1978; McCormick et al., 1994; Jeffree et al., 2001; Storey et al., 2008; Miguel et al., 2014). We are unaware of any studies that do not show at least a local impact. Pollution from mines can reduce genetic diversity within a fish population (Mussali-Galante et al., 2013) and, together with other sources of pollution, is thought to be responsible for ~30% of fish extinctions over the last century (Miller et al., 1989). Non-native species are also more likely to establish and thrive in disturbed environments (Leidy and Fiedler, 1985; Didham et al., 2007). For example, non-native fishes dominated a 70-km stretch of the Agrio and Guadiamar Rivers in Spain ~10 years after all aquatic life was extirpated by a large tailings spill containing heavy metals and sulfides (Miguel et al., 2014).

In the remainder of this section, we highlight in more detail a few of the studies that are relevant to the BWCAW and VNP. These studies document specific effects linking AMD to changes in fish abundance and diversity (beyond 'simple' avoidance). The fact that most of these studies relate to the production, survival, and development of eggs and larvae may indicate that these life stages are particularly vulnerable to AMD. However, this pattern could also result from the relative ease of experimenting with or observing these life stages.

Leis and Fox (1994) found that the mortality of walleye eggs held in incubators in a site in a northern Ontario river following a gold mining tailings spill was significantly higher (81%) than at a reference site (64%), and likely related to metal toxicity (copper and lead), low oxygen from the resuspension and settling of mine tailings, or both. In a related study, Leis and Fox (1996) found that the size of yearling walleye in a tailings reach were comparable to a reference site until they reached ~50 mm in length, but that no yearlings above this size were found in the tailings reach by late July. The walleye in the tailings reach had lower stomach fullness and a higher proportion of empty stomachs, which may have accounted for the smaller size.

Rickwood et al. (2006) assessed egg production and hatching success of fathead minnows (*Pimephales promelas*) in laboratory conditions similar to Copper Cliff mine effluent from the Junction Creek system in Sudbury, Ontario. Total egg production was reduced by ~75% compared to controls. Larval hatching success was ~70% in controls, but only ~2% in the treatments. Deformities were also higher in the treatments (~70%) than in the controls (~10%).

Brook trout, rainbow trout (*Oncorhynchus mykiss*), and cutthroat trout (*O. clarki*) experienced increased mortality near mine waste sources in the Boulder River Watershed, Montana (Farag et al., 2003). Specifically, greater and more rapid mortality was associated with higher concentrations of cadmium, copper, and zinc. Gill tissues of these trout were affected by the elevated concentrations of cadmium, copper, and zinc, and contributed to the mortality of the fish. Trout were observed further downstream, but exhibited impaired health of resident trout, as well as reduced biomass and density.

Rainbow trout fry exposed to sublethal concentrations of copper in controlled laboratory conditions exhibited significantly reduced weight (0.320 g vs 0.578 g) compared to controls after 20 days and had significantly elevated whole-body copper concentrations (18.5μg/l vs 13.1μg/l) after 60 days (Maret al., 1996). Trout fry exposed to copper grew slower than controls over an exposure period of 60 days. Whole-body accumulation rates of copper in fish increased significantly by day 40, but then maintained a steady state thereafter.

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IV. REFERENCES

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SUPPLEMENT:
State of Minnesota, *Regional Copper-Nickel Study*, 1976-1980, a collection of research papers located at the Minnesota Legislative Reference Library, www.leg.state.mn.us/lrl/lrl.asp. Several research papers in this library are referenced in this paper.