SEWRPC Technical Report No. 62

IMPACTS OF CHLORIDE ON THE NATURAL AND BUILT ENVIRONMENT

Chapter 3

IMPACTS OF CHLORIDE ON BIOLOGICAL SYSTEMS

3.1 INTRODUCTION

Good environmental quality is important for the protection of natural systems. Changes in the quality of the environment can cause stresses that impact plants, animals, and other organisms. If these changes are great enough, they can cause major changes in the biological systems of an area. Releases of pollutants into the environment can cause these changes, potentially altering biological systems.

As noted in previous chapters of this report, concentrations of chloride salts and the associated salinity and specific conductance have been increasing in surface waters, both in the Southeastern Wisconsin Region and much of the nation.[1] Chloride salts have also been introduced into other parts of the environment. These chloride related increases have the potential to impact and alter ecological systems. Such effects might impact several levels of biological organization including individual organisms, populations of individual species, biological communities, and ecosystems.

---

[1] *See for example, Richard C. Lathrop, "Chloride and Sodium Trends in the Yahara Lakes,* Research Management Findings, *No.12, Wisconsin Department of Natural Resources, June 1998; S.R. Corsi, L.A. De Cicco, M.A. Lutz, and R.M. Hirsch, "River Chloride Trends in Snow-Affected Urban Watersheds: Increasing Concentrations Outpace Urban Growth Rate and Are Common Among All Seasons,"* Science of the Total Environment,*508:488-497, 2015; and J.A. Thornton, T.M. Slawski, and H. Lin, "Salinization: The Ultimate Threat to Temperate Lakes, with Particular Reference to Southeastern Wisconsin (USA),"* Chinese Journal of Oceanology and Limnology, *33:1-15, 2015.*

This Chapter presents the findings of a literature review of the impacts of chloride salts on biological systems. It discusses the effects of chloride on organisms, biological communities, and ecosystems. It does not address the impact of chloride on human health. Those impacts are discussed in Chapter 5 of this report.

3.2 EFFECTS ON ORGANISMS

Increased concentrations of chloride salts in the environment can adversely affect organisms. At the most extreme level, exposure to high enough concentrations of chloride can lead to the death of organisms. The concentration causing death differs for different types of organisms, reflecting differences in their biology. Exposure to lower concentrations of chloride salts can result in sublethal impacts to organisms such as effects on their growth, reproduction, and physiology.

This section reviews the effects of chloride salts on organisms. It includes discussion of acute and chronic toxicity. This section also discusses sublethal effects that different groups of organisms may experience from exposure to chloride.

This chapter and Appendix B of this report present results from field and experimental studies addressing impacts of chloride salts, salinity, and specific conductance on almost 200 different species and other taxa of organisms. This reflects the species that have been examined in the scientific literature for impacts from chloride salts. Of these species, 57 percent are found within Wisconsin. In addition, another 27 percent of the organisms belong to genera which are found in Wisconsin.

Toxicity

Toxicity is the ability of a substance or a mixture of substances to cause adverse effects to organisms. The types of adverse effects can differ depending on the toxic substance, the organism exposed, and the level of exposure. Examples of adverse effects caused by toxic substances include:

- Reduced somatic and population growth rates
- Reduced adult size
- The presence of developmental abnormalities

- Reduced rates of reproduction including lower rates of egg production, smaller clutch sizes, and lower hatching rates

- Altered behavior such as reduced rates of feeding, reduced swimming speeds, or changes in the amount of time spent on certain activities

- Death

Several factors can influence the type and severity of the effects of exposure to a toxic substance. The level of exposure or dose that an organism receives is a major factor affecting the impacts of a toxin. Higher doses often produce more severe adverse effects. In fact, some substances are non-toxic at lower doses, but become toxic at higher levels. The level of exposure that aquatic organisms are exposed to in water is often expressed as the concentration of the toxic substance in water. The manner of exposure to the toxic substance can also affect the type and severity of the substance's impacts. For instance, a toxic substance may produce different effects depending on whether it is ingested or absorbed through the skin. The number and duration of exposures can also affect the impacts of a toxic substance. Acute toxicity occurs when adverse effects result from a single or small number of exposures over a short period of time. Chronic toxicity occurs when adverse effects result from repeated or constant exposure over a longer period. Finally, environmental factors such as temperature and the presence of other substances can affect toxicity. The specific factors that affect the toxicity of chloride will be discussed later in this Chapter.

The State of Wisconsin has promulgated two surface water quality criteria for chloride, an acute toxicity criterion and a chronic toxicity criterion. These criteria are meant to ensure adequate protection of aquatic organisms from toxic effects. Under the acute toxicity criterion, the maximum daily concentration of chloride is not to exceed 757 milligrams per liter (mg/l)[2] more than once every three years. Under the chronic toxicity criterion, the four-day average of maximum daily concentration of chloride is not to exceed 395 mg/l more than once every three years. Surface waterbodies that exceed either of these criteria are considered impaired under Section 303(d) of the Federal Clean Water Act. In 2022, 35 waterbodies in southeastern Wisconsin were listed as impaired due to exceeding either or both chloride toxicity criteria. The impaired waterbodies are shown on Map 3.1 and listed in Table 3.1.

---

[2] *Acronyms and abbreviations used in this report are defined in Appendix A.*

Acute Toxicity of Chloride

Acute toxicity is often measured by the deaths of test organisms. For freshwater aquatic organisms, acute toxicity is often expressed as the LC50. This is the concentration at which 50 percent of the organisms die over the duration of the test.[3] A higher LC50 indicates lower toxicity to the organism, while a lower LC50 indicates greater sensitivity to the toxin. An LC50 value represents a substantial toxic effect to organism populations. While LC50 values are useful measures of acute toxicity, they do not represent thresholds below which concentrations are safe or harmless in aquatic habitats. It should be kept in mind that appreciable acute toxic effects can be expected to occur at concentrations that are lower than the LC50. In addition, appreciable acute toxicity effects may occur over shorter periods of time than the test period associated with a particular LC50. Because of this, it is important to recognize that evaluations of toxicity that utilize LC50s as an indicator of toxicity refer to concentrations at which substantial incidences of toxic effects are likely to be occurring, as opposed to concentrations at which toxic effects begin to appear. Figure 3.1 shows several aquatic organisms that are commonly used in acute toxicity testing. These organisms are used for testing because they are relatively easy to maintain in a laboratory setting and their biology has been well-studied.

The LC50s cited in the discussion that follows reflect the toxicity of individual, relatively pure chloride salts. The chlorides that are introduced into the environment in forms such as deicers, water softening salts, and fertilizers often contain other substances. For example, commercial deicers contain trace amounts of metals, anticaking ingredients, corrosion inhibitors, and other substances. One study found that sodium chloride-based deicers contained trace amounts of copper, zinc, cyanide, and sulfate.[4] Some of these other substances can cause acute toxicity in aquatic organisms at low concentrations. Unless otherwise stated, toxic effects related to the presence of these substances are not reflected in the LC50 values in the discussion in this section. Because of this, the LC50 values may not reflect the possibility of cumulative effects of mixtures of toxic substances. Despite these caveats, LC50 values are useful for comparing the effects of a toxic substance on different species, populations, or strains of organisms.

---

[3] *It should be noted that other measures of the level of acute toxicity are sometimes used. For example, the LC10 and LC25 endpoints reflect the concentrations at which 10 percent and 25 percent, respectively, of organisms die during the test.*

[4] *B. Mussato and T. Guthrie, "Anti-icers: Chemical Analysis and Toxicity Test Results," Prepared for Insurance Corporation of British Columbia, 2000, cited in Colorado Department of Transportation, "Evaluation of Selected Deicers Based upon a Review of the Literature," Report No. CDOT-DTD-R-2001-15, October 30, 2001.*

Tables 3.2 through 3.5 summarize reported LC50s for chloride for zooplankton, macroinvertebrates, fish, and amphibian species. The tables present results for several exposure times; however, for most groups the majority of the results come from 96-hour (four-day) acute toxicity tests. This is in keeping with standard toxicological procedures. The test results are presented in terms of the concentration of chloride that the organisms were exposed to. This was done to facilitate comparison of the toxicological data to estimates of chloride concentrations in surface waterbodies and to the State acute toxicity criterion for fish and aquatic life. Results from individual toxicity tests are given in Appendix B. In the discussion that follows, the LC50s will be expressed in terms of chloride concentrations.

Some patterns are apparent in values presented in Tables 3.2 through 3.5. There is considerable variation in LC50 values, even for the same species in the same duration test. For example, at test durations ranging between 24 hours and 96 hours, the reported LC50s for the water flea *Daphnia magna* range over two to three orders of magnitude (see Table 3.2). This wide range may be due to several factors, including differences in test conditions, differences in the cation associated with chloride, genetic variation within species, and differences among statistical techniques used to calculate the LC50 value from the raw toxicology data. Potential causes of the wide range of toxic impacts are discussed in the section on factors that can affect the toxicity of chloride.

For individual species, lower LC50s are generally associated with longer periods of exposure. Examples among macroinvertebrates that show this clearly include the brown dun mayfly (*Ameletus* sp.), the pond snail (*Lymnaea* sp.), and the European physa snail (*Physa heterostropha*) (see Table 3.3). This is also shown in the lowest reported LC50s for each test duration for the wood frog (*Lithobates sylvatica*) (see Table 3.5). The lowest reported LC50 values for wood frogs came from the same study and were conducted on tadpoles in the same developmental stage under the same test conditions.[5] It should be noted that the inherent toxicity of a substance like chloride does not change with longer exposure. Instead, longer exposures give the toxin more opportunity to cause damage. As a result, lethal toxic effects occur at lower concentrations with longer exposures.[6]

---

[5] *A.L. Copan,* Acute Toxicity of Deicing Compounds and Personal Care Products to Early Amphibian Life Stages, *Master's Thesis, Saint Mary's University, Halifax, Nova Scotia, Canada, 2016.*

[6] *D.W. Connell, Q.J. Yu, and V. Verma, "Influence of Exposure Time on Toxicity—An Overview,"* Toxicology, *355-356:49-53, 2016.*

Tables 3.2 through 3.5 also show that different groups of organisms have different sensitivities to chloride toxicity. In general, adult fish tend to be less sensitive to acute toxicity from chloride than zooplankton, macroinvertebrates, or amphibians. Among macroinvertebrates, mayflies (Order Ephemeroptera) appear to be more sensitive to acute toxicity from chloride than other insects such as flies (Order Diptera) or caddisflies (Order Trichoptera). Similar variation is seen within these larger groups of organisms. With a 96-hour LC50 of 425, the gray quill mayfly *Callibaetis coloradensis* appears to be particularly sensitive to acute chloride toxicity. Along the same lines, caddisflies in the genus *Hydropsyche* appear to be much less sensitive to acute chloride toxicity in 96-hour tests than other species of caddisflies.

Many of the LC50s shown in Tables 3.2 through 3.5 are lower than Wisconsin's acute toxicity criterion of 757 mg/l. This is seen in several groups such as zooplankton including species of *Ceriodaphnia, Daphnia*, and *Nitocra*; macroinvertebrates including some mayflies (*Centroptilium triangulifer)*, snails (*Lymnaea* sp., and *Melanoides tuberculate)*, and bivalves (*Dreissena polymorpha, Epioblasma torulosa, Lampsilis fascicola, and Lampsilis siliquoidea)*; fish including golden shiners (*Notemigonus crysolucas)* and young walleye (*Stizostedion vitreum*)*,* rainbow trout (*Onchorhynchus mykiss*), and sanger (*Stizostedion canadense*); and amphibians including tadpoles of wood frog (*Lithobates sylvatica*) and boreal toad (*Bufo boreas*). While these LC50s derive from laboratory studies, the results suggest that some species may be experiencing substantial toxic effects at chloride concentrations below Wisconsin's acute toxicity criterion. This in turn suggests that the current acute toxicity criterion in Wisconsin might not be fully protective of aquatic life.

Chronic Toxicity of Chloride

Acute toxicity is not the only toxic effect associated with chloride. While chronic exposures to chloride can result in the deaths of organisms, these exposures have also been shown to produce a variety of sublethal effects in different aquatic organisms. These effects fall into several categories including growth and development, reproduction, and behavior.

Chronic exposure to chloride salts can have several impacts on the growth and development of organisms. The simplest may be that chronic exposure can slow organismal growth. For example, one study found that chronic exposure to high levels of salinity reduced the rate of somatic growth in two mayfly species and one midge species.[7] Reduced growth rates resulting from chronic exposure to chloride salts has also been

---

[7] *K.L. Hassell, B.J. Kefford, and D. Negegoda, "Sub-lethal and Chronic Salinity Tolerances of Three Freshwater Insect:* Cloeon *sp. and* Centroptilum *sp. (Ephemeroptera: Baetidae) and* Chironomus *sp. (Diptera: Chironomidae),"* Journal of Experimental Biology, *209:4,024-4,034, 2006.*

observed as slower growth of portions of an organism, such as plant roots[8] or mussel shells,[9] and lower weights attained after a standard period of growth.[10] Some developmental effects may be more subtle. For instance, chronic exposure to chloride salts can alter the duration of the larval period of some organisms.[11] This can lead to delayed maturity[12] or failure to metamorphose or enter the next life history stage.[13] Finally, chronic exposure to chloride salts can result in organisms having developmental abnormalities.[14]

Chronic exposure to chloride salts can also affect reproduction and several different outcomes have been reported. Chronic exposure to sodium chloride has been reported to inhibit fertilization in some fish species.[15] Chronic exposure to chloride salts has also been reported to reduce hatching success in amphibians.[16] Chronic exposure to chloride salts can also lower the number of offspring produced by a female.[17] This can occur in a number of ways including reducing the number of eggs produced per female,

---

[8] *A. .Joutti, E. Schultz, P. Pessala, T Nysten and P. Hellsten, "Ecotoxicity of Alternative Deicers,"* Journal of Soils and Sediments, *3:269-372, 2003.*

[9] *R.B. Bringolf, W.G. Cope, C.B. Eads, P.R. Lazaro, M.C. Barnhart, and D. Shea, "Acute and Chronic Toxicity of Technical-Grade Pesticides to Glochidia and Juveniles of Freshwater Mussels,"* Environmental Toxicology and Chemistry, *26:2,086-2,093, 2007.*

[10] *J.A. Buckley, K.P. Rustagi, and J.D. Laughlin, "Response of* Lemnia minor *to Sodium Chloride and a Statistical Analysis of Continuous Measurements for EC50 and 95% Confidence Limits Calculation,"* Bulletin of Environmental Contamination and Toxicology, *57:1,003-1,008, 1996.*

[11] *S. Collins,* Toxicity of Deicing Salt Components to Early Amphibian Life Stages, *Master's Thesis, Saint Mary's University, Halifax, Nova Scotia, Canada, March 2010.*

[12] *E.C. Freitas, and O. Rocha, "Effects of Sodium and Potassium on Life History Parameters of Freshwater Cladoceran* Pseudosida ramose," Journal of the Brazilian Society of Ecotoxicology, *7:85-91, 2012.*

[13] *Collins 2010,* op. cit.

[14] *S.J. Collins and R.W. Russell, "Toxicity of Road Salt to Nova Scotia Amphibians,"* Environmental Pollution, *157:320-324, 2009.*

[15] *U. Mahrosh, M. Kleiven, S. Meland, B.O. Rosseland, B. Salbu, and H.-C. Teien, "Single and Multiple Stressor Effect of Road Deicing Salt (NaCl) and Copper (Cu) to Fertilization and Early Development Stages of Atlantic Salmon (*Salmo salar*) Alevins from Hatching to Swim-up,"* Journal of Environmental Sciences, *66:368-378, 2018.*

[16] *Collins 2010,* op. cit.

[17] *A.L. Copan, 2016,* op. cit.

the number of live offspring produced per female, or the number of eggs produced in a single brood.[18] Chronic exposure to chloride salts can also have more subtle effects on reproduction. For example, chronic chloride exposure can result in an increase in the age at which members of a species first reproduce.[19] This tends to lower the total number of offspring that a female produces over her lifetime.

Chronic exposure to chloride can affect behavior in ways that reduce the viability of the affected organisms. For instance, a laboratory study showed that over longer exposures, the level of activity in European grass frog (*Rana temporaria*) tadpoles decreased with increasing sodium chloride concentration.[20] At high concentrations, the tadpoles moved shorter distances and more slowly than at low concentrations. Since low activity levels in tadpoles have been associated with a lower probability of escaping from predators and may limit the foraging capabilities of the tadpoles,[21] this effect could reduce the viability of amphibian populations exposed to chloride in some environments. Other organisms have been reported to reduce feeding when exposed to higher concentrations of chloride salts. For example, *Anodonta anatino*, a freshwater mussel that feeds by filtering particles out of the water, reduces its filtration activity under higher sodium chloride concentrations.[22] Similarly, when exposed to higher concentrations of sodium chloride, Asian clams (*Corbicula fluminea*) closed their shells.[23] This reduces feeding and allows metabolic wastes to build up which can ultimately reduce the viability of the clams.

Chronic toxicity can have many different effects on organisms including lethal and sublethal effects. In addition, different types of organisms can experience different toxic effects from chloride. Sublethal impacts

---

[18] *E.C. Freitas, and O. Rocha, "Acute and Chronic Effects of Sodium and Potassium on the Tropical Freshwater Cladoceran* Psuedosida ramose," Ecotoxicology, *20:88-96, 2011.*

[19] *Frietas and Rocha 2012.* op. cit.

[20] *M. Denoël, M. Bichot, G.F. Ficetola, J. Delcourt, M. Ylieff, P. Kestermont, and P. Poncin, "Cumulative Effects of Road De-Icing Salt on Amphibian Behavior,"* Aquatic Toxicology, *99:275-280, 2010.*

[21] *T.B. Watkins, "Predator-Mediated Selection on Burst Swimming Performance in Tadpoles of the Pacific Tree Frog,* Pseudacris regilla" Physiological Zoology, *69:154-167, 1996; C, Tepelitsky, S. Laenet, J.-P. Lena, N. Mermet, E. Malet, and P. Joly, "Escape Behavior and Ultimate Causes of specific Induced Defenses in an Anuran Tadpole,"* Journal of Evolutionary Biology, *18:180-190, 2005.*

[22] *J.T. Hartmann, S. Beggel, K. Auerswald, B.C. Stoeckle, and J. Geist, "Establishing Mussel Behavior as a Biomarker in Ecotoxicology,"* Aquatic Toxicology, *170:279-288, 2016.*

[23] *K.D. Coldsnow and R.A. Relyea, "Toxicity of Various Road-Deicing Salts to Asian Clams (*Corbicula fluminea*),"* Environmental Toxicology and Chemistry, *37:1,839-1,845, 2018.*

of chloride salts on organisms that are due to or likely due to chronic toxicity are discussed in the sections of this Chapter on the effects of chloride on freshwater and terrestrial organisms.

Factors that Affect the Toxicity of Chloride

As previously mentioned, laboratory data shows that there can be considerable variation in the LC50s for chloride for an individual species from a test of a given duration (see Tables 3.2 through 3.5). This variation reflects differences in test conditions and may be due to environmental factors related to test conditions, other constituents of the salts tested, or biological factors related to the organisms tested. These are described in more detail in the following sections.

Environmental Factors

Several environmental factors can affect the toxicity of chloride. While these factors can be controlled in laboratory toxicity tests, they often vary in nature. As a result, they can alter the impact of chloride on populations of organisms. These factors include temperature, water hardness, the presence of other chemicals, and the nutritional status of the organisms.

Temperature

The water temperature at which aquatic organisms are exposed to chloride salts can influence the toxicity of chloride. For example, Table 3.6 shows LC50 values for four species of mayfly larvae exposed to sodium chloride (NaCl) for 96 hours at seven different water temperatures in a laboratory study.[24] In three species, the maximum LC50, or the greatest tolerance of chloride, occurs at an intermediate temperature. The temperature at which greatest tolerance occurs differs among the species, with *Neocloeon triangulifer* showing its highest tolerance at a relatively cold temperature and *Leptophlebia cupida* showing its highest tolerance at a much warmer temperature. Highest tolerance at an intermediate temperature may not have been observed in *Procloeon fragile* because the toxicity of chloride was not tested at the two lowest temperatures in the study. At temperatures higher than the temperature at which maximum tolerance to chloride was observed, the toxicity of chloride increased rapidly as temperature increased. At the highest temperature tested, the LC50 chloride concentrations represented between 3 and 27 percent of the concentrations at the temperatures where each species showed maximum tolerance.

---

[24] *J.K. Jackson and D.H. Funk, "Temperature Affects Acute Mayfly Responses to Elevated Salinity: Implication for Toxicity of Road De-icing Salts,"* Philosophical Transactions of the Royal Society, Series B, *374:20150084, doi: 10.1098/rstb.2018.0081, 2018.*

Above the temperature of maximum chloride tolerance, the LC50s for sodium chloride for the mayfly species shown in Table 3.6 decreased exponentially as temperature increased. The amount of decrease is consistent with a general pattern in observed in the acute toxicity of many substances in which an 18°F increase in temperature often leads to a two-to-four-fold reduction in the LC50.[25]

It should be noted that most toxicity tests for chloride and chloride salts are conducted at temperatures between 63°F and 77°F, which are recommended as standard temperatures for toxicity testing. Low water temperatures during the winter may reduce the frequency of chloride-related toxicity events below what would be expected with winter deicing activities. Despite this, chloride concentrations during the winter can still get high enough to cause toxic effects even when accounting for the effects of low temperatures.[26]

This effect of temperature on the toxicity of chloride has implications for the well-being of aquatic organisms in southeastern Wisconsin under potential future conditions. There has been a long-term trend toward increasing chloride concentrations in many waterbodies of the Southeastern Wisconsin Region.[27] Climate projections for southeastern Wisconsin indicate that average air temperatures the Region will be warmer in the future, with average air temperatures at the end of the century being five to 10 degrees Fahrenheit warmer than current conditions. This change depends on the carbon emissions scenario used to produce the projection.[28] Projected air temperatures are likely to increase water temperatures in many waterbodies in the Region. For example, projections generated by the U.S. Geological Survey's FishVis decision support tool predict that water temperatures in all modeled streams of the Oak Creek watershed will increase by up to 3.6°F by the end of the century.[29] These projected temperature increases may be further exacerbated by the loss of shade along the Oak Creek Parkway due to the ongoing decline of the

---

[25] *F.L. Mayer Jr., and M.R. Ellersieck, "Experiences with Single-Species Tests for Acute Effects on Freshwater Animals,"* Ambio, *17:367-375, 1988.*

[26] *Jackson and Funk 2018,* op. cit.

[27] *SEWRPC Technical Report No. 63,* Chloride Conditions and Trends in Southeastern Wisconsin, *September 2023.*

[28] *Wisconsin Initiative on Climate Change Impacts,* Wisconsin's Changing Climate: Impacts and Solutions for a Warmer Climate, *2021.*

[29] *J.S Stewart, S.A. Covert, N.J. Estes, S.M. Westenbroek, D. Krueger, D.J. Wieferich, M.T. Slattery, J.D. Lyons, J.E. McKenna, Jr, D.M. Infante, and J.L. Bruce,* FishVis, A Regional Decision Support Tool for Identifying Vulnerabilities of Riverine Habitat and Fishes to Climate Change in the Great Lakes Region, *U.S. Geological Survey Scientific Investigations Report No. 2016-5124, 2016.*

ash tree canopy and subsequent spread of invasive buckthorn and reed canary grass.[30] Should these predicted water temperature increases occur, it could result in the incidence and severity of chloride toxicity impacts being greater than what would be expected otherwise.

Water Hardness

The hardness of the water in which aquatic organisms are exposed to chloride can affect the toxicity of chloride. Hardness is an indicator of the mineral content of water and measures the combined concentrations of ions of calcium, magnesium, and several other metals. The main components of hardness are calcium and magnesium ions. Because the relative concentrations of the constituents of hardness can vary, measurements of hardness are often reported as an equivalent concentration of milligrams per liter of calcium carbonate (mg/l as $CaCO_3$).

Table 3.7 shows an example of the effect of hardness on the acute toxicity of chloride from a laboratory study.[31] In 48-hour toxicity tests using the water flea *Ceriodaphnia dubia*, the LC50 increased with increasing hardness, indicating that the water fleas were less sensitive to chloride in harder water. The same study observed a similar effect of hardness on acute chloride toxicity in 96-hour tests using the freshwater clam, *Sphaerium simile*, and the annelid sludge worm, *Tubifex tubifex*. The study did not observe a difference using the snail *Gyraulus parvus*; however, the range of hardness used in these tests was narrow, extending between 56 and 212 mg/l as $CaCO_3$.

The mechanism through which higher hardness reduces sodium chloride toxicity is not known. Calcium ions have been shown to reduce the permeability of cell membranes to both water and other ions. This has been

---

[30] *SEWRPC Community Assistance Planning Report No. 330,* A Restoration Plan for the Oak Creek Watershed, *December 2021.*

[31] *D.J. Soucek, T.K. Linton, C.D. Tarr, A. Dickinson, N. Wickramanayake, C.G. Delos, and L.A. Cruz, "Influence of Water Hardness and Sulfate on the Acute Toxicity of Chloride to Sensitive Freshwater Invertebrates,"* Environmental Toxicology and Chemistry, *30:930-938, 2011.*

shown in studies with fish[32] and invertebrates.[33] Such an effect may reduce passive diffusion of chloride into the cells of these animals or reduce the energy that they require to regulate their ionic content. Alternatively, the decrease in chloride toxicity that occurs with increasing hardness may be related to the maintenance of a tolerable ratio of cations within the organism rather than any mechanistic effect of hardness itself.[34]

Presence of Other Chemicals

The concentrations of other chemicals in the water may also affect the toxicity of chloride to aquatic organisms. Table 3.8 shows that the toxicity of chloride to the water flea *Ceriodaphnia dubia* increased slightly as the concentration of sulfate in the water increased.[35] A similar effect of chloride concentration on the toxicity of sulfate to both *Ceriodaphnia dubia* and the amphipod *Hyalella azteca* has been reported in which higher chloride concentrations resulted in lower LC50s for sulfate.[36] This suggests that the toxic effects of these two ions to these organisms are additive, with the magnitude of the total impact being equal to the sum of the individual effects of the ions.

The toxicity of chloride salts may sometimes increase with declining concentrations of other substances. Studies with the water flea *Daphnia magna* showed that the toxicity of both sodium chloride and calcium chloride increased as the concentration of dissolved oxygen in the water decreased.[37] This increase in toxicity likely reflects the organisms experiencing the combined effects of the two stressors.

---

[32] *F.B. Eddy, "The Effect of Calcium on Gill Potentials and on Sodium and Chloride Fluxes in the Goldfish,* Carassium auratus*,"* Journal of Comparative Physiology, *96:131-142, 1975; P. Pic and J. Maetz, "Role of External Calcium in Sodium and Chloride Transport in the Gills of Seawater-adapted* Mugil capito*,"* Journal of Comparative Physiology B, *141:511-521, 1981; W.T.W. Potts and W.R. Flemming," The Effects of Prolactin and Divalent Ions on the Permeability to Water of* Fundulus kansae*,"* Journal of Experimental Biology, *53:317-327, 1970.*

[33] *J.D. Robertson, "The Function and Metabolism of Calcium in the Invertebrata,"* Biological Reviews, *16:106-133, 1941.*

[34] *J.R.F. Elphick, K.D. Bergh, and H.C. Bailey, "Chronic Toxicity of Chloride to Freshwater Species: Effect of Hardness and Implications for Water Quality Guidelines,"* Environmental Toxicology and Chemistry, *30:239-246, 2011.*

[35] *Soucek et al. 2011,* op. cit.

[36] *D.J. Soucek, "Comparison of Hardness- and Chloride-Regulated Acute Effect of Sodium Sulfate on Two Freshwater Crustaceans,"* Environmental Toxicology and Chemistry, *26:773-779, 2007.*

[37] *E.J. Fairchild II,* Effects of Lowered Oxygen Tension on the Susceptibility of *Daphnia magna* to Certain Inorganic Salts, *Ph.D. Dissertation, Louisiana State University, Baton Rouge, Louisiana, 1954.*

Test Organism Nutritional Status

In general, the toxicity of a chemical to an aquatic organism is influenced by the availability of its food supply.[38] At the same concentration of the toxicant, LC50s and other toxicology endpoints show greater toxicity when food is less available or when organisms are under nutritional stress than at higher levels of food availability.

A laboratory study using a uniclonal hybrid of two *Daphnia* species found a strong positive linear relationship between the concentration for available food and the LC50 for chloride in 14-day toxicity tests.[39] The study also found similar linear relationships between food availability and chronic toxicity endpoints such as age at first reproduction, clutch size, total egg production, and growth rate. Similar food supply impacts were seen when the organisms were exposed to sodium chloride and calcium chloride, though at equivalent concentrations of chloride the LC50s were lower for calcium chloride than sodium chloride. The range of food levels used in this laboratory study spanned the ranges seen in lakes, including concentrations that are similar to those in oligotrophic, mesotrophic, and eutrophic lakes. The findings of the study suggest that organisms living in oligotrophic systems might be particularly vulnerable to the toxic effects from chloride.

The relationship between the toxicity of chloride and the availability of food may reflect the impact of nutritional status on the ability of an organism to repair itself. An organism that is exposed to a toxicant such as chloride needs energy to fuel repair mechanisms and compensate for changes in osmotic balance.[40] The greater the exposure to the toxicant, the more energy, and thus the more food, the organism needs to counteract damage. Thus, the increased toxicity of chloride to *Daphnia* at low food levels may be the result of a decreased ability of the organism to pay the energy costs needed to repair itself at low food levels.

---

[38] *E.H.W. Heugens, A.J. Hendrick, T. Dekker, N.M. van Straalen, and W. Admiraal, "A Review of the Effects of Multiple Stressors on Aquatic Organisms and Analysis of Uncertainty Factors for Use in Risk Assessment,"* Critical Reviews in Toxicology, *31:247-284, 2001.*

[39] *A.H. Brown and N.D. Yan, "Food Quantity Affects the Sensitivity of* Daphnia *to Road Salt,"* Environmental Science and Technology, *49:4,673-4,680, 2015.*

[40] *H.J. Geyer, C.E. Steinberg, I. Scheunert, R. Brüggemann, W. Schütz, A. Kettrup, and K. Rozman, "A Review of the Relationship between Acute Toxicity (LC50) of g-Hexachorocyclohexane (g-HCH, Lindane) and Total Lipid Content of Different Fish Species,"* Toxicology, *83:169-179, 1993.*

Other Salt Ingredients and Impurities

Other substances in chloride salts may affect their overall toxicity. Chloride salts produced for some uses are relatively pure. Examples of this include sodium chloride produced for use as table salt or water softener salt. Other chloride salts may include additives or impurities that can produce their own toxic effects. Examples of these include the cation associated with the chloride ion, chemicals added to a salt to prevent caking or to reduce the salt's corrosive effects, and impurities in the salt.

Cations

The cation associated with chloride can have a major effect on the toxicity experienced by organisms. Reviews of laboratory toxicity studies have found that magnesium chloride and calcium chloride are far more toxic to many aquatic organisms than sodium chloride and can produce impacts at lower concentrations.[41] For example, a review found that sodium chloride-based deicers have lower toxicity to rainbow trout (*Onchrhynchys mykiss)*, the water flea *Ceriodaphnia dubia*, and the alga *Selenastrum capricornatum* than other chloride-based deicers such as calcium chloride and magnesium chloride and acetate-based deicers.[42] Most of the studies reviewed showed that magnesium chloride was particularly toxic to many organisms. The reviews found that the general order of toxicity of chloride salts from least toxic to most toxic is sodium chloride, calcium chloride, magnesium chloride, and potassium chloride. It is important to note that species differ in their tolerance to chloride associated with various cations and some deviate from the patterns discussed in the review articles. Table 3.9 shows that the relative toxicities experienced by Asian clam *Corbicula fluminea* when exposed to different chloride salts follow the pattern described in the reviews. Tables 3.10 and 3.11 show that patterns of LC50s for chloride associated with different cations for the water flea *Daphnia magna,* and the diatom, *Nitzchia*, respectively are different than those seen in the Asian clam.

In addition to the cation associated with chloride, the toxicity of different chloride salts can be affected by the presence of other cations in the water. A laboratory study of the toxicity of major ions to the water flea

---

[41] *D.A. Benoit, and C.E. Stephan,* Ambient Water Quality Criteria for Chloride, *U.S. Environmental Protection Agency EPA 440/5-88-001, February 1988; M. Evans and C. Frick,* The Effects of Road Salt on Aquatic Ecosystems, *National Water Research Institute Contribution Series No. 02-038, Saskatoon, Saskatchewan, Canada, 2001.*

[42] *B. Mussato and T. Guthrie 2000,* op. cit.

Ceriodaphnia dubia found a complex relationship among the toxicities of different cations.[43] This study found that the toxicity of sodium ions and magnesium ions to this water flea decreased as the concentration of calcium ions in the water increased. In addition, the toxicity of potassium ions to this species decreased as the concentration of sodium ions increased. As with hardness, these relationships among the toxicities of different cations may be related to maintaining a tolerable internal ratio of cations;[44] however, the mechanisms responsible for toxicity appears to be different among salts with different cations.[45] The toxicity of potassium and magnesium salts may be primarily due to the toxicity of the cation. The toxicity of sodium salts appears to be due to both the toxicities of the cation and the anion. In *Ceriodaphnia dubia*, this appears to be related, at least in part, to osmotic stress caused by the salt. It is also likely that the toxicity of calcium salts is due to the cation; however, since the presence of calcium may ameliorate chloride toxicity, this is less certain.

As previously noted, these comparisons of the effects of the cations associated with chloride are based on toxicity assessments conducted in the laboratory. An important caution to note in interpreting these toxic effects to organisms is that they do not account for any differences in how these compounds are used. For example, in a highway deicing situation it is possible that a more toxic salt may be used in lower amounts which might produce fewer toxic effects. The difference in the amount of chloride salts with different cations that are used should always be considered when evaluating potential toxic effects.

Anticaking Additives

When deicing salt is exposed to air with fluctuating humidity, it can form large clumps that make spreading difficult.[46] When the relative humidity exceeds about 70 percent, a brine solution forms on the surface of the salt crystals.[47] This brine evaporates when the relative humidity drops, causing the salt in the brine to recrystallize which causes the salt crystals to clump together.

---

[43] *D.R. Mount, R.J. Erickson, T.L. Highland, J.R. Hockett, D.J. Hoff, C.Y. Jenson, T.J. Norberg-King, K.N. Peterson, Z.M. Polaske, and S. Wisniewski, "The Acute Toxicity of Major Ion Salts to* Ceriodaphnia dubia*: I. Influence of Background Water Chemistry,"* Environmental Toxicology and Chemistry, *35:3,039-3,057, 2016.*

[44] *Elphick et al. 2011,* op. cit.

[45] *Mount et al. 2016,* op. cit.

[46] *H.W. Fiedelman,* Prussian Blue and Sodium Ferrocyanide Additives for Deicing Salt, *Report No. 4, 930-012, Morton Salt Company, Woodstock, Illinois, 1971.*

[47] *F. Gotzfried,* Ferrocyanides as Anticaking Agents in Road Salt, *German Salt Industry Association, Bonn, Germany, 1995.*

Anticaking agents are often added to deicing salts to prevent clumping. While chromate and phosphate compounds are occasionally added to deicing salts for this purpose, the most common additives consist of iron cyanide compounds that are either sprayed onto the salt crystals or added to the brine solutions used to manufacture deicing salts. These iron cyanide compounds include sodium ferrocyanide ($Na_4Fe(CN)_6$), also known as yellow prussiate of soda, and ferric ferrocyanide ($Fe(Fe_3(CN)_6)_3$), also known as Prussian blue. These substances reduce the solubility of sodium chloride in the moisture adsorbed to the deicer, reducing recrystallization of the salt as humidity drops.

When road salt is stored or applied, ferrocyanide anticaking agents will dissolve with the salt. As a result, surface waters adjacent to salt storage facilities or heavily salted highways can be contaminated with ferrocyanides. These compounds are relatively nontoxic;[48] however, exposure to light can induce ferrocyanides in water to dissociate to release free cyanide in the forms of hydrogen cyanide (HCN) and cyanide ions (CN^-).[49] Ultimately, all of the cyanide contained in the iron-cyanide compounds can be released as free cyanide[50] which is highly toxic.

Cyanide contamination has been reported in runoff from salt piles. One study found that concentrations of total cyanide in runoff from road salt piles ranged from below the limit of detection to 200 micrograms per liter (µg/l).[51] Total cyanide includes both ferrocyanides and free cyanide. The same study reported that concentrations of free cyanide in runoff from salt piles ranged from below the limit of detection to 96 µg/l.

Contamination of runoff with cyanide compounds can lead to contamination of surface waterbodies and groundwater. For example, a study found that concentrations of total cyanide in Lincoln Creek in the City

---

[48] *Agency for Toxic Substances and Disease Registry,* Toxicological Profile for Cyanide, *U.S. Department of Health and Human Services, July 2006.*

[49] *J.L. Meeussen, M.G. Keizer and F.A.M. de Haan, "Chemical Stability and Decomposition Rate of Iron Cyanide Complexes in Soil Solutions,"* Environmental Science and Technology, *26:511-516, 1992.*

[50] *D. Kuhn and T.C. Young, "Direct Photolysis of Hexacyanoferrate (II) Under Conditions Representative of Surface Waters,"* Chemosphere, *60:1,222-1,230, 2005.*

[51] *T. Ohno,* Determination of Levels of Free Cyanide in Surface and Ground Waters Affected by MDOT Salt Storage Facilities, *Maine Department of Transportation Technical Report 86 C, 1989.*

of Milwaukee ranged from below the limit of detection to 130 µg/l.[52] This study did not assess concentrations of free cyanide in the Creek.

Free cyanide is highly soluble in water and highly toxic. It is readily taken up by aquatic organisms through skin and gills.[53] Free cyanide can cause both acute and chronic toxic effects in aquatic organisms. Geometric means of LC50s for freshwater animal species in 96-hour acute toxicity tests ranged between 59 and 330 µg CN^-/l.[54] Coldwater fish were the most sensitive species to acute cyanide toxicity. This was followed in order of decreasing toxicity, by warmwater fish, cladoceran zooplankton, and aquatic insects. While some exceptions have been reported, organisms receiving a sublethal acute dose of free cyanide generally recover because they are able to detoxify it by converting the cyanide ion to thiocyanate (SCN^-).[55]

Cyanide toxicity occurs because it interferes with cellular respiration. Cyanide binds to and inactivates cytochrome c oxidase, an important enzyme in respiration. This results in cellular hypoxia which leads to respiratory arrest and death. Cyanide does not bioaccumulate nor is it magnified through the food web.

Chronic exposure to cyanide can also cause adverse effects in freshwater organisms. Chronic exposure to cyanide concentrations of 5-10 µg/l interfered with reproduction in trout and salmon.[56] Exposure to cyanide

---

[52] *V. Novotny, D. Muehring, D.W. Smith, and R. Facey, "Impact of Urban Nonpoint Source Snowmelt on Receiving Waters," Paper Presented at the 70th Annual Water Environment Federation Conference, Chicago, Illinois, 1997.*

[53] *R. Eisler,* Cyanide Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review, *Biological Report No. 85, U.S. Fish and Wildlife Service, 1991.*

[54] *R.W. Gensemer, D.K. DeForest, A.J. Stenhouse, C.J. Higgins, and R.D. Cardwell, "Aquatic Toxicity of Cyanide," Chapter 14 In: D.A. Dzombak, R.S. Ghosh, and G.M. Wong-Chong, (editors),* Cyanide in Water and Soil: Chemistry, Risk, and Management, *Taylor & Francis, Boca Raton, Florida, 2006.*

[55] Ibid.

[56] *A. Szabo, S.M. Ruby, F. Rogan, and Z. Amit, "Changes in Brain Dopamine Levels, Oocyte Growth and Spermatogenesis in Rainbow Trout,* Onchorhyncus mykiss, *Following Sublethal Cyanide Exposure,"* Archives of Environmental Contamination and Toxicology*: 21152-157, 1991.*

led to reduced egg production in fathead minnows.[57] Cyanide exposure also reduced the length of time over which yearling coho salmon were able to swim against a current.[58]

The State of Wisconsin has established acute and chronic toxicity water quality criteria for free cyanide. These criteria are meant to ensure adequate protection of aquatic organisms from toxic effects and are shown in Table 3.12. Surface waterbodies that exceed either of these criteria are considered to be impaired under Section 303(d) of the Federal Clean Water Act. In 2022, no waterbodies in southeastern Wisconsin were listed as impaired for exceeding cyanide water quality standards.

Anti-Corrosion Additives

Corrosion inhibitors are added to some formulations of chemical deicers to reduce or prevent corrosion of metallic material that the deicers come into contact with. An analysis of the composition of 11 deicing compounds found that these additives mostly consist of organic compounds; however, the exact composition of some is not certain because of the proprietary nature of the formulations.[59] Some corrosion inhibitors are derived from sugar cane, sugar beets, corn, barley, or milk. Others contain organic amines such as triethanolamine. Many of these corrosion inhibitors are nontoxic, but some contain compounds that contribute to high biochemical oxygen demand (BOD). Reported levels of BOD associated with corrosion inhibitors in some deicers were as high as 83,000 milligram BOD per kilogram deicer (mg/kg).[60] Microbial decomposition of these compounds can reduce oxygen concentrations in surface waters, which can result in adverse impacts to aquatic organisms.

Decomposition of those corrosion inhibitors that contain amines can lead to the release of ammonia, which is toxic to aquatic organisms. The State of Wisconsin has promulgated acute and chronic water quality criteria for ammonia. The values of these are based on a formula that takes ambient pH and water temperature into account. This formula can be found in Chapter NR 105, Surface Water Quality Criteria and Secondary Values for Toxic Substances, of the *Wisconsin Administrative Code*.

---

[57] *D.T. Lind, L.L. Smith, and S.J. Broderius, "Chronic Effects of Hydrogen Cyanide on the Fathead Minnow,* Journal of the Water Pollution Control Federation, *49:262-268, 1977.*

[58] *S.J. Broderius,* Determination of Molecular Hydrocyanic Acid in Water and Studies of the Chemistry and Toxicity to Fish of the Nickelcyanide Complex," *Master's Thesis, Oregon State University, Corvallis Oregon, 1970.*

[59] *M. Fischel,* Evaluation of Selected Deicers Based on a Review of the Literature, *Report No. CDOT-DTD-R-2001-15 to the Colorado Department of Transportation, October 30, 2001.*

[60] Ibid.

Impurities

Some chloride salts used for deicing also contain various impurities. A review examining the chemical composition of 11 deicers found several contaminants that in high enough concentrations could have adverse effects on aquatic biota.[61] For example, the review found that a solid, sodium chloride-based deicer contained 0.29 mg/kg copper and 10 mg/kg zinc. Copper and zinc were also found in a brine deicer consisting of 23 percent sodium chloride, at concentrations of 0.78 mg/l and 2 mg/l, respectively.

The review also examined four magnesium chloride and calcium chloride deicers. High concentrations of arsenic, cadmium, copper, lead, and zinc were found in some magnesium chloride-based deicers. Some of these deicers also contained detectible amounts of chromium and barium, and others contained high levels of nitrates and/or ammonia. While the one calcium chloride-based deicer tested contained low concentrations of metals, it contained high concentrations of nitrates.

There have been relatively few studies of the combined toxic effects of chloride salts and heavy metals on organisms.[62] Depending on the underlying mechanisms of toxicity, chloride salts and heavy metals could have additive toxic effects in which the total effect is the sum of the effects of the two toxicants, synergistic effects in which the total effect is greater than the sum of the individual effects of the two toxicants, or antagonistic effects in which the total effect is less than the sum of the effects of the two toxicants. A study of salmon egg development found that the combined toxic effects of road salts and copper were more severe than the effects of each alone.[63] It is unknown if this result applies to other organisms.

Toxic impacts of heavy metal impurities in salts would be in addition to the impacts of any metals that were mobilized from soil or sediment by the salts. This topic was discussed in Chapter 2 of this Report.

The State of Wisconsin has promulgated acute and chronic water quality criteria for several of the metals that were detected in these deicer formulations. The criteria are based on formulas that take ambient water

---

[61] Ibid.

[62] *M.S. Schuler and R.A. Relyea, "A Review of the Combined Threats of Road Salts and Heavy Metals to Freshwater Systems,"* BioScience*, 68:327-335, 2018.*

[63] *U. Mahrosh, M. Kleiven, S. Meland, B.O. Rosseland, B. Salbu. And H.-C. Teien, "Toxicity of Road Deicing Salt (NaCl) and Copper (Cu) to Fertilization and Early Development Stages of Atlantic Salmon* (Salmo salar*),"* Journal of Hazardous Materials, *280:331-339, 2014.*

hardness into account. These formulas can be found in Chapter NR 105, Surface Water Quality Criteria and Secondary Values for Toxic Substances, of the *Wisconsin Administrative Code*.

Biological Factors

Biological factors can also affect the toxicity of chloride to organisms. Within a species, some developmental or life history stages may be more sensitive to the effects of chloride salts than others. In addition, genetic variation among organisms within a species may make some individuals more sensitive to and other individuals more tolerant of chloride salts.

Developmental Stage

The toxicity of chloride to many organisms is affected by the developmental or life history stage that the organism is in when it is exposed. Organisms in younger stages are often more sensitive to chloride than older individuals. For example, younger and smaller mayflies are more sensitive to toxic effects from sodium chloride than older individuals.[64] Similarly, juvenile water fleas in the species *Daphnia carinata* showed higher mortality rates than adults when exposed to chloride concentrations between 100 mg/l and 1,500 mg/l.[65]

The developmental stage during which aquatic vertebrates are exposed can have a major effect on the toxicity of chloride. Table 3.13 shows LC50s for five developmental stages of wood frog (*Lithobates sylvatica*) tadpoles that were exposed to sodium chloride for 72 hours.[66] Gosner stages are levels in tadpole development. Stage 19 tadpoles are late-stage embryos that have begun to develop gills and tails. They still have a large yolk sac and have not begun to feed. Stage 33 tadpoles are actively feeding tadpoles. They have reduced gills and prominent hind limb buds. Toes are beginning to form on these limb buds. LC50s for wood frog tadpoles increase over the course of development indicating that that the older stages are less sensitive to chloride than the younger stages.

[64] *J.M. Diamond, E.L. Winchester, D.G. Macker, and D. Gruber, "Use of the Mayfly* Stenonema modestum *(Heptageniidae) in Subacute Toxicity Assessment,"* Environmental Toxicology and Chemistry, *11:415-425, 1992.*

[65] *C.J. Hall and C.W. Burns, "Mortality and Growth Responses of* Daphnia carinata *to Increases in Temperature and Salinity,"* Freshwater Biology, *47:451-458, 2002.*

[66] *A.L. Copan, 2016,* op. cit.

Early life history stages of freshwater fish are also more sensitive to chloride. Table 3.14 shows LC50s for four stages of Rohu carp (*Labeo rohita*) exposed to calcium chloride for 96 hours.[67] Spawn are larval fish that have hatched, but still have a yolk sac and do not feed. Fry are young fish that have absorbed their yolk sacs and have begun feeding. Calcium chloride is highly toxic to eggs of this fish species. As the fish develops after hatching, its tolerance of calcium chloride increases. Fingerling Rohu carp are much more tolerant of chloride than earlier life stages.

Under some circumstances, the sensitivity of early life history stages of some aquatic organisms to chloride salts could reduce the viability of their populations. High chloride concentrations in a waterbody at times when these early life stages are present could reduce the number of individuals that successfully complete these stages. This could reduce or limit the recruitment of additional individuals into the adult population. Even though adults might be relatively tolerant of chloride, this could lead to an overall decline in the size, and ultimately the viability, of the aquatic organism population.

Genetic Variation

Genetic variation within a species may affect the sensitivity or tolerance of individuals to a toxic substance like chloride. This can be most clearly observed in clonal organisms. Such clonality occurs in cladoceran zooplankton. Water fleas, such as those in the genera *Daphnia* and *Ceriodaphnia* are cyclic parthenogens. Under normal conditions, they reproduce asexually.[68] The eggs that they produce contain exact duplicates of their mothers' genes. As a result of this, the population of a water flea species in a waterbody consists of several clones. The individuals within a clone are genetically identical to one another and would be expected to respond similarly to environmental stressors such as exposure to chloride.

Table 3.15 shows LC50s from laboratory studies of five different clones of the water flea *Daphnia longispina* that were exposed to sodium chloride for 48 hours.[69] The five clones show subtle differences in their sensitivity to chloride. The presence of these genetic differences in a population in the field can lead to

---

[67] *A. Mallick, B.C. Mohapatra, and N. Sarangi, "Acute Toxicity of Calcium Chloride on Different Stages (Egg, Spawn, Fry and Fingerling) of Rohu (*Labeo rohita*, Hamilton)," *Research Journal of Animal, Veterinary and Fishery Science*, 2:11-16, 2014.*

[68] *Sexual reproduction in water fleas occurs during periods of environmental stress and produces resting eggs which sink to the sediment and hatch the following spring.*

[69] *J. Leitao, R. Ribero, A.M.V.M. Soares, and I. Lopes, "Tolerance to Copper and Salinity in* Daphnia longispina*, Implications within a Climate Change Scenario,"* PLoS One, *8:e68702, 2013.*

changes in the population. Exposure to chloride could reduce the amount of genetic variation in a population by eliminating the most sensitive genotypes. This could have substantial effects on the population if it is also exposed to other toxic substances. Depending on the relationship of tolerance to the various toxicants, exposure to multiple toxic substances could lead to further reductions in genetic variation. The worst case would be that each toxic substance heavily impacts different clones. This could lead to extirpation of the species from the waterbody.[70]

Effects on Freshwater Organisms

General Impacts of Salt on Freshwater Organisms

The addition of chloride salts and/or other salts alters the osmotic balance between freshwater organisms and their surrounding environment.[71] Normally, the concentration of salts in freshwater and soil water is much less than those within the cells and tissues of organisms. This means that freshwater organisms must have ways to keep salts from diffusing out of or compensate for salts diffusing out of their bodies and to keep water from diffusing into their bodies as a result of osmotic pressure generated by this concentration difference. Most freshwater organisms actively regulate their osmotic pressure.[72] This regulation has metabolic costs, and freshwater organisms expend energy to accomplish it.

The osmotic balance freshwater organisms experience changes as the concentration of salts in water increases. When the concentration of salts in the water exceeds that in their bodies, organisms face the opposite challenge to the one described in the previous paragraph: they must have ways to keep salts from or compensate for salts diffusing into their bodies and keep water from diffusing out. Because they are adapted to environments in which their internal concentration of salt is higher than that of their environment, some aquatic organisms may lack mechanisms to accomplish these functions. In addition, these tasks also pose energy costs on the organisms that could reduce their viability. When external salinity gets too high, the osmoregulatory mechanisms of an organism may collapse.[73] This can lead to cellular damage. If the damage is severe enough, it can lead to death.

---

[70] Ibid.

[71] *S.E.G. Findlay and V.R. Kelly, "Emerging Indirect and Long-term Road Salt Effects on Ecosystems,"* Annals of the New York Academy of Sciences, *1,223:58-68, 2011.*

[72] *M. Cañedo-Argüelles, B.J. Kefford, C. Piscart, R.B. Schäfer, and C.-J. Schulz, "Salinization of Rivers" An Urgent Ecological Issue,"* Environmental Pollution, *173:157-167, 2013.*

[73] Ibid.

The above description of the impact of the effects of a change in salinity on freshwater organism osmotic balance is highly simplified. Osmoregulation is a complex process and involves regulation of many aspects of the organismal internal environment.[74] These aspects include the regulation of several properties such as total osmotic pressure, internal concentrations of individual ions, differences in ion concentrations within cells and the fluids surrounding cells, and internal acid-base balance.

Bacteria

Bacteria have important roles in aquatic, soil, and sediment communities. Some bacterial species degrade organic matter while other species mediate important processes such as nutrient cycling. In addition, bacteria serve as a food source for protozoa and some animals, including small zooplankton.

Several studies have reported the impacts of chloride salts on bacteria. Because of the difficulties identifying bacteria species in nature, most of these studies focused on bacterial communities as discussed below.

Chloride was found to have mixed effects on denitrifying bacteria. One study found that chloride concentrations of 2,000 mg/l and 5,000 mg/l inhibited denitrification in forested wetlands.[75] The same study found much less inhibition on denitrification in roadside wetlands that had historically been exposed to road salts. In addition, this study found that chloride concentrations of 2,000 mg/l and 5,000 mg/l increased the density of denitrifying bacteria in roadside wetlands. As discussed in Chapter 2 of this report, the increased ionic strength caused by salt introductions can suppress the bacterial enzymes responsible for denitrification.

Chloride salts can affect bacteria that are present in biofilms. In a microcosm study of biofilm communities, bacterial densities that were exposed to sodium chloride at concentrations of about 26 mg/l for 72 hours decreased relative to unexposed controls.[76] This occurred whether the exposure was constant or came in pulses that lasted 30 minutes. Oxygen consumption was also lower in the biofilm treatments exposed to sodium chloride than in the controls. Changes in salinity can affect the nature of a freshwater biofilm. The

---

[74] Ibid.

[75] *N.A. Lancaster, J.T. Bushey, C.R. Tobias, B. Song, and T.M. Vadas, "Impact of Chloride on Denitrification Potential in Roadside Wetlands,"* Environmental Pollution*, 212:216-223, 2016.*

[76] *J. Cochero, M. Licursi, and N. Gómez, "Effects of Pulse and Press Additions of Salt on Biofilms of Nutrient-rich Streams,"* Science of the Total Environment, *579:1,496-1,503, 2017.*

release of the extracellular substances that make up such films has been observed to increase with higher salinity.[77] This release may act to protect the cells and their processes.

Increased salinity may enhance the viability of some bacterial species in freshwater. For example, at specific conductance below 1,500 microSiemens per centimeter (µS/cm), the survival of *Escherichia coli* (*E. coli*) rose with increasing salinity in laboratory microcosms.[78] The increase in survival happened when the bacteria were exposed to either sodium chloride or a mixture of calcium chloride, magnesium chloride, and potassium chloride. Other experiments in the same study showed that *E. coli* exposed to magnesium chloride survived longer than those exposed to either sodium chloride or magnesium chloride at the same levels of specific conductance. High salinities associated with brackish water and seawater are known to reduce the survival of *E. coli*.[79] Because of gaps in the available data, it is not clear where the thresholds for salt concentration and the survival of *E. coli* lies.[80] Given that the largest increase in survival occurred at the lower level of the range of specific conductance examined, these results suggest that small increases in salinity can dramatically affect bacterial water quality. Since *E. coli* is used as an indicator of fecal pollution and suitability of water for human contact, higher concentrations of chloride salts could lead to recreational use impairments of additional waterbodies by promoting survival of *E. coli*.

Algae

Algae is a term that refers to a diverse group of photosynthetic organisms. Many algal groups are only distantly related to each other. Forms of algae include prokaryotic single-celled, filamentous, and colonial cyanobacteria, which are also referred to as blue-green algae. Algae also include eukaryotic single-celled, filamentous, colonial, and multicellular forms in several different groups. Some groups, such as the red algae and brown algae are found mostly in marine environments, but others occur in freshwater environments and soils. Some examples of freshwater algae are shown in Figure 3.2.

---

[77] *D.J. Steele, D.J. Franklin, and G.J. Underwood, "Protection of Cells from Salinity Stress by Extracellular Polymeric Substances in Diatom Biofilms,"* Biofouling*, 308:987-998, 2014.*

[78] *S.E. DeVilbiss, M.K. Steele, L.-A.H. Krometis, and B.D. Badgley, "Freshwater Salinization Increases Survival of* Escherichia coli *and Risk of Bacterial Impairment,"* Water Research, *191: 116812, 2021.*

[79] *A.F. Carlucci, and D. Pramer, "An Evaluation of Factors Affecting the Survival of* Escherichia *coli in Seawater. II. Salinity, pH, and Nutrients,"* Applied Microbiology, *8:247-250, 1960; I.C. Anderson, M. Rhodes, and H. Kator, "Sublethal Stress in* Escherichia coli*: A Function of Salinity,"* Applied and Environmental Microbiology, *38:1,147-1,152, 1979.*

[80] *DeVilbiss et al 2021,* op. cit.

Algae grow in several different places in the environment. Phytoplankton consists of unicellular, filamentous, and colonial algae that are suspended in the water column. Algal periphyton consists of unicellular, filamentous, and colonial algae that grow on surfaces in aquatic environments. Surfaces supporting algal growth include rocks, soft sediments, and plants. Macroalgae consist of multicellular algae that superficially resemble aquatic plants.

As primary producers, algae constitute part of the base of the food web. They are ubiquitous, abundant, and diverse. Because algae grow rapidly, they respond quickly to stresses in the environment and they are among the first organisms to respond to environmental changes.

Limited information is available on the impacts of chloride and chloride salts on freshwater algae. Detailed information on their salinity tolerance is lacking because only a few species in a few groups have been examined. There is a similar lack of detailed knowledge on the effects of chloride salts on algal physiology.

Phytoplankton and periphyton are commonly found in lake, pond, stream, river, and wetland environments. They serve as food sources to protozoa, zooplankton, macroinvertebrates, tadpoles, and fish. Most of the available information on the effects of chloride salts and salinity on algae comes from three groups: the blue-green algae or cyanobacteria, the diatoms or Bacillariophyta , and the green algae or Chlorophyta. The green algae include some macroalgae such as stoneworts in the genera *Nitella* and *Chara*.

Some studies have found that higher salinity reduces the concentration of planktonic algae in the water column.[81] Other studies have reported increases in phytoplankton concentrations with increasing salinity.[82] Increases in salinity were also found to reduce the photosynthetic efficiency of periphytic algae growing on

---

[81] *J.C. Batterton and C. Baalen, "Growth Responses of Blue-green Algae to Sodium Chloride Concentration,"* Archives of Microbiology*, 76:151-165, 1971; L.M. Kipriyanova, N.I. Yermolaeva, D.M. Bezmaternykh, S.Y. Dvurechenskaya, and E.Y. Mitrofanova, "Change in the Biota of Chany Lake Along a Salinity Gradient,"* Hydrobiologia*, 576:83-93, 2007; E. Coring and J. Bäthe, "Effects of Reduced Salt Concentration on Plant Communities in the River Werra (Germany),"* Limnologica*, 41:134-142, 2011.*

[82] *W.D. Hintz, B.M. Mattes, M.S. Schuler, D.K. Jones, A.B. Stoler, L. Lund, and R.A. Relyea, "Salinization Triggers a Trophic Cascade in Experimental Freshwater Communities with Varying Food-chain Length,"* Ecological Applications, *27:833-844, 2017.*

rock.[83] This may be due to chloride ions inhibiting the activity of carbonic anhydrase, an enzyme that is important in preparing carbon dioxide for use in photosynthesis.[84] This inhibition may also account for the toxicity of chloride to some algal species.

Limited information is available on the effects of chloride on cyanobacteria. Higher salinity has been reported to favor the growth of some cyanobacteria species because they require sodium ions for growth.[85]

Concentrations of chloride and chloride salts can affect the presence and growth of diatoms. A paleolimnological study of 309 lakes in the northeastern U.S. characterized the optimal chloride concentrations for the presence of 235 common diatom species.[86] The study found that optimal chloride concentrations for diatoms ranged between 0.3 mg/l and 39 mg/l. Through the disappearance of diatom species with optima at low chloride concentrations and the appearance of species with optima at higher chloride concentrations in sediment cores, this study was able to document changes in chloride concentrations in many of the lakes examined. Another study characterized optimal chloride concentrations and levels of specific conductance for 191 species of periphytic diatoms in samples from 1,109 sites on rivers in the United States.[87] Optimum concentrations of chloride for periphytic diatoms ranged between 1.1 mg/l and 58.5 mg/l and optimum levels of specific conductance ranged between 40 μS/cm and 902 μS/cm. These optimal concentrations suggest that impacts to diatoms might occur at relatively low chloride concentrations.

Periphytic diatom species are important indicators of environmental stress. Because they are found in shallow areas near the shore or bank, periphytic diatoms are in the first areas to receive materials from

---

[83] *E.I.L. Silva and R.W. Davies, "The Effects of Simulated Irrigation Induced Changes in Salinity on the Metabolism of Lotic Biota."* Hydrobiologia*, 416:193-202, 1999.*

[84] *M.L. Dionisio-Sese and S. Miyachi, "The Effect of Sodium Chloride on Carbonic Anhydrase Activity in Marine Microalgae,"* Journal of Phycology*, 28:619-624, 1992.*

[85] *M.B. Allen and D.I. Arnon, "Studies on Nitrogen Fixing Blue-green Algae. II. The Sodium Requirements of* Anabaena cylindrica*,"* Physiologia Plantarum*, 8:653-660, 1955.*

[86] *S.S. Dixit, J.P. Smol, R.M. Hughes, S.G. Paulsen, and G.B. Collins, "Assessing Water Quality Changes in Lakes of the Northeastern United States Using Sediment Diatoms,"* Canadian Journal of Fisheries and Aquatic Sciences, *56:131-152, 1999.*

[87] *M. Potapova and D.F. Charles, "Distribution of Benthic Diatoms in U.S. Rivers in Relation to Conductivity and Ionic Composition,"* Freshwater Biology, *48:1,311-1,328, 2003.*

anthropogenic stressors. Significant changes in the species of periphytic diatoms present have been reported with increases in chloride concentration in both lakes[88] and streams.[89] One study sampled diatom communities in 41 streams with chloride concentrations ranging from 5 mg/l to 502 mg/l.[90] It found a strong association between salinity and species composition of the diatom community. In addition, substantial changes occurred in the species making up diatom communities at a threshold chloride concentration of about 35 mg/l. Taxonomic changes also occurred as chloride concentrations increased above this threshold, but the changes were more gradual. Measures of diatom community diversity did not change with increasing chloride concentrations, suggesting that sensitive species dropped out of the community and were replaced by more tolerant species as chloride concentration rose.

Impacts of chloride to diatom species in other parts of the freshwater environment have also been reported. Shifts in the species composition of diatom assemblages were observed following salt pollution in the River Wipper in Germany.[91] Salinization has also been reported to reduce the density of diatom cells in freshwater biofilms.[92] Higher salinity has also been reported to result in changes to the external morphology of diatom cells.[93]

---

[88] *M.J. MacDougall, A.M. Patterson, J.G. Winter, F.C. Jones, L.A. Knopf, and R.I. Hall, "Response of Periphytic Diatom Communities to Multiple Stressors Influencing Lakes in the Muskoka River Watershed, Ontario, Canada,"* Freshwater Science*, 36:77-89, 2017.*

[89] *E.R. Porter-Goff, P.C. Frost, and M.A. Xenopoulos, "Changes in Riverine Benthic Diatom Community Structure along a Chloride Gradient,"* Ecological Indicators*, 32:97-106, 2013.*

[90] Ibid.

[91] *H. Ziemann, L. Kies, and C.-J. Schulz, "Desalinization of Running Waters. III. Changes in the Structure of Diatom Assemblages Caused by a Decreasing Salt Load and Changing Ion Spectra in the River Wipper (Thuringia, Germany),"* Limnologica*, 31:257-280, 2001.*

[92] *S. Busse, R. Jahn, and C.-J. Schulz, "Desalinization of Running Waters. II. Benthic Diatom Communities: A Comparative Field Study on Responses to Decreasing Salinities,"* Limnologica*, 29:465-474, 1999.*

[93] *R. Trobajo, L. Rovira, D.G. Mann, and E.J. Cox, 'Effects of Salinity on Growth and Valve Morphology of Five Estuarine Diatoms,"* Phycological Research*, 592:83-90, 2011.*

Differing effects of chloride have been reported for green algae. One study found that increased salinity favors the growth of some species in the genera *Chlorella, Ankistrodesmus, and Scenedesmus*.[94] In addition, laboratory experiments with two green algal species showed that growth was enhanced by increasing the concentration of sodium chloride over that which was found in the pond from which they were isolated.[95] Growth of *Chlorococcum humicola* was enhanced at chloride concentrations ranging between 182 and 2,914 mg/l. Similar enhancement was observed in *Scenedesmus bijugatus* at chloride concentrations ranging between 182 and 1,457 mg/l. In both species inhibition of growth occurred at a chloride concentration of 11,654 mg/l.

Other studies have found that increasing chloride concentrations caused adverse effects in some species of green algae. One study exposed *Scenedesmus obliquus* to sodium chloride at chloride concentrations ranging between 2,340 and 11,690 mg/l.[96] It found that the growth rate of the cells, their dry weight, their total protein content, and their cellular concentration of photosynthetic pigments all decreased with increasing sodium chloride concentration. Another study found that higher salt concentrations resulted in reduced abundance of filamentous algae.[97]

Few data were available on the effects of chloride on multicellular freshwater algae. One study found that increased chloride concentrations resulted in reduced biomass and chlorophyll-a content of the Charophyte alga *Nitella*.[98]

---

[94] *E. Kessler, "Physiological and Biochemical Contributions to the Taxonomy of the General* Ankistrodesmus *and* Scenedesums*. IV. Salt Tolerance and Thermophily,"* Archives for Microbiology*, 119:13-16, 1977; E. Kessler, "Mass Culture of* Chlorella *Strains under Conditions of High Salinity, Acidity, and Temperature,* Archiv für Hydrobiologie, Supplement *60:80-86, 1980.*

[95] *R.C. Dash, P.K. Mohapatra, and R.C. Mohanty, "Salt Induced Changes in the Growth of* Chlorococcum humicola *and* Scenedesmus bijugatus *under Nutrient Limited Cultures,"* Bulletin of Environmental Contamination and Toxicology*, 54:695-702, 1995.*

[96] *A.A. Mohammet and A.A. Shafea, "Growth and Some Metabolic Activities of* Scenedesmus obliquus *under Different NaCl Concentrations,"* Biologia Plantarum*, 34:423-430, 1992.*

[97] *L. Lind, M.S. Schuler, W.D. Hintz, A.B. Stoler, D.K Jones, B.M. Mattes, and R.A. Relyea, "Salty Fertile Lakes: How Salinization and Eutrophication Alter the Structure of Freshwater Communities,"* Ecosphere*, 9:e02383, 2018.*

[98] Ibid.

Aquatic Plants (Macrophytes)

Aquatic plants, or macrophytes, include both mosses and vascular plants. These form an integral part of the aquatic food web, converting carbon dioxide and inorganic nutrients present in the water and sediments into organic compounds that are directly available as food for other aquatic organisms. In this process, known as photosynthesis, plants utilize energy from sunlight and release oxygen required by other aquatic life forms. Macrophytes provide food and habitat for fish and other aquatic organisms, produce oxygen, and may remove nutrients and pollutants from the water that could otherwise cause algal blooms or other problems. Examples of aquatic macrophytes are shown in Figure 3.3.

Aquatic plants are often described using the terms submerged, floating-leaf, free-floating, and emergent, depending on where the plant is found in the lake, stream, or wetland system. Emergent plants, such as bulrushes and cattails, are rooted in the substrate and have leaves that emerge above the water. They are commonly found in shallow areas such as along the shoreline areas of a lake. Submerged plants such as coontail (*Ceratophyllum demersum*), are rooted in the bottom substrate and grow entirely under water. They can grow in deeper water than emergent plants but are restricted to depths in which light can penetrate. Floating-leaf plants, such as water lilies, are rooted in the substrate and generally have large, floating leaves. They are usually found in shallow water areas of a few feet in depth or less that contain loose bottom sediments. Free-floating plants, such as duckweed (*Lemna* spp.), have small leaves, are not rooted to the sediment, and are often blown around the waterbody by wind. All four macrophyte types play significant roles in aquatic systems.

Limited information is available on the impacts of chloride and chloride salts on freshwater aquatic plants. Detailed information on their salinity tolerance is lacking because few species have been examined.

Chloride Tolerance of Aquatic Plants

Most freshwater aquatic plant species cannot tolerate concentrations of dissolved salts greater than 10,000 mg/l.[99] Some freshwater macrophyte species die when chloride concentrations are between 1,000 mg/l and 2,000 mg/l[100] and a large proportion of them are sensitive to salinity that produces specific conductance in

---

[99] *P. Lacoul and B. Freedman, "Environmental Influences on Aquatic Plants in Freshwater Ecosystems,* Environmental Reviews, *14:89-136, 2006.*

[100] *B.T. Hart, P. Bailey, R. Edwards, K. Hortle, K. James, A. McMahon, C. Meredith, and K. Swadling "A Review of the Salt Sensitivity of the Australian Freshwater Biota,"* Hydrobiologia*, 210:105-144, 1991.*

the range of 1,500 µS/cm to 3,000 µS/cm.[101] Woody aquatic plant species are often sensitive to salinity.[102] Young plants may also be particularly sensitive to the effects of chloride salts because of the role of water in plant growth and development.[103] The expansion of newly divided plant cells occurs through the cells taking up water and using the pressure from this water to increase cell size prior to synthesis of the final layers of the cell wall. Higher salt concentrations in the environment may interfere with this growth process.

The tolerance to chloride salts varies among aquatic plant species and by the type of salt and the duration and intensity of exposure to the salt.[104] For example, a field study found that almost all the endemic plant species were absent from the portion of an Indiana bog impacted by road salt when the concentration of chloride reached 1,215 mg/l.[105] Some macrophyte species disappeared at lower concentrations. For example, the peat moss *Sphagnum recurvum* was not seen in this wetland when chloride concentrations exceeded 500 mg/l.[106] Exotic species were less affected and many of the endemic species returned following a 50 percent reduction in the salt concentration.

Impacts of Chloride Salts on Aquatic Plants

High concentrations of salts can induce several types of sublethal effects in aquatic plants. Both submergent and emergent plants may experience these. Examples of these sublethal effects include reductions in height, length, or overall size; biomass; leaf size or proliferation; and flowering as well as displaying signs of injury

---

[101] *K.R. James, B. Cant, and T. Ryan "Responses of Freshwater Biota to Rising Salinity Levels and Implications for Saline Water Management: A Review,"* Australian Journal of Botany, *51:703-713, 2003 and L.M. Kipriyanova, N.I. Yermolaeva, and D.M. Bezmaternykh, "Change in the Biota of Chany Lake Along a Salinity Gradient,"* Hydrobiologia, *576:83-93, 2007.*

[102] *W. Conner, K. McLeod, and J. McCarron, "Flooding and Salinity Effects on Growth and Survival of Four Common Forested Wetland Species,"* Wetlands Ecology and Management*, 5:99-109, 1997 and K.W. Krauss, J.L. Chambers, and D. Creech, "Selection for Salt Tolerance in Tidal Freshwater Swamp Species: Advances Using Baldcypress as a Model for Restoration," pages 385-410 in W.H. Conner, T.W. Doyle, and K.W. Krauss (editors),* Ecology of Tidal Freshwater Forested Wetlands of the Southeastern United States, *Springer, Dordrecht, The Netherlands, 2007.*

[103] *M. Rahman, U.A. Soomoro, M.Z. Haq, and S. Gul, "Effects of NaCl Salinity on Wheat (*Triticum aestivum *L.) Cultivars,"* World Journal of Agricultural Sciences*, 4:398-403, 2008.*

[104] *Lacoul and Freedman 2006,* op. cit.

[105] *D.A. Wilcox, "The Effects of Deicing Salts on Vegetation in Pinhook Bog, Indiana,"* Canadian Journal of Botany, *64:865-874, 1986.*

[106] *D.A Wilcox, "The Effects of NaCl Deicing Salts on* Sphagnum recurvum *P. Beauv.,"* Environmental and Experimental Botany, *24:295-304, 1984.*

such as leaf burn, wilting, and discoloration.[107] Sublethal effects of salinity are discussed in the following paragraphs.

Higher concentrations of chloride and other salts can lead to reductions in growth of aquatic plants. These changes can be observed as reductions in size or reductions in biomass. For example, a laboratory study found that growth of the peat moss *Sphagnum recurvum* was reduced at chloride concentrations between 300 mg/l and 1,500 mg/l.[108] Based on the growth during exposure to other salts, chloride ions appear to be stronger inhibitors of growth to this moss than sodium ions. The moss also showed signs of injury from salts. As water evaporated from *Sphagnum* fronds, salt was deposited on the tips of the plant. This led to the death of the plant within three weeks, unless the salt was washed off.

Increases in chloride concentration have also been reported to decrease the biomass of aquatic plants. A laboratory study found that the wet weight of the duckweed *Lemna minor* decreased as the concentration of chloride increased.[109] This accompanied a decrease in the somatic growth rate of this plant. The surface area of leaves on the plants also decreased with increasing chloride concentration.

Similar effects on growth were observed in a study of pond weeds in the genus *Potamogeton*. The biomass of three *Potamogeton* species was lower when grown at a chloride concentration of 250 mg/l than in controls.[110] The somatic growth rates of these plants were also lower at a chloride concentration of 250 mg/l. The same study found no reduction of growth in the fan-leaved water crowfoot (*Rannunculus circinatus*) at the 250 mg/l level. This study also found that the number of leaves on individual *Potamogeton* plants and the surface areas of those leaves were reduced when exposed to the higher chloride concentration. This may be related to reduced expansion of leaf cells during growth and may result in reduced photosynthesis by the plants. This leaf impact was not observed in *R. circinatus*.

---

[107] *K. James and B. Hart, "Effect of Salinity of Four Freshwater Macrophytes,"* Marine and Freshwater Research, *44:769-777, 1993.*

[108] *Wilcox 1984*, op. cit.

[109] *Ł. Sikorski, A.I. Piortrowicz-Cieślak, and B. Adomas, "Phytotoxicity of Sodium Chloride Towards Common Duckweed (*Lemna minor *L.) and Yellow Lupin (*Lupinus luteus *L.),"* Archives of Environmental Protection*, 39:117-128, 2013.*

[110] *F.W.B. van den Brink and G. van der Velde, "Growth and Morphology of Four Freshwater Macrophytes Under the Impact of the Raised Salinity Level of the Lower Rhine"* Aquatic Botany, *45:285-297, 1993.*

In vascular plants, salinization can lead to reduced carbon fixation through photosynthesis.[111] For example, photosynthetic production begins to decrease in common waterweed (*Elodea canadensis*) when chloride concentration rises to 100 mg/l.[112] Reduced photosynthesis can lead to reductions in plant growth, reproduction and viability. In part, reduction in photosynthesis may reflect the changes in the number and morphology of leaves that occur at higher concentrations of salts. It may also result from changes to the plant's photosynthetic apparatus. Decreases in plant concentrations of chlorophyll-a and other photosynthetic pigments with increasing salinity and sodium chloride concentration have been reported for *Lemna minor*.[113] Changes in cellular photosynthesis pigment content has also been reported in water thyme (*Hydrilla verticullata*), guppy grass (*Najas indica*), and rice field water nymph (*Najas gramenia*).[114]

Increases in the concentration of chloride and chloride salts can also reduce reproduction of aquatic plants which can happen in several ways. High concentrations of chloride can lead to reduced flower production in some aquatic plants. For example, the number of flowers produced by shining pondweed (*Potamogeton lucens*), redhead pondweed (*Potamogeton perfoliatus*), and long-leaf pondweed (*Potamogeton nodosus*) was lower in cultures with chloride concentration of 250 mg/l than in controls.[115] Exposure to saline conditions can also reduce seed germination of aquatic plants.[116] Sodium chloride concentrations of 3,000 mg/l were reported to reduce seed germination in sago pondweed (*Potamogeton pectinatus*).[117] Similarly, a three-

---

[111] *W.E. Odum, "Comparative Ecology of Tidal Freshwater and Salt Marshes,"* Annual Review of Ecology and Systematics, *19:147-176, 1988; Hart et al. 1991,* op. cit.*; and James et al. 2003,* op. cit.

[112] *H. Zimmerman-Timm, "Salinization in Inland Waters," pages 133-136 in: J. Lozan, H. Graßl, P. Hupfer, L. Menzel, and C. Schönwiese (editors),* Water Uses and Human Impacts on the Water Budget*, Verlag Wissenshaftliche Auswertungen/GEO, Hamburg, Germany, 2007.*

[113] *E.C. Keppeler, "Toxicity of Sodium Chloride and Methyl Parathion on the Macrophyte* Lemna minor *(Linnaeus, 1753) with Respect to Frond Number and Chlorophyll,"* Biotemas*, 22:27-33, 2009; J.A. Simmons, "Toxicity of Major Cations and Anions (Na^+, K^+ Ca^{2+}, C^{l-}, and SO_4^{2-}) to a Macrophyte and an Alga,"* Environmental Toxicology and Chemistry*, 31:1,370-1,374, 2012.*

[114] *N.P. Rout and B.P Shaw, "Salt Tolerance in Aquatic Macrophytes: Ionic Relation and Interaction,"* Biologia Plantarum*, 44:95-99, 2001.*

[115] *van den Brink and van der Velde 1993,* op. cit.

[116] *D.L. Nielsen, M.A. Brock, K. Crosslé, K. Harris, M. Healy, and M. Jarosinski, "The Effects of Salinity on Aquatic Plant Germination and Zooplankton Hatching from Two Wetland Sediments,"* Freshwater Biology, *48:2,214-2,223, 2003.*

[117] *J.W. Teeter,* The Influence of Sodium Chloride on the Growth and Reproduction of the Sago Pondweed (*Potamogeton pecinatus* L.), *Master's Thesis, Utah State University, Logan, Utah, 1963.*

month experiment testing the emergence of plants from a wetland seed bank found that constant exposure to salinity of 1,000 mg/l and 5,000 mg/l reduced both the abundance and diversity of seeds germinating.[118] Reduction in germination was not observed when seeds were exposed to a 14-day pulse of saline water followed by freshwater. This suggests that some seeds may be able to tolerate shorter exposures to chloride salts. Finally, vegetative reproduction in aquatic plants can be favored over sexual reproduction in salinized environments.[119] This can reduce a waterbody's genetic diversity in species, potentially making them more vulnerable to other stressors.

<u>Mechanisms Underlying Chloride Salt Impacts on Aquatic Plants</u>

Several mechanisms likely underlie the effects of chlorides and chloride salts on aquatic plants. First, excessive concentrations of sodium chloride can impede the ability of plants to absorb water.[120] Unlike animal cells, plant cells have a rigid cell wall and a large central vacuole. Under normal conditions, water flows into the cell and into the vacuole, causing the cell membrane to press against the cell wall. This is referred to as turgor. Higher than normal concentrations of salts in the environment can cause the cell to lose water, reducing turgor. With enough water loss, the cell membrane may pull away from the cell wall. Non-turgid conditions adversely affect the functioning of the plant and if they persist, they can eventually lead to death of the plant.

Chloride-associated cations in energy metabolism may also play a role in the impacts of chloride salts on aquatic plants. During energy metabolism, positively charged ions are passed across internal membranes within cells. Excessive sodium ions in the environment can lead to changes in a plant's internal balance between sodium ions and potassium ions. This could require that the plant expend energy to compensate for this change, leading to slower growth or chlorophyll production.[121] In any case, the physiological mechanisms that mitigate salt stress in plants come at a cost of reduced growth, reproduction, and competitive ability.[122]

---

[118] *D.L. Nielsen, M.A. Brock, R. Petrie, and K. Crosslé, "The Impact of Salinity Pulses on the Emergence of Plant and Zooplankton from Wetland Seed and Egg Banks,"* Freshwater Biology, *52:784-795, 2007.*

[119] *R.W. Robinson, E.A. James, and P.I. Boon, "Population Structure in the Clonal, Woody Wetland Plant* Melaleuca ericifolia *(Myrtaceae): An Analysis Using Historical Aerial Photographs and Molecular Techniques,"* Australian Journal of Botany, *60:9-19, 2012.*

[120] *J.K. Zhu, "Plant Salt Tolerance,"* Trends in Plant Science, *6:66-71, 2001.*

[121] *Simmons 2012,* op. cit.

[122] *R. Munns, and M. Tester, "Mechanisms of Salinity Tolerance,"* Annual Review of Plant Physiology, *59:651-668, 2008.*

Zooplankton

Zooplankton are free-floating animals that can be found in the water columns of lakes, wetlands, and streams. They are typically small, with lengths less than 2 millimeters. Zooplankters are consumers of phytoplankton, bacteria, and protists and key grazers on algal populations.[123] Zooplankton, in turn, are significant prey for invertebrate and fish predators.[124] As such, they provide a critical link in aquatic food webs, passing production from primary producers to higher trophic levels. Most zooplankton in freshwater systems are members of four groups of organisms: Cladocera, copepods, ostracods, and rotifers. The first three groups are members of the crustacea, and the fourth group is a separate phylum of invertebrates. Examples of zooplankton are shown in Figure 3.4

Among zooplankton, the impacts of chloride and chloride salts have been studied the most in the Cladocera, and especially in water fleas of the family Daphniidae. Many studies have focused on *Ceriodaphnia dubia* and species in the genus *Daphnia* because they are readily cultured in laboratory settings and are commonly used in toxicology studies. Less information is available on impacts of chloride and chloride salts on predatory Cladocera, copepods, rotifers, and ostracods.[125]

<u>Impacts of Chloride Salts on Zooplankton Abundance</u>

High salinities and high concentrations of chloride salts are associated with lower abundance of zooplankton. For example, a field survey of 14 waterbodies with salinities ranging from 157 mg/l to 31,774 mg/l found that the densities and species richness of rotifers decreased with increasing salinity.[126] Reductions in zooplankton abundance with increasing chloride concentrations have been observed in mesocosm experiments in which communities have been exposed to different concentrations of chloride. Densities of cladocerans, copepods, and rotifers decreased with increasing concentrations of chloride in

---

[123] *W. Lampert, W. Fleckner, H. Rai, and B.E. Taylor, "Phytoplankton Control by Grazing Zooplankton: A Study on the Spring Clear-water Phase,"* Limnology and Oceanography, *31:478-490, 1986.*

[124] *P. Larsson and S. Dodson, "Invited Review—Chemical Communication in Planktonic Animals,"* Archive für Hydrobiologie*, 129:129-155, 1993.*

[125] *W.D. Hintz and R.A. Relyea, "A Review of the Species, Community and Ecosystem Impacts of Road Salt Salinization in Freshwaters,"* Freshwater Biology*, 64:1,081-1,097, 2019.*

[126] *I. Bielanska-Grajner and A. Cudak, "Effects of Salinity on Species Diversity of Rotifers in Anthropogenic Waterbodies,"* Polish Journal of Environmental Studies, *23:27-34, 2014.*

one experiment in which chloride concentrations ranged between 120 mg/l and 1,500 mg/l.[127] The impacts of chloride on zooplankton abundance differ among groups. Another mesocosm study found 85 percent reductions in the density of adult copepods and 94 percent reductions in the density of cladocerans relative to controls at a chloride concentration of 645 mg/l.[128] This experiment did not detect an effect of chloride on the abundance of rotifers, ostracods, or juvenile copepods.

The diverse chloride response of zooplankton groups may reflect differences in the biology of the groups, but also may reflect differences among individual species. Another study that exposed rotifer species to concentrations of sodium chloride ranging between 0 mg/l and 4,500 mg/l (chloride concentrations of 0 mg/l to 2,730 mg/l) found that rotifer abundance decreased with increasing sodium chloride concentration; however, the chloride concentration at which effects became apparent differed among species.[129] Reductions in the densities of populations of the species *Anuraeopsis fissa, Brachionus calyciflorus,* and *Brachionus havanaensis* were detected at sodium chloride concentrations above 1,500 mg/l or chloride concentrations of about 910 mg/l, while reductions in the species *Brachionus patulus,* and *Brachionus rubens* were detected at sodium chloride concentrations above 3,000 mg/l or chloride concentrations of 1820 mg/l. Greater reductions typically occur at higher chloride concentrations. For example, the abundance of *Daphnia pulex* in a mesocosm study decreased by 40 percent relative to controls at chloride concentration of 860 mg/l.[130] The decrease relative to controls in a treatment with chloride concentration of 1,300 mg/l was about 79 percent. The reductions in zooplankton abundance are partially due to the impacts of acute toxicity that were discussed earlier in this chapter, but sublethal factors may also play a major role.

---

[127] *D.A. Greco, S.E. Arnott, I.B. Fournier, and B.S. Schamp, "Effect of Chloride and Nutrients on Freshwater Plankton Communities,"* Limnology and Oceanography Letters, *doi: 10.1002/lol2.10202, 2022.*

[128] *R.J. van Meter, C.M. Swan, J. Lepis, and J.W. Snodgrass, "Road Salt Stress Induces Novel Food Web Structure and Interactions,"* Wetlands*, 31:843-851, 2011.*

[129] *S.S.S. Sarma, S. Nandina, J. Morales-Ventura, I. Delgado-Martínez, and L. González-Valverde, "Effects of NaCl Salinity on the Population Dynamics of Freshwater Zooplankton (Rotifers and Cladocerans),"* Aquatic Ecology*, 40:349-360, 2006.*

[130] *W.D. Hintz and R.A. Relyea, "A Salty Landscape of Fear: Responses of Fish and Zooplankton to Freshwater Salinization and Predatory Stress,"* Oecologia*, 185:147-156, 2017.*

Impacts of Chloride Salts on Zooplankton Population Growth

Population growth rates of three *Daphnia* species have been reported to decrease with higher concentrations of sodium chloride.[131] In addition, the longevity of zooplankton is also affected by chloride. A life table analysis showed that the average lifespan and life expectancy at birth in *Daphnia magna* decreased with increasing concentration of sodium chloride.[132] Similar reductions in longevity were observed in the water flea *Pseudosida ramosa* when it was exposed to either sodium chloride or potassium chloride.[133]

The lower population growth rates reflect the fact that individual zooplankters may grow more slowly under higher salinity and higher concentrations of chloride. One study measured the body size of *Daphnia carinata* grown in chloride concentrations between 100 mg/l and 1,500 mg/l.[134] The body lengths attained by these water fleas decreased with increasing chloride concentrations. This indicates that the water fleas experienced reduced somatic growth rates with higher chloride concentrations. Similarly, the mean length, wet weight, and dry weight attained by *Daphnia magna* were lower with increasing sodium chloride concentration.[135] The ratio of body width to body length in *Daphnia pulex* decreased with rising sodium chloride concentration, indicating slower growth.[136] This was accompanied by a decrease in lipid content in the organisms, suggesting that they had lower energy reserves. The maximum body size attained by *Pseudosida ramosa* was also shorter when it was cultured at higher concentrations of sodium.[137] Finally,

---

[131] *A.M.M. Gonçalves, B.B. Castro, M.A. Pardal, and F. Gonçalves, "Salinity Effects on Survival and Life History of Two Freshwater Cladocerans (*Daphnia magna and Daphnia longispina*)," *Annales de Limnologie—International Journal of Limnology*, 43:13-20, 2007; G. Bezrici, S.B. Akkas K. Rinke, F. Yildirim, Z. Kalaylioglu, F. Severcam, and M. Beklioglu, "Impacts of Salinity and Fish-exuded Kairomone on the Survival and Macromolecular Profile of* Daphnia pule*,"* Ecotoxicology*, 21:601-614, 2012.*

[132] *F. Martínez-Jerónimo and L. Martínez-Jerónimo, "Chronic Effects of NaCl Salinity on a Freshwater Strain of* Daphnia magna *Straus (Crustacea: Cladocera): A Demographic Study,"* Ecotoxicology and Environmental Safety*, 67:411-416, 2007.*

[133] *E.C. Freitas and O. Rocha, "Effects of Sodium and Potassium on Life History of the Freshwater Cladoceran* Pseudosida ramosa*,"* Journal of the Brazilian Society of Ecotoxicology*, 7:85-91, 2012.*

[134] *Hall and Burns 2002,* op. cit.

[135] *M.M. El-Deeb Ghazy, M.M Habashy, F.I. Kossa, and E.Y. Mohammady, "Effects of Salinity on Survival, Growth and Reproduction of the Water Flea,* Daphnia magna*,"* Nature and Science*, 7:28-42, 2009.*

[136] *Bezrici et al. 2012,* op. cit.

[137] *Freitas and Rocha 2012,* op. cit.

lower somatic growth rates with increasing salinity[138] and sodium chloride concentration[139] have been reported in *Daphnia pulicaria*.

The reduction in somatic growth rates associated with higher chloride concentrations may be due to salinity or chloride acting to inhibit feeding by zooplankton. One study found that feeding rates in six clonal lineages of *Daphnia longispina* decreased as salinity rose.[140] Similarly, one out of five experimental populations of *Daphnia dentifera* collected from different lakes showed reductions in feeding when exposed to sodium chloride concentrations of 600 mg/l.[141] Reduced feeding rates result in the organism obtaining fewer nutrients and less matter to fuel growth, resulting in slower somatic growth rates and ultimately slower population growth rates.

<u>Impacts of Chloride Salts on Zooplankton Mobility</u>

Exposure to chloride salts can also affect the ability of zooplankton to move. For example, swimming velocity in many zooplankton species is dependent on size. In experiments that controlled for the effects of size, exposure of *Daphnia magna* to salinity decreased their swimming velocity.[142] Related experiments showed that this effect could not be accounted for by reduced food intake. The swimming velocity gradually returned to normal under saline conditions, but this was accompanied by considerable mortality in the test animals. The authors concluded that increased salinity temporarily impaired *Daphnia* physiology. This temporary reduction in swimming velocity could lead to greater exposure of daphnids to predators.

Exposure to chloride salts can also affect another type of zooplankton movement. Many zooplankton show phototactic responses, moving away from light. Exposure of *Daphnia magna* to sublethal concentrations of

---

[138] *C. Venâncio, R. Ribero, A.M.V.M. Soares, and I. Lopes, "Multigenerational Effects of Salinity, in Six Clonal Lineages of* Daphnia Longispina*,"* Science of the Total Environment, *519-620:194-202, 2018.*

[139] *Gonçalves et al. 2007,* op. cit.

[140] *C. Venâncio et al. 2018,* op. cit.

[141] *C.L. Searle, C.L. Shaw. K.K. Hunsberger, M. Prado, and M.A. Duffy, "Salinization Decreases Population Densities of the Freshwater Crustacean* Daphnia dentifera*,"* Hydrobiologia, *770:165-172, 2016.*

[142] *M Baillieul, B. DeWachter, and R. Blust, "Effect of Salinity on the Swimming Velocity of the Water Flea* Daphnia magna*,"* Physiological Zoology, *71:703-707, 1998.*

sodium chloride led to disruption of phototactic responses in their offspring.[143] This can disrupt the typical vertical migration in zooplankton populations.

Many freshwater zooplankton species migrate vertically through the water column on a daily basis. Typically, they move downward into darker bottom waters at dawn and upward into surface layers at dusk.[144] This is likely a means of avoiding predation by visually feeding predators such as many fish, with dark bottom waters serving as a refuge during the day when feeding rates of visual predators are higher.[145] Disruption of this response to light by sodium chloride can lead to greater exposure of zooplankton to predators.

Impacts of Chloride Salts on Zooplankton Reproduction

Exposure to chloride and chloride salts can also reduce zooplankton reproduction. Several studies found lower reproductive output by the water flea *Ceriodaphnia dubia* begins to be observed at chloride concentrations in the range of about 150 mg/l to 520 mg/l and 50 percent reductions in reproductive output were seen at chloride concentrations in the range of about 350 mg/l to 965 mg/l.[146] Similar reductions in reproductive output have been reported in *Daphnia ambigua*,[147] *Daphnia magna*,[148] and the rotifer *Brachionus calyciflorus.*[149]

Chloride salts and salinity affect several aspects of zooplankton reproduction. First, exposure to chloride salts can increase the age at which reproduction first occurs. For example, exposure to potassium chloride

---

[143] *M.A. Kolkmeier and B.W. Brooks, "Sublethal Silver and NaCl Toxicity in* Daphnia magna*: A Comparative Study of Standardized Chronic Endpoints and Progeny Phototaxis,"* Ecotoxicology, *22:693-706, 2013.*

[144] *H.-B. Stitch and W. Lampert, "Predator Evasion as an Explanation of Diurnal Vertical Migration by Zooplankton,"* Nature*, 293:396-398, 1981; E.V. Van Gool and J. Ringelberg, "Quantitative Effects of Fish Kairomones and Successive Light Stimuli on Downward Swimming Responses of* Daphnia*,"* Aquatic Ecology*, 291:291-296, 1998.*

[145] *W. Lampert, "The Adaptive Significance of Diel Vertical Migration,"* Functional Ecology*, 3:21-27, 1989.*

[146] *M.A. Aragão and E.V. Pereira, "Sensitivity of* Ceriodaphnia dubia *of Different Ages to Sodium Chloride,"* Bulletin of Environmental Contamination and Toxicology,*" 70:1,247-1,250, 2003; S.H. Harmon, W.L. Specht, and G.T. Chandler, "A Comparison of the Daphnids* Ceriodaphnia dubia *and* Daphnia ambigua *for Their Utilization in Routine Toxicity Testing in the Southeastern United States,"* Archives of Environmental Contamination and Toxicology,*" 45:79-85, 2003; Elphick et al. 2011,* op. cit.

[147] *Harmon et al. 2003,* op. cit.

[148] *Elphick et al. 2011,* op. cit.

[149] Ibid.

increased the number of days needed for *Pseudosida ramosa* to reach maturity from 8.75 to 11.2.[150] Exposure to sodium chloride concentrations between 1,670 mg/l and 2,660 mg/l increased the time to first reproduction in *Daphnia magna* from seven days in controls to nine days in all treatments with added salt.[151] Increases in the age at first reproduction with increasing salinity or sodium chloride concentration have also been reported in *Daphnia longispina*[152] and the copepod *Gladioferens imparipes*.[153]

Second, exposure to chloride salts can reduce the size of reproductive broods. Many zooplankton, including most cladocerans and copepods, produce offspring in clutches or broods of a few to several eggs. Decreases in the average number of eggs produced per brood with increasing concentration of sodium chloride has been reported in *Daphnia longispina*[154] and *Daphnia pulex.*[155] In addition, the time between production of broods increased and the total number of broods produced decreased with higher sodium chloride concentrations have been observed in *Daphnia magna*.[156]

Third, exposure to chloride salts can reduce the total number of offspring produced per female zooplankter. One study that exposed *Ceriodaphnia dubia* and *Daphnia ambigua* to concentrations of sodium chloride ranging between 210 mg/l and 2,200 mg/l found that the total number of offspring produced over a female's lifetime was lowered with increasing chloride concentration.[157] A similar effect occurred when *Daphnia longispina* and *Daphnia magna* were exposed to sodium chloride.[158] Reductions in total lifetime reproductive output have also been reported for zooplankton exposed to potassium chloride. In one study, *Daphnia magna* exposed to a potassium chloride concentration of 8 mg/l produced an average of

---

[150] *Freitas and Rocha 2012,* op. cit.

[151] *El-Deeb Ghazy et al. 2009,* op. cit.

[152] *Gonçalves et al. 2007,* op. cit.

[153] *M. Grzesiuk and A. Mikulski, "The Effect of Salinity on Freshwater Crustaceans,"* Polish Journal of Ecology, *54:669-674, 2006.*

[154] *Gonçalves et al. 2007,* op. cit.

[155] *Bezrici et al. 2012,* op. cit.

[156] *Martínez-Jerónimo and Martínez-Jerónimo 2007,* op. cit.

[157] *Harmon et al. 2003,* op. cit.

[158] *Gonçalves et al. 2007,* op. cit.; *Martínez-Jerónimo and Martínez-Jerónimo 2007,* op. cit.

74 offspring per female, while *Daphnia magna* exposed to concentration of 24 mg/l produced an average of 49 offspring per female.[159]

Impacts of Chloride Salts on Zooplankton Resting Eggs

Many zooplankton species produce two types of eggs. Their normal mode of reproduction is through subintaneous eggs that hatch within a few days of being developed. In addition to this, most cladoceran species and many species of copepods and rotifers produce resting eggs. These eggs are an adaptation that allows the species to weather unfavorable conditions. They are resistant to harsh environmental conditions and capable of extended periods of dormancy. Once formed, resting eggs sink to the substrate. Maximum concentrations of resting eggs in the substrate have been reported to range from 1,000 to 1,000,000 resting eggs per square meter. Hatching of resting eggs often serves to reestablish populations within a waterbody after they disappear due to reduced food levels, predation, or unfavorable environmental conditions.[160]

Studies have found that exposure to salinity and chloride salts can inhibit hatching of zooplankton resting eggs. One study collected three species of Great Lakes zooplankton, the water fleas *Bosmina liederi* and *Daphnia longiremis* and the rotifer *Brachionus calyciflorus*, from ballast sediments in ships and exposed them to several levels of salinity.[161] It found that the proportion of hatched eggs decreased with increasing salinity. At most, only 10 percent of the eggs that were exposed to elevated salinity hatched. In addition, the development of embryos in *Bosmina* and *Daphnia* eggs terminated at salinities equal to or greater than 8,000 mg/l. No hatching was seen at salinities higher than 8,000 mg/l, although some eggs that were exposed to this salinity hatched when placed into freshwater following exposure.

A second study exposed a mixture of resting eggs from different zooplankton species to levels of constant salinity ranging between 1,000 mg/l and 5,000 mg/l for a three-month period.[162] The abundance and diversity of zooplankton that hatched decreased with increasing salinity. Reduction in hatching was not

---

[159] *G.A. Leblanc and D.C. Suprenant, "The Influence of Mineral Salts on Fecundity of the Water Flea* Daphnia magna *and the Implication on Toxicity Testing of Industrial Wastewater,"* Hydrobiologia, *108:25-31, 1984.*

[160] *N.G. Hairston, Jr., "Zooplankton Egg Banks as Biotic Reservoirs in Changing Environments,"* Limnology and Oceanography, *41:1,087-1,092, 1996.*

[161] *S.A. Bailey, I.C. Duggan, C.D.A. van Overdijk, T.H. Johengen, D.F. Reid, and H.J. MacIsaac, "Salinity Tolerance of Diapausing Eggs of Freshwater Zooplankton,"* Freshwater Biology, *49:286-295, 2004.*

[162] *Nielsen et al. 2007,* op. cit.

observed when resting eggs were exposed to a 14-day pulse of saline water followed by freshwater. This suggests that some resting eggs may be able to tolerate exposure to chloride salts for a relatively short period of time.

Inhibition of resting egg hatching with increased salinity and concentrations of chloride salts may act as an additional stressor on zooplankton populations. This could reduce the likelihood of populations in salinized waterbodies reestablishing after experiencing other environmental stresses.

Factors that May Mitigate Zooplankton Sensitivity to Chloride Salts

In some instances, sensitivity of zooplankton to chloride and chloride salts may potentially be mitigated by other characteristics of the aquatic environment. For example, if food availability can modify sensitivity to higher chloride concentrations, zooplankton in environments with higher concentrations of food might be more tolerant of chloride.[163] An example of such an environment might be a eutrophic lake, although mitigation may also depend on the quality of the food. An indication that such mitigation can occur was found in a 14-day experiment that exposed a uniclonal hybrid of *Daphnia pulex* and *Daphnia pulicaria* to sodium chloride and calcium chloride.[164] At any given chloride concentration, zooplankton survival, brood size, egg production, and growth rate increase and age at first reproduction decreased with increasing food concentration. This suggests that food availability may mitigate some impacts of chloride in at least some zooplankton species.

Reduced impacts of chloride and chloride salts on zooplankton with greater food availability suggests that some impacts are related to energy demands placed upon the organism by higher salt concentrations. Regulating internal salt concentration in a salinizing freshwater environment requires that zooplankters increase their production of osmoprotectant compounds and cellular ion transporters.[165] This is energetically costly and requires the use of resources that would otherwise be allocated to somatic growth and reproduction. This idea is supported by the previously mentioned finding that *Daphnia pulex* exposed to sodium chloride had lower energy reserves than those that were not exposed.

---

[163] *Greco et al. 2022*, op. cit.

[164] *Brown and Yan 2015*, op. cit.

[165] *L.C. Latta, L.J. Weider, J.K. Colbourne, and M.E. Pfrender, "The Evolution of Salinity Tolerance in* Daphnia*: A Functional Genomics Approach,"* Ecology Letters*, 15:794-802, 2012.*

The presence of other ions in the water also affects the impact of chloride on zooplankton. For example, one study found that less severe reductions in reproduction in *Ceriodaphnia dubia* occur in water with greater hardness or alkalinity.[166] This suggest that chloride may impair osmoregulatory functions and/or acid base balance within the organism. It should be noted that this potential mechanism and the one discussed in the previous paragraph are not mutually exclusive.

Macroinvertebrates

Freshwater aquatic macroinvertebrates are animals without backbones that are large enough to be seen without a microscope. They are typically larger than one or two millimeters. Macroinvertebrates consist of several groups of animals, each with their own biological characteristics. Macroinvertebrates include bivalves such as mussels and clams; gastropods such as snails; annelids such as worms and leeches; crustaceans such as crayfish, amphipods, and isopods; and insects. Members of most of these groups spend their entire life cycle within water; however, only juvenile stages of many aquatic insect species are found in water. The adults in many aquatic insect species are terrestrial. Macroinvertebrates are important consumers in aquatic food webs. Many species process organic matter, breaking down large organic material such as leaves into smaller pieces. Others feed on small organic particles in the water column or sediment. Still others graze on algae and fungi that grow on the surfaces of rocks or aquatic plants. Macroinvertebrates in turn are significant prey for larger organisms such as fish, amphibians, birds, and mammals. Some examples of macroinvertebrates are shown in Figure 3.5.

Freshwater Macroinvertebrate Sensitivity to Chloride and Salinity

Some general features of the biology of many freshwater aquatic macroinvertebrates can make them sensitive to impacts from chloride salts and salinity. As freshwater organisms, they are adapted to an environment in which the salt concentration is lower than that of their internal fluids. Normally, the main problem that they face relative to salt concentration is keeping enough ions in their bodies.[167] Macroinvertebrate physiology is adapted to address this problem and not the opposite problem of keeping ions out. This can be an issue because they typically maintain the osmotic balance of their internal fluids within a narrow range. There are limits relative to external salt concentrations over which macroinvertebrates can do this. Outside of these limits, their ability to regulate osmotic pressure, or the tendency of water to

[166] *P.J. Lasier and I.R. Hardin, "Observed and Predicted Reproduction of* Ceriodaphnia dubia *Exposed to Chloride, Sulfate, and Bicarbonate,"* Environmental Toxicology and Chemistry*, 29:347-358, 2010.*

[167] *A. Tiwari and J.W. Rachlin, "A Review of Road Salt Ecological Impacts,"* Northeaster Naturalist*, 25:123-142, 2018.*

flow into their bodies, breaks down.[168] In particular, they are generally incapable of maintaining body solute concentrations below that of the water in which they live. As the salinity of the environment increases, macroinvertebrates tend to take up more ions and lose water from cells until the cells can no longer function properly.[169] This can disrupt their metabolism and if the disruption is severe enough or prolonged, it can lead to organism death.

Freshwater organisms have two broad strategies for dealing with the osmotic challenges created by increased salinity.[170] First, they can adjust their intracellular osmotic pressure to meet that of the environment by synthesizing compatible solutes, such as amino acids and proteins. Some macroinvertebrates, such as salt marsh mosquitoes, are capable of this, but many others lack this ability.[171] Second, they can maintain the volume of their body fluids and osmotic pressure through changes in permeability of cell membranes and ion transport within different tissues.[172] This involves active transport of salt ions. For example, some aquatic insects, especially among mayflies, stoneflies, and caddisflies, have chloride cells that can transport chloride and sodium ions through active transport; however, in insects these cells only enable them to bring the ions into their bodies. They do not enable the insects to excrete them.

There are two consequences to either of these strategies to regulate chloride intake. First, they are better at enabling the organisms to tolerate conditions in which the salinity of the environment is lower than their internal salt concentration. For example, laboratory studies show that mayflies, stoneflies, and caddisflies

---

[168] *B.J. Kefford, D. Nugegoda, L. Zalizniak, E.J. Fields, and K.L. Hassell, "The Salinity Tolerance of Freshwater Macroinvertebrate Eggs and Hatchlings in Comparison to Their Older Life-Stages: A Diversity of Responses,"* Aquatic Ecology, *41:335-348, 2007.*

[169] *B.T. Hart, P. Bailey, R. Edwards, K. Hortle, K. James, A. McMahon, C. Meredith, and K. Swadling, "A Review of the Salt Sensitivity of Australian Freshwater Biota,"* Hydrobiologia*, 210:105-144, 1991.*

[170] *J. Velasco, C. Gutiérrez-Cánovas, M. Botella-Cruz, D. Sánchez-Fernández, P. Arribas, J.A. Carbonell, A. Millán, and S. Pallarés, "Effects of Salinity Changes on Aquatic Organisms in a Multiple Stressor Context,"* Philosophical Transactions of the Royal Society Series B*, 374:20180011, 2018.*

[171] *M.A Chadwick, H. Hunter, J.W. Feminella, and R.P Henry, "Salt and Water Balance in* Hexagenia limbate *(Ephemeroptera: Ephemeridae) When Exposed to Brackish Water,* The Florida Entomologist*, 85:650-651, 2002.*

[172] *H. Komnick, "Chloride Cells and Chloride Epithelia of Aquatic Insects,"* International Review of Cytology*, 49:285-328, 1977.*

better tolerate conditions in which environmental salinity is lower than their internal salt concentrations.[173] Second, using either of these strategies requires expenditure of energy by the organism. This reduces the energy that is available for growth and reproduction and can lead to a variety of impacts on the organism which will be discussed later in this section.

The specific habitats in which many macroinvertebrates live is a second aspect of their biology which can make them sensitive to impacts from chloride salts. Many macroinvertebrates live on or within the bed substrates of streams, lakes, or wetlands. As described in Chapter 2 of this report, introduction of chloride salts into a waterbody can result in the formation of a dense layer of water containing a relatively high concentration of chloride immediately above the bottom of the waterbody. This can expose organisms living on and in the substrate to higher chloride concentrations than would be experienced by organisms living higher in the water column.[174] Freshwater mussels are a good example of macroinvertebrates that may experience higher exposure to chloride salts due to their living in benthic habitat. In addition, since juvenile mussels in many species remain burrowed during early life stages, they may also experience higher exposure to chloride if groundwater or water in the hyporheic zone beneath the bed surface is contaminated with salt.[175]

Macroinvertebrates differ in their sensitivity to chloride salts and salinity. Mayflies and stoneflies in the insect orders Ephemeroptera and Plecoptera and air-breathing (pulmonate) snails are particularly sensitive to salinity.[176] For example, in field settings species richness in mayfly, stonefly, and caddisfly taxa decreases with increasing salinity.[177] Similarly, the number of genera of mayflies present at sites was found to decrease with increasing chloride concentration.[178] On the other hand, larval beetles, dragonflies, and damselflies in

---

[173] *N.N. Kapoor, "Osmotic Regulation and Salinity Tolerance of the Stonefly Nymph,* Paragnetina media*,"* Journal of Insect Physiology*, 25:17-20, 1979.*

[174] *C. Ellis, R. Champlin, and H.G. Stefan, "Density Current Intrusions in and Ice-covered Urban Lake,"* Journal of the American Water Resources Association, *33:1,363-1,374, 1997.*

[175] *Hintz and Relyea 2019,* op. cit.

[176] *B.J. Kefford, G.L. Hickey, A. Gasith, E. Ben-David, J.E. Dunlop, C.G. Palmer, K. Allan, S.C. Choy, and C. Piscart, "Global Scale Variation in the Salinity Sensitivity of Riverine Macroinvertebrates: Eastern Australia, France, Israel and South Africa,"* PLoS One*, 7:e.35224, 2012; Hassell et al. 2006,* op. cit.

[177] *Cañedo-Argüelles et al. 2013,* op. cit. *and references therein.*

[178] *S. Stranko, R. Bourquin, J. Zimmerman, M. Kishiwagi, M. McGinty, and R. Klauda,* Do Road Salts Cause Environmental Impacts? *Maryland Department of Natural Resources, 2013.*

the insect orders Coleoptera and Odonata are more tolerant of salinity. This is also the case of some crustaceans and some true flies in the insect order Diptera, including some mosquito and biting midge species.[179]

Tolerance of aquatic macroinvertebrate groups to chloride salts and salinity may be based upon specific biological or life history traits. Field studies conducted in rivers in Germany and France that had low salinity upstream and high salinity downstream due to point source discharges showed clear differences in the traits that were associated with different levels of specific conductance. These differences accounted for about 30 percent of the variation in the data.[180] Macroinvertebrates found in high specific conductance reaches of these rivers tended to incubate their eggs internally, conduct gas exchange through gills, produce multiple broods of offspring over a year, feed by shredding leaves and other large particulate organic matter, and have a longer life cycle. Macroinvertebrates found in the low specific conductance reaches tended to deposit their eggs in clutches in the environment, exchange gases through their body walls, produce only one brood per year, and have a shorter life cycle. The same studies also saw taxonomic differences that were associated with the level of specific conductance, with crustacea and some mollusks being more common and mayflies, stoneflies, and caddisflies being less common in stream reaches with higher specific conductance.

Possession of certain traits may account for the sensitivity of freshwater mussels and mussel populations to chloride salts and salinity for several reasons. First, they maintain the lowest internal salt concentration of any animal.[181] Although this results in their having lower energy requirements for ion regulation, it makes them more sensitive to environmental concentrations of salts than other animals.[182] They are also sessile and cannot easily escape to another part of the waterbody when exposed to high salt concentrations. Their ability to close their shells to avoid elevated salt concentrations is a temporary solution. Ultimately,

---

[179] *Cañedo-Argüelles et al. 2013,* op. cit. *and references therein.*

[180] *C. Piscart, P.Usseglio-Poatera, J.-C. Moreteau, and J.-N. Beisel, "The Role of Salinity in the Selection of Traits of Freshwater Invertebrates,"* Archiv für Hydrobiologie*, 166:185-198, 2006; E. Szocz, E. Coring, J. Bathe, and R.B. Schafer, "Effects of Anthropogenic Salinization on Biological Traits and Community Composition, of Stream Macroinvertebrates,"* Science of the Total Environment, 468-469:943-949, 2014.

[181] *P. Wilmer, G. Stone, and I. Johnson,* Environmental Physiology of Animals (second edition), *Blackwell, Malden, Massachusetts, 2005.*

[182] *L.F. Gainey and M.J. Greenberg, "Physiological Basis of the Species Abundance-Salinity Relationship in Molluscs: A Speculation,"* Marine Biology*, 40-41-49, 1977.*

metabolic requirements such as the need to acquire oxygen or excrete wastes will force them to reopen their shells.[183] As a result, closing their shell does not provide protection from extended periods of increased salinity or chloride concentration. Freshwater mussels also have a complicated life history. Their larvae, called glochidia, are obligate parasites on fish hosts.[184] Changes in the abundance of these hosts as a result of increased chloride concentration can affect recruitment of new mussels into the adult population.[185] Finally, they are long lived, with some species having life spans in excess of a century. Because of this, adult mussels can persist in a waterbody for decades despite a lack of recruitment. This makes it hard to recognize that a population is declining.[186]

Types of Effects of Chloride Salts and Salinity on Macroinvertebrates

Exposure to chloride, chloride salts, or salinity has been found to cause a variety of sublethal effects in macroinvertebrates. While different effects have been observed in different species, these effects fall into three broad categories: changes in behavior, changes in growth and development, and impacts on reproduction.

Effects of Chloride Salts and Salinity on Macroinvertebrate Behavior

Behavioral effects of exposure to chlorides include changes to feeding rates, changes in locomotion, and induction of invertebrate drift. Examples of changes in feeding rates have been observed in clams and mussels. The fingernail clam *Musculium transversum* beats its lateral cilia to bring food and oxygenated water to itself and carry wastes away. Impairment of ciliary beating is a sign of stress and can be detrimental to these clams. In eight-day experiments, exposure to potassium chloride impaired ciliary beating.[187] The rate of beating decreased as the concentration of potassium chloride increased. The impact of exposure may be permanent. When large clams were exposed to potassium chloride and subsequently placed in

---

183 *C.J. Blakeslee, H.S. Galbraith, L.S. Robertson, and B. St. John White, "The Effects of Salinity Exposure on Multiple Life Stages of a Common Freshwater Mussel,* Elliptio complanata*,"* Environmental Toxicology and Chemistry*, 32:2,849-2,854, 2013.*

184 *M.C. Barnhart, W.R. Haag, and W.N. Roston, "Adaptations to Host Infection and Larval Parasitism in Unionoida,"* Journal of the North American Benthological Society*, 27:370-394, 2008.*

185 Ibid.

186 *D.L. Strayer, J.A. Downing, W.R. Haag, T.L. King, J.B. Layzer, T.J. Newton, and S.J. Nichol, "Changing Perspectives on Pearly Mussels, North America's Most Imperiled Animals,"* BioScience*, 54:429-439, 2004.*

187 *K.B. Anderson, R.E. Sparks, and A.A. Paparo,* Rapid Assessment of Water Quality Using the Fingernail Clam *Musculium transversum*, *University of Illinois Water Resources Center Report No, 133, April 1978.*

freshwater, the beating rates did not recover. A more complicated effect of chloride salts on feeding rates was observed with exposure of a unionid mussel to sodium chloride.[188] Over shorter exposure periods or intermittent exposures at low or moderate concentrations, the mussel *Anodonta anotina* increased its filtration rate, but over longer exposure periods at higher concentrations its filtration rate decreased. The authors suggested that the increase in filtration rates at lower concentrations may reflect a need for mussels to flush salt from their bodies. Prolonged reductions in feeding rates by macroinvertebrates can reduce the energy that the organisms have available for growth and reproduction.

Exposure to chloride salts can also adversely affect locomotion in macroinvertebrates. For example, the swimming performance of the amphipod *Gammarus subaegensis* exposed to calcium chloride was reduced relative to that of untreated animals.[189] Performance decreased as calcium chloride concentration increased. About half the ampipods showed reduced swimming performance at a calcium chloride concentration of 2,850 mg/l.

Increases in chloride concentrations alter invertebrate drift behavior in streams and rivers. Drift is a common behavior in which macroinvertebrates living on or in the substrate or on aquatic macrophytes enter the water column and are transported downstream in flow. Normally, this serves as a means of avoiding predators and dispersing into downstream areas. Higher chloride concentrations can lead to an increase in drift behavior. One field study found that drift behavior increased when chloride concentrations exceeded 1,000 mg/l.[190] A mesocosm experiment examining a mixed macroinvertebrate community found that the number of organisms drifting increased with higher sodium chloride concentration.[191] An example of this is seen in another study in which chloride concentrations of 606 mg/l, 1,516 mg/l, and 6,066 mg/l led to 13 percent, 45 percent, and 58 percent increases, respectively, in drift in the amphipod *Gammarus*

---

[188] *J.T. Hartmann, S. Beggel, K. Auershald, B.C. Stoeckle, and J. Geist, "Establishing Mussel Behavior as a Biomarker in Ecotoxicology,"* Aquatic Toxicology, *170:297-288, 2016.*

[189] *M.J. Baek, T.J. Yoon, D.G. Kim, C.Y. Lee, K. Cho, and Y.J. Bae, "Effects of Road Deicer Runoff on Benthic Macroinvertebrate Communities in Korean Freshwaters with Toxicity Tests of Calcium Chloride ($CaCl_2$),"* Water, Air, and Soil Pollution, *225:e1961, 2014.*

[190] *R.A. Crowther and H.B.N. Hynes, "Effect of Road Deicing Salt on Drift of Stream Benthos,"* Environmental Pollution, *14:113-126, 1977.*

[191] *M. Cañedo-Argüelles, T.E. Grantham, I. Perrée, M. Rieradevall, R. Céspedes-Sánchez, and N. Prat, "Response of Stream Invertebrates to Shot-term Salinization: A Mesocosm Approach,"* Environmental Pollution, *166:144-151, 2012.*

pseudolimnaeus.[192] This chloride-induced increase in drift behavior could potentially reduce the abundance of macroinvertebrates in reaches of streams and rivers impacted by elevated chloride concentrations.

Behavioral changes induced by exposure to chloride salts can potentially affect the viability of macroinvertebrate populations. Reductions in feeding rate can lead to slower growth and less reproduction, which can ultimately reduce the number of new individuals recruited into the population. In addition, impairment of locomotion and alteration of drift behavior can increase exposure of the affected organisms to predators. Increases in drift behavior above normal levels can lead to removal of some macroinvertebrate species from chloride-impacted stream reaches. If the effects are great enough, the abundance of sensitive species of macroinvertebrates in chloride-impacted habitats may be greatly reduced. In some instances, sensitive species may be extirpated from highly impacted habitats.

Effects of Chloride Salts and Salinity on Macroinvertebrate Development

Chloride and chloride salts can affect growth and development of freshwater macroinvertebrates. Impacts that have been reported include reductions in growth, lengthening the period needed to complete larval development, reductions in the size of adults, introduction of developmental deformities, and alterations of sexual dimorphism and sex ratios. Increased concentrations of chloride or salinity and increased specific conductance have been reported to reduce growth in several species of macroinvertebrates. For example, reductions in the final biomass attained by larvae of the midge *Chironomus dilutus* after 28 days of growth were observed at a chloride concentration of 2,133 mg/l, with a 50 percent reduction seen at a concentration of 3,047 mg/l.[193] A similar reduction in growth with increasing chloride concentration has been reported in mayflies. Over 90 days, first instar nymphs of the burrowing mayfly *Hexagenia limbata* achieved higher biomass at a salinity of 0 mg/l than at 2,000 mg/l or 4,000 mg/l.[194] Growth reductions in mayflies have been reported occurring as a result of several different chloride salts. A series of 14-day growth experiments found 25 percent reductions in growth in the mayfly *Neocloeon tringulifer* when exposed to sodium chloride

---

[192] *B.J. Blasius and R.W. Merritt, "Field and Laboratory Investigations on the Effects of Road Salt (NaCl) on Stream Macroinvertebrate Communities,"* Environmental Pollution*, 120:219-231, 2002.*

[193] *Elphick et al. 2011,* op. cit.

[194] *M.A. Chadwick and J.W. Feminella, "Influence of Salinity and Temperature on the Growth and Production of a Freshwater Mayfly in the Lower Mobile River, Alabama,"* Limnology and Oceanography*, 46:532-542, 2001.*

concentrations of 229 mg/l or potassium chloride concentrations of 356 mg/l.[195] Similarly, 20-day growth experiments found reductions in daily growth rates when the same mayfly species was exposed to a mixture of sodium chloride and calcium chloride.[196] Reductions began to be seen when specific conductance was above 363 μS/cm. Finally, the lengths, widths, and masses attained by the snail *Heliosoma trivovlis* decreased with increasing specific conductance.[197]

Chloride- and salinity-induced reductions in growth can lengthen the time required for freshwater macroinvertebrates to complete larval development. For example, over 7-day experiments the number of molts undergone by the mayfly *Isonychia bicolor* decreased with increasing chloride concentration. This effect appeared to be dose dependent, with higher chloride concentrations leading to fewer molts.[198] Since the number of molts is an indication of the amount of growth, this result indicates that increasing chloride concentrations led to slower growth. Similarly, the time needed for first instar larvae of the mosquito *Culiseta incidens* to complete larval development and form pupae got longer with increasing concentration of sodium chloride.[199]

In some instances, slower growth and longer developmental periods resulting from exposure to chloride salts may occur despite increased food consumption. An experiment exposing the isopod *Lirceus* sp. to sodium chloride found that the amount the isopod grew was 12 percent lower at a chloride concentration of about 215 mg/l than it was at a concentration of about 4 mg/l despite the isopods in the high salt

---

[195] *K.A. Struewing, J.M. Lazorchak, P.C. Weaver, B.R. Johnson, D.H Funk, and D.B. Buchwalter, "Part 2: Sensitivity Comparisons of the Mayfly* Centroptilum triangulifer *to* Ceriodaphnia dubia *and* Daphnia magna *Using Standard Reference Toxicants; NaCl, KCl, and $CuSO_4$,"* Chemosphere*, 139:597-603, 2015.*

[196] *B.R. Johnson, P.C. Weaver, C.T. Nietch, J.M. Lazorchak, K.A. Struewing, and D.H. Funk, "Elevated Major Ion Concentrations Inhibit Larval Mayfly Growth and Development,"* Environmental Toxicology and Chemistry*, 34:167-172, 2015.*

[197] *J.G. Suski, C.J. Solace, and R. Patiño, "Species-Specific and Transgenerational Responses to Increasing Salinity in Sympatric Freshwater Gastropods,"* Environmental Toxicology and Chemistry*, 31:2,517-2,524, 2012.*

[198] *B.S. Echols, R.J. Currie, D.S. Cherry, and J.R. Voshell, "Seasonal Availability and Sensitivity of Two Field-Collected Mayflies for the Development of a Standardized Toxicity Test,"* Environmental Monitoring and Assessment*, 185:1,341-1,353, 2013.*

[199] *F.C. Lee, "Effect of Various Sodium Chloride Concentrations on the Development of the Mosquito* Culiseta incidens *(Thompson),"* Mosquito News*, 33:78-83, 1973.*

treatment eating about 74 percent more.[200] At the same time, there appeared to be no change in the efficiency of the isopods' assimilation of food. This suggests that the increased consumption was respired, potentially to accommodate higher metabolic demands for dealing with osmotic stress. The reduction in growth rate may reflect diversion of energy in the organism from growth to meeting these metabolic demands.

In insects that undergo complete metamorphosis, increasing salinity can also affect the size and weight of the insects when they pupate. For example, a study found that the dry weight of pupae of the mosquito *Aedes aegypti* decreased with increasing salinity.[201] Pupation does not generally occur until insects gain a critical mass of internal nutrient stores.[202] Because adult insects are encased in an exoskeleton and cannot molt, adult size and weight are determined by the weight of the pupa. The size that pupae attain is based on the ability of larvae to gather and retain nutrients during the larval period. As a result, increased energy expenditures or reduced nutrient assimilation due to physiological impacts from higher salt concentrations require insect larvae to either increase their feeding rates, experience longer larval periods, or attain reduced adult body sizes.

Developmental deformities in macroinvertebrates have also been associated with increased salinity. For example, wing deformities were observed in *Chironomus* midges emerging from pupae that were incubated a specific conductance equal to or greater than 2,500 μS/cm.[203] Similarly, malformations were seen in hatchlings of the snail *Heliosoma trivolvis* at specific conductance greater than 1,500 μS/cm.[204] The percentage of malformed snails increased with specific conductance, reaching 17 percent at a specific conductance of 3,750 μS/cm.

---

[200] *M. Tyree, N. Clay, S. Polaskey, and S. Entrekin, "Salt in Our Streams: Even Small Sodium Additions Can Have Negative Effects on Detritivores,"* Hydrobiologia*, 775:109-122, 2016.*

[201] *T.M. Clark, B.J. Flis, and S.K. Remold, "Differences in the Effects of Salinity on Larval Growth and Developmental Programs of a Freshwater and a Euryhaline Mosquito Species (Insecta: Diptera, Culicidae)*, The Journal of Experimental Biology*, 207:2,289-2,295, 2004.*

[202] *A.N. Clements,* The Biology of Mosquitoes. Volume 1. Development, Nutrition, and Reproduction*, CABI Publishing, Wallingford, U.K., 2000.*

[203] *Hassell et al. 2006,* op. cit.

[204] *Suski et al. 2012,* op. cit.

Exposure to chloride salts can induce other developmental effects. High concentrations of chloride salts can affect sex ratios and sexual dimorphism in some macroinvertebrate species. An example of this comes from an experiment in which larvae of the midge *Chironomus riparius* were exposed to different concentrations of sodium chloride while under a temperature regime simulating a spring thaw.[205] When larvae were exposed to 0 mg/l sodium chloride, emerging adult females were larger than emerging adult males. This is normal for this species. The ratio of males to females in the emerging midges was one to one. The average time to complete development was about 48 days. When larvae were exposed to 5,000 mg/l sodium chloride, emerging adult females were the same size as emerging adult males. The ratio of males to females was two to one. The average time to complete development was about 59 days.

Impacts of chloride and chloride salts on growth and development can potentially affect the viability of macroinvertebrate populations. This can happen in a variety of ways. In many macroinvertebrate species, the number of eggs a female can produce is a function of her size, with larger females producing more eggs. In these species, reductions in the size of females resulting from increased ambient concentrations of chloride will lead to fewer eggs being laid. Coupled with other impacts of chloride, this can lead to decreases in the abundance of impacted macroinvertebrate species. For those species that reproduce only once during the year, larval forms such as aquatic insect nymphs have a limited period in which to achieve adulthood and reproduce. If the developmental period of a species is lengthened too much, there may not be adequate time remaining in the season for adults to emerge and reproduce before the onset of winter. This can also lead to fewer eggs being laid and a decrease in the abundance of the species. Similar reductions in abundance may result from a large enough percentage of the population having deformities or a skewed sex ratio.

Effects of Chloride Salts and Salinity on Macroinvertebrate Reproduction

Chloride and chloride salts can affect freshwater macroinvertebrate reproduction. Examples of impacts include reductions in reproductive output which consists of the number of offspring produced, delay or prevention of reproduction, reduction in the motility of sperm, slower egg development, reduced hatching success, and unsuccessful attachment of mussel glochidia to fish hosts. For example, in 28-day tests, exposure to chloride resulted in reductions in reproductive output in two annelid worm species.[206] Reductions were observed in the blackworm *Lumbriculus variegatus* at a chloride concentration of 366 mg/l,

---

[205] *D.W. Lob and P. Silver, "Effects of Elevated Salinity from Road Deicers on* Chironomus riparius *at Environmentally Realistic Springtime Temperatures,"* Freshwater Science*, 31:1,078-1,087, 2012.*

[206] *Elphick et al. 2011,* op. cit.

with 50 percent of the exposed animals showing lower reproduction at 958 mg/l. Similarly, reductions were observed in the sludge worm *Tubifex tubifex* at a chloride concentration of 462 mg/l, with 50 percent of the exposed animals showing reproductive reductions at 752 mg/l. Another study showed that the snail *Heliosoma trivolvis* did not reproduce at specific conductance above 3,000 μS/cm and that the onset of reproduction was delayed at specific conductance levels above 2,000 μS/cm.[207]

Exposure to sodium chloride with chloride concentrations of about 580 mg/l has also been reported to suppress motility of sperm in the zebra mussel, *Dreissena polymorpha*.[208] Such suppression prevents sperm from reaching eggs and limits fertilization.

Exposure to chloride salts and salinity can also increase egg development time and reduce hatching of macroinvertebrate eggs. For instance, eggs of the snail *Heliosoma trivolvis* took longer to hatch at specific conductance levels above 250 μS/cm than they took at lower values.[209] This suggests that exposure to higher salinity delayed embryonic development. The fraction of eggs of the snail *Glyptophysa gibbosa* that hatched decreased with increasing specific conductance.[210] Similarly, hatching of the eggs of the common backswimmer, *Notonecta glauca* decreased with increasing specific conductance.[211]

Exposure to chloride salts can also reduce attachment of freshwater mussel glochidia to fish hosts. As previously described, mussel glochidia must attach to a fish host in order to continue development. Attachment is controlled by an ion gradient which can induce clamping behavior, which is important for attaching.[212] Elevated salinity can induce premature clamping and can reduce the number of glochidia that

---

[207] *Suski et al. 2012,* op. cit.

[208] *A. Ciereszko, K. Dabrowski, B. Piros, M. Kwasnik, and J. Glogowski, "Characterization of Zebra Mussel (*Dreissena polymorpha*) Sperm Motility: Duration of Movement, Effects of Cations, pH and Gossypol,"* Hydrobiologia*, 452:225-232, 2001.*

[209] *Suski et al. 2012,* op. cit.

[210] *Kefford et al. 2007,* op. cit.

[211] *H. Komnick and W. Wichard, "Chloride Cells of Larva of* Notonecta glauca *and* Naucoris cimicoides *(Hemiptera, Hyrocorisae) Fine Structure and Cell Counts at Different Salinities,"* Cell and Tissue Research*, 156:539-549, 1975.*

[212] *G. Lefeve and W.C. Cutis, "Studies on the Reproduction and Artificial Propagation of Freshwater Mussels,"* Bulletin of the United States Bureau of Fisheries*, 30:105-201, 1912.*

are able to successfully attach to fish hosts.[213] For example, the percentage of glochidia in the species *Anodonta anatina* that successfully attached to fish decreased from 40 percent at a chloride concentration of 130 mg/l to 7 percent at a chloride concentration of 2,909 mg/l.[214] Similarly, at a sodium chloride concentration of 3,000 mg/l, attachment of glochidia of the mussel *Elliptio complanata* was only 11 percent of the level seen in controls.[215]

Impacts of chloride and chloride salts on reproduction can potentially affect the viability of macroinvertebrate populations. Lower reproductive output, reductions in the motility of sperm, slower egg development, reduced hatching success, and reduced attachment of mussel glochidia to fish hosts can all reduce the recruitment of adults into the population. Over the long term, this can reduce species abundance.

Fish

Freshwater fish are animals with backbones that live all life stages entirely in water. Freshwater fish vary in size. For example, the least darter (*Etheostoma microperca*) is one of the smallest fish in Wisconsin. Typically, its length is only one to one-and-a-half inches. The lake sturgeon (*Acipenser fulvescens*) is one of the largest fish in the State and typical lengths range between 59 and 71 inches. The maximum length for a lake sturgeon reported in Wisconsin is 98 inches. Some examples of fish reported to be impacted by chloride salts are shown in Figure 3.6.

Fish perform several roles in aquatic communities. They occupy all consumer categories in aquatic food webs. This includes detritivores; herbivores that feed on plankton, periphyton, or macrophytes; and predators that feed on zooplankton, macroinvertebrates, or other fish. Some fish are generalists that feed on an array of food items. Fish continue to grow throughout their entire lives. As a result, the diet of some fish species changes with age and size. For example, some species shift from feeding on zooplankton to macroinvertebrates to other fish as they grow. This reflects an aspect of fish biology that most fish consume their prey whole. The largest prey fish can consume is limited by the size of their gape and organisms that are larger than this are not susceptible to predation. As fish grow their gape gets larger as does the size of the prey they can ingest.

---

[213] *S. Beggel and J. Geist, "Acute Effects of Salinity Exposure on Glochidia Viability and Host Infection of the Freshwater Mussel* Anodonta anatine*, (Linnaeus, 1758),* Science of the Total Environment*, 502:659-665, 2015.*

[214] Ibid.

[215] *Blakeslee et al. 2013,* op. cit.

Fish can also serve as prey in aquatic communities and the major predator of fish is other fish. Large predatory invertebrates may also feed on fish larvae and fry. In addition, some mammals and birds specialize as fish predators.

Fish also have an important role in outdoor recreation in Wisconsin. People in the State commonly fish for trout and salmon; other sport fish such as largemouth and smallmouth bass, northern pike, walleye, and muskellunge; panfish such as bluegill, yellow perch, and crappies; and whitefish. In addition, people also fish for rough fish such as suckers and carp. Recreational fishing has a large economic impact on Wisconsin. The Wisconsin Department of Natural Resources (WDNR) estimates that fishing generates almost $2.3 billion in economic activity annually.

Fish Sensitivity to Chloride and Salinity

Some general features of the biology of fish can make them sensitive to impacts from chloride salts and salinity in general. Freshwater fish generally maintain their body fluids at an ionic concentration of about 10,000 mg/l, which is far higher than that in the environment.[216] They gain water and lose ions passively, with sodium and chloride ions passing through their gills, oral membranes, intestinal surface, and skin. Fish conduct osmotic regulation through active transport of ions into their cells across their oral membranes and gill surfaces and the excretion of large amounts of dilute urine. Fish must expend energy to use these regulatory processes. This reduces the energy that is available for growth and reproduction and can lead to a variety of impacts on the organism that will be discussed later in this section.

Life stage is an important consideration when examining the effects of chloride salts and salinity on fish.[217] Early life stages of fish are often more sensitive to chloride salts and salinity than adults. Some examples of early life stages in salmon are shown in Figure 3.7. Larval fish lack adult osmoregulatory capabilities. Trout and salmon, for example, enter a nonfeeding alevin stage after hatching. These larvae lack fully developed structures such as gills and kidneys that help fish cope with environmental contaminants and regulate the ion concentration of their internal fluids.[218] This is typical of newly hatched fish of all species. In addition, larval fish have a greater surface to volume ratio than adult fish that provides a relatively greater area for movement of water and ions across their skin. Larval fish also have fewer gill filaments. These structures are

---

[216] *Hart et al. 1991,* op. cit.

[217] *W.D. Hintz and R.A. Relyea, "Impacts of Road Deicing Salts on the Early-Life Growth and Development of a Stream Salmonid: Salt Type Matters,"* Environmental Pollution*, 223:409-415, 2017.*

[218] *Hintz and Relyea* Environmental Pollution *2017,* op. cit.

involved in the transfer of ions and water across the gills.[219] Larval fish are unable to move away from contaminated habitats. As a result of all these factors, larval fish are less tolerant of chloride salts and salinity than either eggs or adults.

Timing of reproduction can also affect the sensitivity of some fish species to chloride salts and salinity. Larval fish and fry of some species are present in streams or lakes during spring snowmelt and are therefore exposed to high salinity and concentrations of chloride.

Types of Impacts of Chloride Salts on Fish

Exposure to chloride, chloride salts, or salinity has been found to cause a variety of sublethal effects in fish. While different effects have been observed in different species, these effects fall into four broad categories: changes in metabolism, changes in behavior, changes in growth, and impacts on reproduction. It is also important to recognize that relatively few fish species have been examined and most of those are species that are easily reared or kept in a laboratory setting. For example, when adult rainbow trout (*Oncorhynchus mykiss*) are properly acclimated, they may be able to physiologically compensate for higher salinity.[220] Other obligate freshwater salmonids may lack this ability.[221]

Impacts of Chloride Salts and Salinity on Fish Metabolism

Increases in salinity can lead to changes in metabolic rates in fish. One study found that over one week of exposure, the metabolic rates of redbelly dace (*Phoxinus erythrogaster*) and northern studfish (*Fundulus catentus)* rose with increasing salinity.[222] The authors suggested that the increase in metabolism may reflect energy use by the fish to repair damage from salinity or to excrete salt. Another study that exposed goldfish (*Carassius auratus*) to a range of salinities found that the conversion of food mass to fish mass was lower at

---

[219] *Hart et al. 1991,* op. cit.

[220] *J.D. Morgan and G.K. Iwama, "Effects of Salinity on Growth, Metabolism, and Ion Regulation in Juvenile Rainbow and Steelhead trout (*Oncorhynchus mykiss*) and Fall Chinook Salmon (*Onchorhynchus tshawyscha*),"* Canadian Journal of Fisheries and Aquatic Sciences*, 48:2,083-2,094, 1991.*

[221] *S.D. Blair, D. Matheson, Y. He, and G.G. Goss, " Reduced Salinity Tolerance in Arctic Grayling (*Thymallus arcticus*) Is Associated with Rapid Development of a Gill Interlamellar Cell Mass: Implications of High-Saline Spills on Native Freshwater Salmonids,"* Conservation Physiology, *4: doi: 10.1093/conphys/cow010, 2016.*

[222] *C. Toepfer and M. Barton, "Influence of Salinity on the Rates of Oxygen Consumption in Two Species of Freshwater Fishes,* Phoxinus erythrogaster *(Family Cyprinidae) and* Fundulus catenatus *(Family Fundulidae*)," Hydrobiologia, *242:149-154, 1992.*

salinities greater than about 4,000 mg/l.[223] It is likely that the additional food these fish consumed was respired to provide energy to compensate for higher salinity. A more complicated relationship between salinity and metabolic rates was seen in a study with fathead minnows (*Pimephales promelas*).[224] In a 24-hour test, the minnows' metabolic rate was lower at salinities of 200 mg/l to 400 mg/l than it was at lower salinities. The authors suggested that this reflected reduced activity by the fish at higher salinities. Researchers observed a different result in a 96-hour test for fathead minnows.[225] At lower salinities, the minnows' metabolic rate increased with increasing salinity, plateauing at a salinity of 100 mg/l. This plateaued rate then lowered when salinities exceeded 400 mg/l. The increase in metabolic rate with salinity is what would be expected if the fish required more energy to maintain osmoregulation. The decrease in metabolic rate at higher salinities may have resulted from the fish reaching the upper limits of their ability to compensate for stresses related to salinity.[226] While the fathead minnows were able to survive, they may not have been able to maintain their metabolic rate in the presence of higher salinity. These studies indicate that increases in salinity, including those imposed by higher chloride concentrations, can impose additional metabolic costs on fish which can reduce the scope of activity for fish and may lead to reductions in growth or reproduction.

Impacts of Chloride Salts and Salinity on Fish Behavior

Behavioral impacts of salinity that have been reported in fish include general decreases in activity, impaired locomotion, and reductions in antipredator behaviors. Levels of activity in goldfish (*Carassius auratus*) during the day were reduced as compared to controls at all salinities equal to or greater than 2,000 mg/l.[227] Interestingly, exposure to salinity up to 10,000 mg/l had no effect on the goldfish level of activity during night-time. In a seven-day experiment, fathead minnow (*Pimephales promelas*) larvae that were exposed to sodium chloride showed impaired swimming behavior at concentrations of 4,000 mg/l and 8,000 mg/l.[228]

---

[223] *R.K Luz, R.M. Martínez-Álvarez, N. DePedro, and M.J. Delgado, " Growth, Food Intake Regulation, and Metabolic Adaptations in Goldfish* (Carassius auratus*) Exposed to Different Salinities,"* Aquaculture*, 276:171-178, 2008.*

[224] *D. H. Pistole, J.D. Peles, and K. Taylor, "Influence of Metal Concentrations, Percent Salinity, and Length of Exposure on the Metabolic Rate of Fathead Minnows (*Pimephales promelas*),"* Comparative Biochemistry and Physiology, Part C*, 148:48-52, 2008.*

[225] Ibid.

[226] *P. Calo, "Physiological Costs of Combating Chemical Toxicants: Ecological Implications,"* Comparative Biochemistry and Physiology, Part C*, 100:3-9, 1991.*

[227] *Luz et al. 2008*, op. cit.

[228] *Baek International, "Ecotoxicity Test Results," 1999, cited in Evans and Frick 2001*, op. cit.

Finally, the amounts of antipredator behavior exhibited by fathead minnow exposed to salinity decreased when salinity reached and exceeded 8,000 mg/l.[229]

Impacts of Chloride Salts and Salinity on Fish Growth

Chloride salts and salinity can affect the growth of freshwater fish. Several early life stages can be affected. For example, over a 470-degree day exposure,[230] Atlantic salmon (*Salmo salar*) alevins (nonfeeding larvae) exposed to road salt consisting of over 98 percent sodium chloride at a concentration 1,000 mg/l showed less growth than those in a control treatment.[231] The rate at which these larvae used their yolk sacs also decreased with salt concentration, suggesting slower conversion of stored food mass to fish mass. Similar reductions in growth with increased chloride concentrations have been reported in newly hatched rainbow trout (*Onchorhynchus mykiss*).[232] Reductions in growth with higher sodium chloride concentrations have also been reported for fathead minnow (*Pimephales promelas*) larvae[233] and fry[234] and goldfish (*Carassius auratus*).[235]

Growth reductions related to chloride salts may be occurring in local streams. When fathead minnow (*Pimephales promelas*) larvae were grown in seven-day bioassays using water from 14 Milwaukee-area streams, the mean weight of the larvae decreased with increasing chloride concentration.[236] These effects began to be seen above chloride concentrations of 2,940 mg/l. A bioassay using water collected on different

---

[229] *Z. Hoover, M.C.O. Ferrari, and D.P. Chivers, "The Effects of Sub-Lethal Salinity Concentrations on the Anti-Predator Responses of Fathead Minnows,"* Chemosphere*, 90:1,047-1,052, 2013.*

[230] *Degree days are a measure of total temperature exposure over time. The cited study measured exposure using degree days to control for the effect of temperature on alevin growth rates.*

[231] *Mahrosh et al. 2018,* op. cit.

[232] *Elphick et al. 2011,* op. cit.

[233] Ibid.*; Baek International 1995,* op. cit.

[234] *W.J. Birge, J.A. Black, A.G. Westerman, T.M. Short, S.B. Taylor, D.M. Bruser, and E.D. Wallingford,* Recommendations on Numerical Values for Regulating Iron and Chloride Concentrations for the Purpose of Protecting Warmwater Species of Aquatic Life in the Commonwealth of Kentucky, *Memorandum of Agreement No. 5429, Kentucky Natural Resources and Environmental Protection Cabinet, 1985.*

[235] *Luz et al. 2008,* op. cit.

[236] *S.R. Corsi, D.J. Grazcyk, S.W. Geis, N.L. Booth, and K.D. Richards, "A Fresh Look at Road Salt: Aquatic Toxicity and Water Quality Impacts on Local, Regional, and National Scales,"* Environmental Science & Technology*, 44:7,376-7,382, 2010.*

dates from Wilson Park Creek in the City of Milwaukee showed similar results, with growth reductions appearing above a chloride concentration of 2,920 mg/l.[237]

The cation associated with chloride can influence whether an effect on fish growth occurs and the magnitude of any effect. A 25-day experiment examined growth of newly hatched rainbow trout (*Onchorhynchus mykiss*) exposed to three chloride salts with chloride concentrations ranging from 25 mg/l to 3,000 mg/l.[238] Magnesium chloride had no effect on growth at any concentration tested. Sodium chloride reduced growth at concentrations greater than 860 mg/l. A sodium chloride concentration of 3,000 mg/l resulted in a 9 percent reduction in length and a 27 percent reduction in mass of young rainbow trout. as compared to controls. Calcium chloride reduced growth at concentrations equal to or greater than 860 mg/l. A calcium chloride concentration of 3,000 mg/ resulted in an 11 percent reduction in length and a 31 percent reduction in mass of young rainbow trout as compared to controls.

It should be noted that the growth of some fish species may be stimulated by moderate concentrations of chloride salts. For example, the growth of bridle shiner minnows (*Notropis bifrenatus*) was enhanced by chloride concentrations of 500 mg/l to 1,000 mg/l.[239] Larvae of common carp (*Cyprinus carpio*) show increasing rates of growth with increasing salinity over the range 0 mg/l to 3,000 mg/l.[240] Similarly, the highest hatching rate of channel catfish (*Ictalurus punctatus*) eggs occurred at a sodium chloride concentration of 1,000 mg/l.[241]

There are potential ecological consequences that could result from reductions in growth rates in fish due to elevated chloride concentrations or salinity.[242] A result of reduced growth rates is that fish will spend more time in early life stages which could lead to increased risk of predation due to the fish being small for a longer period. Reduced growth rates could also affect the availability of food to young fish. Size is an

---

[237] Ibid.

[238] *Hintz and Relyea 2017* Oecologia, op. cit.

[239] *Hintz et al. 2017,* op. cit.

[240] *T.J. Lam and R. Sharma, "Effects of Salinity and Thyroxine on Larval Survival, Growth and Development in the Carp,* Cyprinus carpio*,"* Aquaculture*, 44::201-212, 1985.*

[241] *C.R. Weirich and T.R. Tierach, "Effects of Environmental Sodium Chloride on Percent Hatch, Yolk Utilization, and Survival of Channel Catfish Fry,"* Journal of the World Aquaculture Society*, 28:289-296, 1997.*

[242] *Hintz and Relyea* Environmental Pollution *2017,* op. cit.

important factor that determines what food is available to fish. Fry can only feed on prey that are within a narrow range of sizes. In some instances, slower growth could result in fry depleting available food in the proper size range as development proceeds. These two impacts could potentially reduce recruitment of fish into the adult population; however, fish have the capacity to accelerate growth at other life stages to compensate for poor growth at early stages.[243] As a result, growth could accelerate as fish age or water quality improves.

Impacts of Chloride Salts and Salinity on Fish Reproduction

Chloride and chloride salts can affect freshwater fish reproduction. Impacts include prevention of fertilization, low survival of eggs, delays in egg hatching, and interference with early development. Elevated salinity can prevent fertilization of freshwater fish eggs by reducing the motility of their sperm. Depending on species, the upper limit of salinity for successful fertilization in freshwater fish is between 9,000 mg/l and 15,000 mg/l.[244]

Exposure to chloride salts can also affect egg survival. For example, exposure of Atlantic salmon (*Salmo salar*) eggs to sodium chloride at concentrations ranging between 5,000 mg/l and 10,000 mg/l during fertilization resulted in low survival of the eggs.[245] Hatching was also delayed in those eggs that did survive. Higher egg survival was observed when the chloride exposure occurred after fertilization. These effects on eggs may be due, in part, to impacts of salinity on early development. For example, embryos in zebrafish (*Danio rerio*) eggs incubated at salinities greater than 4,000 mg/l were unable to undergo gastrulation.[246]

Elevated salinity can also induce production of deformed embryos in developing fish eggs. One way that this happens is through salinity's effects on early egg development. Once fish eggs are fertilized, they undergo a process of hardening of their outer membrane. This is accompanied by swelling of the egg. The swelling forms a perivitelline space within the egg that provides space for embryo formation and growth.

---

[243] *M. Ali, A. Nicienza, and R.J. Wootton, "Compensatory Growth in Fishes: A Response to Growth Depression,"* Fish and Fisheries*, 4:147-190, 2003.*

[244] *A.E. Hogan and J.C. Nicholson, "Sperm Motility of Sooty Grunter* Hephaestus fulginosus *(Macleay) and Jungle Perch* Kujlia rupestris *(Lacepede) in Different Salinities,"* Australian Journal of Marine and Freshwater Research*, 38:523-528, 1987.*

[245] *Mahrosh et al. 2014,* op. cit.

[246] *M.S. Sawant, S. Zhang, and L. Li, "Effect of Salinity on Development of Zebrafish* Brachydanio rerio*,"* Current Science*, 81:1,347-1,350, 2001.*

These processes can be disrupted by high salinity. For example, in salmonid species hardening of the outer membrane and formation of the perivitelline space is inhibited when salinity is higher than about 3,000 mg/l.[247] Similarly, Atlantic salmon (*Salmo salar*) eggs showed reduced swelling at sodium chloride concentrations above 5,000 mg/l.[248] Since the volume of a fish embryo increases during development, a small perivitelline space can restrict development and lead to more deformities in hatched fish.[249] In some instances, the percentage of fish hatching that showed deformities rose with increasing salt concentration. This was seen in Atlantic salmon (see Table 3.16).[250] Examples of deformities observed included scoliosis, conjoined twins, coiled tails, and deformed yolk sacs.

Impacts of chloride and chloride salts on reproduction can potentially affect the viability of fish populations. Reductions in the motility of sperm and fertilization, slower egg development, reduced hatching success, and inducement of deformities can all reduce the recruitment of adults into the fish population. Over the long term, this can reduce the species' abundance.

Amphibians

Amphibians are four-limbed cold-blooded animals with backbones that have life histories that require the use of both aquatic and terrestrial habitats. Typically, adult amphibians lay their eggs in water and have aquatic larvae. As these larvae grow, they undergo metamorphosis to assume their adult form. The adults are either terrestrial or semiaquatic. Adult amphibians continue to grow throughout their lives. Life stages of two amphibian species are shown in Figure 3.8.

Two groups of amphibians are found in Wisconsin, anurans and caudates. Anurans include frogs, tree frogs, and toads. Adult anurans lack tails; these structures disappear during metamorphosis. Caudates include salamanders, newts, and mudpuppies and adults have tails throughout their lives. Examples of Wisconsin amphibians are shown in Figure 3.9.

---

[247] *Hart et al. 1991,* op. cit.

[248] *Mahrosh et al. 2014,* op. cit.

[249] *X. Li, E. Jenssen, and H.J. Fyhn, "Effects of Salinity on Egg Swelling in Atlantic Salmon (*Salmo salar*),"* Aquaculture, *76:317-334, 1989; M. Keinanen, C. Tigerstedt, S. Peuranen, and P.J. Vuorinen, "The Susceptibility of Early Developmental Phases of an Acid-Tolerant and Acid-Sensitive Fish Species to Acidity and Aluminum,"* Ecotoxicology and Environmental Safety, *58:160-172, 2004.*

[250] *Mahrosh et al. 2018,* op. cit.

Amphibians generally prefer cool, damp habitats. Because they can easily dry out, amphibians are generally restricted to high moisture environments. Many are also nocturnal for this reason.

Amphibians perform several roles in aquatic and terrestrial communities. An important feature of their biology is that they change trophic position over their life cycle.[251] Anuran larvae are primarily herbivorous or omnivorous, feeding on plankton, periphyton, and detritus. Grazing by tadpoles is an important process regulating the growth of aquatic algae. Caudate larvae are carnivorous, feeding on small aquatic animals such as macroinvertebrates, tadpoles, and other salamander larvae. Salamander larvae serve as the top predator in many ephemeral ponds. Adult amphibians are carnivorous, feeding on insects and other invertebrates. The prey organisms of many adult amphibians are terrestrial.

Amphibians also serve as prey to other organisms. They are high quality prey for predators such as birds, mammals, fish, and reptiles. Because they move between aquatic and terrestrial habitats during their life cycle, amphibians serve to link aquatic food webs to terrestrial food webs.

Factors Affecting Amphibian Sensitivity to Chloride Salts and Salinity

Some general features of the biology of amphibians can make them sensitive to impacts from chloride salts and salinity in general. The skin of amphibians lacks scales or other coverings and is highly permeable. The exchange of gases, water, and ions occurs across the skin. Amphibians constantly transport ions across their skin to maintain ion and water balance.[252] Beginning at metamorphosis, the skin of adult amphibians is capable of actively transporting both sodium and chloride ions into the body.[253] While diffusion of some ions may occur through their skin, in larvae the gills are the site of active transport of sodium and chloride ions.[254] This active transport requires that the organism expend energy.

---

[251] *H.M. Wilbur, "Experimental Ecology of Food Webs: Complex Systems in Temporary Ponds,"* Ecology*, 78:2,279-2,302, 1997.*

[252] *G.R. Ultsch, D.F. Bradford, and J. Freda, "Physiology: Coping with the Environment," pages 189-214 in: R.W. McDiarmid and R. Altig (editors),* Tadpoles: The Biology of Anuran Larvae*, University of Chicago Press, Chicago, Illinois, 1999.*

[253] *V.H. Shoemaker and K.A. Nagy, "Osmoregulation in Amphibians and Reptiles,"* Annual Review of Physiology, *39:449-471, 1977.*

[254] Ibid.

To maintain internal ion concentrations, amphibian kidneys produce large amounts of dilute urine at high rates.[255] This involves considerable reabsorption of chloride and sodium ions. Anurans typically reabsorb about 99 percent of the sodium ions from fluids passing through their kidneys.[256] While the rates of reabsorption are not as high in caudates, they have been reported as being in the range of 90 to 95 percent.[257]

The osmoregulatory mechanisms of amphibians are adapted to dilute freshwater environments. These mechanisms work to move ions into the amphibian body, keep them there, and to move water out of the body. Amphibians counteract higher external salinity by increasing their internal salinity through water loss.[258] While this can compensate for small increases in salinity, larger increases in external salinity can lead to dehydration and organ failure.

Amphibian egg membranes are also highly permeable and sensitive to osmotic changes.[259] As will be described below, increased salinity can lead to changes in developing eggs that reduce hatching success and promote deformities.

The breeding locations used by amphibians also make them sensitive to chloride salts and salinity. Most species breed in waterbodies or wetlands. The timing of breeding is typically triggered by water temperature. Some species start breeding in the early spring when considerable salt is likely to be present

---

[255] *Ultsch et al. 1999,* op. cit.

[256] *H.O. Garland and I.W. Henderson, "Influence of Environmental Salinity on Renal and Adrenocortical Function in the Toad* Bufo marinus*,"* General and Comparative Endocrinology*, 27:136-143, 1975.*

[257] *L.B. Kirschner,T. Kerstetter, D. Porter, and R.H. Alvarado, "Adaptation of Larval* Ambystoma tigrinum *to Concentration Environments,"* American Journal of Physiology*, 220:1,814-1,819, 1971; D.F. Stiffler and R.H. Alvarado, "Renal Function in Response to Reduced Osmotic Load in Larval Salamander,"* American Journal of Physiology*, 266:1.243-1.249, 1974.*

[258] *J.B. Baliniski, "Adaptation of Nitrogen Metabolism to Hyperosmotic Environments in Amphibia,"* The Journal of Experimental Zoology*, 215:335-350, 1981.*

[259] *F.R. Hunter and O. De Luque, "Osmotic Studies of Amphibian Eggs. II. Ovarian Eggs,"* Biological Bulletin*, 117:468-481, 1959.*

in the environment.[260] In Wisconsin for example, the breeding seasons of four species of frogs and five species of salamanders typically begin in late March.[261]

Amphibians potentially face high risk of exposure to contaminants like chloride due to their breeding and living part of their lives in waterbodies. Adults may potentially escape increases in salinity by dispersing to lower salinity environments or selecting more favorable oviposition sites,[262] but this might be limited by short dispersal ranges. For example, salamanders in the genus *Ambystoma* typically return to their natal pools to breed.[263] Similarly, most anurans have small migratory ranges compared to other vertebrates, often dispersing no more than 1,100 yards from their natal sites.[264] The limited dispersal ranges may not allow these organisms to move an adequate distance to escape impacts from chloride salts. Once eggs are laid, they are restricted to the aquatic environment in which they were laid and therefore cannot escape exposure to contaminants.[265] This restriction lasts until metamorphosis allows the young amphibians to enter the terrestrial environment.

The amount of time amphibians spend in water can affect the impacts they experience from chloride salts, with species that spend more time in water facing higher risk of exposure. This amount of time differs among species.[266] Larval wood frogs (*Lithobates sylvatica)* are typically in ponds for about five to nine weeks. Similarly, larval spotted salamanders (*Ambystoma maculatum*) spend 13 to 18 weeks in ponds. Green frog (*Rana clamitans*) larvae, on the other hand, spend over a year in ponds. Amphibian species also differ in the amount of time adults spend in water. Adult wood frogs and spotted salamanders live in terrestrial habitats,

---

[260] *Collins and Russell 2009,* op. cit*.*

[261] *R. Christoffel, R. Hay, and M. Wolfgram,* Amphibians of Wisconsin*, Wisconsin Department of Natural Resources PUB-ER-105-2001, 2001.*

[262] *B. Viertel, "Salt Tolerance of* Rana temporaria*: Spawning Site Selection and Survival During Embryonic Development (Amphibia, Anura),"* Amphibia-Reptilia*, 20:161-171, 1999.*

[263] *C.R. Shoop, "Orientation of* Ambystoma maculatum*: Movements Near Breeding Ponds and Displacements of Migrating Individuals,"* Biological Bulletin*, 135:230-238, 1968; S.L. Stenhouse, "Migratory Orientation and Homing in* Ambystoma maculatum *and* Ambystoma opacum*,"* Copeia*, 1985:631-637, 1985.*

[264] *U. Sinsch, "Migration and Orientation in Anuran Amphibians,"* Ethology, Ecology & Evolution*, 2:65-79, 1990.*

[265] *K. Chinathamby, R.D. Reina, P.C.E. Bailey, and B.K. Lees, "Effects of Salinity on the Survival, Growth and Development of Tadpoles of the Brown Tree Frog,* Litoria ewingii*,"* Australian Journal of Zoology*, 54:97-105, 2006.*

[266] *N.E. Karraker, "Are Embryonic and Larval Green Frogs (*Rana clamitans*) Insensitive to Road Deicing Salt?"* Herpetological Conservation and Biology*, 2:35-41, 2007.*

and only spend a few weeks during the breeding season in water. While adult green frogs may disperse over land, they spend most of their adult lives in water.

Utilization of certain habitats may pose additional risks of exposure to amphibians. For example, some amphibian species breed in ephemeral ponds that are created by snowmelt and spring rains. These pools lack inlets and outlets and may or may not be hydraulically connected to groundwater. They are desirable breeding sites for amphibians because they lack fish, which are major predators of amphibian eggs and larvae. Contaminants can enter these temporary waterbodies through overland flow. The concentrations of these substances may increase as water evaporates. Constructed stormwater retention ponds also serve as habitats for many amphibian species.[267] These ponds may have high concentrations of chloride due to runoff from roads and other impervious surfaces.[268] Chemical stratification can occur in retention ponds, with salt concentrations near the bottom being two to five times higher than that at the surface.[269] This can pose special risks to amphibian species such as spring peepers (*Pseudacris crucifer*) that deposit their eggs at the bottom.[270]

<u>Types of Effects of Chloride Salts and Salinity on Amphibians</u>

Exposure to chloride, chloride salts, or salinity has been found to cause a variety of sublethal effects in amphibians. While different effects have been observed in different species, these effects fall into four broad categories: changes in behavior, changes in growth and development, impacts on reproduction, and increases in levels of stress hormones. It is also important to recognize that relatively few species have been examined and most of those are species that are easily reared or kept in a laboratory or mesocosm setting.

---

[267] *J.W. Snodgrass, J. Simon, and R. Casey, "Ecotoxicology of Amphibians and Reptiles in Urban Environments," Chapter 12, pages 177-196, In: J.C. Mitchell and R.E. Jung Brown (editors),* Urban Herpetology*, Society for the Study of Amphibians and Reptiles, Salt Lake City, Utah, 2008; E.C. Ostergaard, K.O. Richter, and S.D. West, "Amphibian Use of Stormwater Ponds in the Puget Sound Lowlands of Washington, Chapter 17, pages 259-270, In: J.C. Mitchell and R.E. Jung Brown (editors),* Urban Herpetology*, Society for the Study of Amphibians and Reptiles, Salt Lake City, Utah, 2008.*

[268] *N.E. Karraker, "Impacts of Road Deicing Salts on Amphibians and Their Habitats," Chapter 14, pages, 183-196, In: J.C. Mitchell and R.E. Jung Brown (editors),* Urban Herpetology*, Society for the Study of Amphibians and Reptiles, Salt Lake City, Utah, 2008.*

[269] *P.M. Marsalek, W.E. Watts, J. Marsalek, and B.C. Anderson, "Winter Flow Dynamics of an On-Stream Stormwater Management Pond,"* Water Quality Research Journal of Canada, *35:505-523, 2000.*

[270] *E.K. Dobbs, M.G. Brown, J.W. Snodgrass, and D.R. Ownby, " Salt Toxicity to Treefrogs (*Hyla chrysoscelis*) Depends on Depth,"* Herpetologica*, 68:22-30, 2012.*

In addition, most of the research has been conducted on amphibian eggs, embryos, and larvae. Much less work has examined impacts of chloride salts on adult amphibians.

Impacts of Chloride Salts and Salinity on Amphibian Behavior

Increases in the concentrations of chloride salts and salinity can lead to changes in amphibian behavior. Examples of this include salinity affecting amphibian activity levels, the amount of anti-parasite behavior amphibians display, and the choice of amphibian breeding locations.

Studies have reported reductions in general activity levels of larvae in several amphibian species with increasing chloride concentration or salinity.[271] Tadpoles of the frog *Rana temporaria* exposed to sodium chloride over periods of 42 and 56 days showed reductions in the amount of movement and the speed at which they moved with increasing chloride concentration.[272] At high sodium chloride concentrations, the tadpoles also moved shorter distances than at low concentrations. Less effect was seen over 14- and 28-day exposures. Similarly, the activity levels in larvae of American toad (*Anaxyrus americanus*) exposed to sodium chloride at a concentration 780 mg/l were reduced by 13 percent.[273] Wood frog (*Lithobates sylvatica*) larvae exposed to sodium chloride also showed lower activity levels. [274] At higher chloride concentrations the larvae also swam in tight circles. By inducing these sorts of behavioral changes, chloride salts and salinity may lower the foraging and predator avoidance abilities of amphibian larvae, leading to increased mortality.[275]

Exposure to chloride salts can also affect behaviors that reduce the likelihood that amphibian larvae will become infected with parasites. In one experiment, wood frog (*Lithobates sylvatica*) tadpoles were exposed

---

[271] *Collins and Russell 2009,* op. cit.

[272] *M. Denoël, M. Bichot, G.F. Ficetola, J. Delcourt, M. Ylieff, P. Kestermant, and P. Poncin, "Cumulative Effects of Road Deicign Salt on Amphibian Behavior,"* Aquatic Toxicology*, 99:275-280, 2010.*

[273] *D.K. Jones, B.M. Mattes, W.D. Hintz, M.S. Schuler, A.B. Stoler, L.A. Lind, R.O. Cooper, and R.A. Relyea, "Investigations of Road Salts and Biotic Stressors on Freshwater Wetland Communities,"* Environmental Pollution*, 221:159-167, 2017.*

[274] *D. Sanzo and S.J. Hecnar, "Effects of Road Deicing Salts on Larval Wood Frogs (*Rana sylvatica*),"* Environmental Pollution*, 140:247-256, 2006.*

[275] *Karraker 2008,* op. cit.; C. *Teplitsky, S., Plenet, J.-P., Léna, N., Mermet, E. Malet, and P. Joly, P., "Escape Behaviour and Ultimate Causes of Specific Induced Defences in an Anuran Tadpole,"* Journal of Evolutionary Biology, *18:180–190, 2005.*

to three concentrations of road salt consisting mostly of sodium chloride.[276] Higher intensities of infection with trematode flukes were seen at sodium chloride concentrations of 400 mg/l and 800 mg/l than at 160 mg/l. This correlated with the amount of anti-parasite behavior observed in the tadpoles. Less anti-parasite behavior occurred at the two higher concentrations than at the lowest one.

Concentrations of chloride salts and salinity can affect the choice of breeding locations by amphibians. This is important because the distribution of amphibians depends on their ability to locate suitable sites for egg laying and the ability of embryos and larvae to survive, develop, and undergo metamorphosis at these sites.[277] Some amphibian species choose breeding and egg laying sites based partly on salinity.[278] A field study in Nova Scotia, Canada found significant differences in the chloride concentrations of ponds occupied and not occupied by spotted salamanders (*Ambystoma maculatum*) and spring peepers (*Pseudacris crucifer*), with ponds that had higher chloride concentrations being unoccupied.[279] A second field study found wood frogs (*Lithobates sylvatica*) and spring peepers calling only in wetlands with chloride concentrations below 250 mg/l.[280] A third study found that wood frogs did not breed in stormwater ponds with chloride concentrations above 260 mg/l, whereas American toads (*Anaxyrus americanus*) were found breeding in these stormwater ponds. [281] Given the short dispersal ranges of many amphibian species, contamination of ponds with chloride could restrict breeding opportunities.

Impacts of Chloride Salts and Salinity on Amphibian Growth and Development

Exposure to chloride salts and salinity can affect the growth and development of amphibians. Three main effects have been reported: slower growth of larvae that leads to them requiring a longer period to reach

---

[276] *D. Milotic, M. Milotic, and J. Koprivnikar, "Effects of Road Salt on Larval Amphibian Susceptibility to Parasitism through Behavior and Immunocompetence,"* Aquatic Toxicology*, 189:42-49, 2017.*

[277] *L.G. Alexander, S.P. Lailvaux, J.H.K. Pechmann, and P.J. DeVries, "Effects of Salinity on Early Life Stages of the Gulf Coast Toad* Incilius nebulifer *(Anura: Bufonidae),"* Copeia*, 2012:106-114, 2012.*

[278] *T. Haramura, "Salinity Tolerance of Eggs of* Buergeria japonica *(Amphibia, Anura) Inhabiting Coastal Areas,"* Zoological Science, *24:820-823, 2007.*

[279] *Collins and Russell 2009,* op. cit.

[280] *E. Sadowski, "The Impact of Chloride Concentrations on Wetlands and Amphibian Distributions in the Toronto Region,"* Prairie Perspectives*, 5:144-162, 2002.*

[281] *M.T. Gallagher, J.W. Snodgrass, A.B. Brand, R.E. Casey, S.M. Lev, and R.J. Van Meter, "The Role of Pollutant Accumulation in Determining the Use of Stormwater Ponds by Amphibians,"* Wetlands Ecology and Management*, 22:551-564, 2014.*

metamorphosis, smaller size and lower weight at metamorphosis, and impacts on sex determination and alteration of sex ratios.

Exposure of amphibian eggs and larvae to chloride salts and salinity can lead to slower growth and extend the time needed for larvae to reach metamorphosis. For example, spotted salamander (*Ambystoma maculatum*) larvae in reciprocal transplants of egg masses between pools unimpacted and impacted with chloride grew more slowly in impacted pools than in unimpacted pools, regardless of the type of pool from which they originated.[282] In laboratory experiments, spotted salamander larvae grown at a chloride concentration of 8 mg/l required 34 days to reach metamorphosis, while those grown at a chloride concentration of 900 mg/l required 74 days.[283] Similar effects have been reported in gulf coast toad (*Incilius vallaiceps)*,[284] natterjack toad (*Bufo calamita*),[285] and brown tree frog (*Litoria ewingii*).[286] Interestingly, metamorphosis occurred earlier in wood frogs (*Lithobates sylvatica*) that were exposed to higher concentrations of sodium chloride, but the weight of the new adults was lower than those grown at lower concentrations.[287]

Slower growth of amphibian larvae and delays reaching metamorphosis can lead to fewer adults entering amphibian populations. First, a longer larval period exposes the organisms to higher risk of predation. Many predators of amphibian larvae are gape limited—the size of the prey that they can eat is limited by the size of their mouths. Because of this, smaller larvae are more likely to be eaten. In fact, at least one study has shown that some predators of anuran larvae prefer smaller tadpoles as prey.[288] As a result, being smaller for a longer time increases their risk of being predated.[289] Second, some amphibians lay their eggs in

---

[282] *S.P. Brady, "Road to Evolution? Local Adaptation to Road Adjacency in an Amphibian (*Ambystoma maculatum*),"* Scientific Reports*, 2:235, doi: 10.1038/srep00235, 2012.*

[283] *Collins 2010,* op. cit.

[284] *Alexander et al. 2012,* op. cit.

[285] *I. Gomez-Mestre, M. Tejedo, E. Demayo, and J. Estepa, "Developmental Alterations and Osmoregulatory Physiology of a Larval Anuran under Osmotic Stress,"* Physiological and Biochemical Zoology*, 77:267-274, 2004.*

[286] *Chinathamby et al. 2006,* op. cit.

[287] *Sanzo and Hecnar 2006,* op. cit.

[288] *L. Richards and C.M. Bulls, "Size Limited Predation on Tadpoles of Three Australian Frogs,"* Copeia*, 1990:1,041-1,046, 1990.*

[289] *Chinathamby et al. 2006,* op. cit.

ephemeral ponds. These species need to complete larval development and metamorphose before the ponds dry out. Longer development periods induced by chloride salts could result in the larval habitat disappearing before these amphibians have reached metamorphosis.[290]

Exposure to chloride salts and salinity can result in amphibian larvae being smaller and having lower weight at metamorphosis. Spotted salamander (*Ambystoma maculatum*) larvae in reciprocal transplants of egg masses between pools unimpacted and impacted with chloride were 11.3 percent smaller in impacted pools than in unimpacted pools, regardless of the type of pool from which they originated.[291] A second study found that spotted salamander larvae reared in media with higher concentrations of chloride had lower weight at metamorphosis.[292] Similarly, the weight of newly metamorphosized gulf coast toads (*Incilius vallaiceps)* decreased with the salinity that their eggs and tadpoles were reared in.[293] The reduction in size at metamorphosis can have impacts on both individual amphibians and amphibian populations. Size at metamorphosis is a major factor influencing survival and fitness of juvenile and adult amphibians.[294] Smaller amphibian individuals have less resistance to dehydration than larger ones because they have greater surface area to volume ratios. Small size increases the relative amount of surface area over which evaporation of water from their bodies can occur, potentially increasing evaporation. In addition, smaller size at metamorphosis can lead to less egg production, both through smaller individuals beginning to reproduce at an older age and smaller individuals producing fewer eggs.

Exposure to chloride salts can also affect sex determination in some amphibians. A mesocosm study found that exposure of wood frog (*Lithobates sylvatica*) larvae to chloride concentrations of 867 mg/l reduced the female to male sex ratio in the frogs by 10 percent in comparison to controls.[295] The fact that sex ratios in

---

[290] *Collins 2010,* op. cit.

[291] *S.P. Brady, 2012,* op. cit.

[292] *Collins 2010,* op. cit.

[293] *Alexander et al. 2012*, op. cit.

[294] *D.C. Smith, "Adult Recruitment in Chorus Frogs: Effects of Size and Date at Metamorphosis,"* Ecology, *68:344-350, 1987; R.D. Semlitsch, D.E. Scott, and J.H.K. Pechmann, "Time and Size at Metamorphosis Related to Adult Fitness in* Ambystoma talpoideum*,"* Ecology, *69:184-192, 1988.*

[295] *M.R. Lambert, A.B. Stoler, M.S. Smylie, R.A. Relyea, and D.K. Skelly, "Interactive Effects of Road Salt and Leaf Litter on Wood Frog Sex Ratios and Sexual Size Dimorphism,"* Canadian Journal of Fisheries and Aquatic Life*, 74:141-146, 2016.*

mesocosms were not correlated with survivorship indicates that this effect is likely due to the masculinization of frogs and not due to sex-based mortality.

Impacts of Chloride Salts and Salinity on Amphibian Reproduction

Chloride salts and salinity can affect amphibian reproduction. Examples of impacts include reducing egg production, interfering with egg physiology and survival, reducing egg hatching success, and interfering with early development leading to deformities and abnormalities in larvae.

Exposure to chloride salts can lead to reduced egg production in amphibians. One study found that egg masses of spotted salamander (*Ambystoma maculatum*) in roadside pools with higher specific conductance and chloride concentrations contained about 30 percent fewer eggs than those in woodland pools with lower specific conductance and chloride concentrations.[296] Chloride may only produce this impact in some amphibian species. The authors noted that that no difference was seen between the number of eggs in wood frog (*Lithobates sylvatica*) egg masses in the two pool types.

Exposure to chloride salts and salinity can also affect the physiology and survival of amphibian eggs. After amphibian egg masses are laid, their size increases due to water uptake. The size increase can be quite dramatic. For example, the size of spotted salamander (*Ambystoma maculatum*) egg masses can increase by a factor of four due to water uptake.[297] This water uptake provides several benefits to the eggs. It improves respiration within the clutch;[298] decreases the risk of disease to individual eggs;[299] and protects eggs against ultraviolet light,[300] desiccation,[301] and predation.[302]

---

[296] *N.E. Karraker and J.P. Gibbs, "Contrasting Road Effect Signals in Reproduction of Long- Versus Short-Lived Amphibians,"* Hydrobiologia*, 664:213-218, 2011.*

[297] *N.E. Karraker and J.P. Gibbs, "Road Deicing Salt Irreversibly Disrupts Osmoregulation of Salamander Egg Clutches,"* Environmental Pollution*, 159:833-835, 2011.*

[298] *Karraker 2008,* op. cit.

[299] Ibid.

[300] *K.P. Grant and L.E. Licht, "Effects of Ultraviolet Radiation on Life-History Stages of Anurans from Ontario, Canada,"* Canadian Journal of Zoology*, 73:2,292-2,301, 1995.*

[301] *W.E. Duellman, and L. Trueb,* Biology of Amphibians*, John Hopkins University Press, Baltimore, Maryland, 1986.*

[302] *S.C. Richter, "Larval Caddisfly Predation on the Eggs and Embryos of* Rana capito *and* Rana sphenocephala*,"* Journal of Herpetology*, 34:590-593, 2000.*

In one study examining the impact of chloride and salinity on egg expansion, newly laid egg masses of spotted salamanders (*Ambystoma maculatum*) were incubated for nine days in solutions with three different chloride concentrations.[303] Following this incubation, all the egg masses were transferred to the solution with the lowest concentration of chloride for eight days. During the first phase of the experiment, the weight of egg masses incubated at a chloride concentration of 1 mg/l increased by 27 percent. Those eggs masses incubated in solutions with chloride concentrations of 145 mg/l and 945 mg/l lost 18 percent and 33 percent, respectively, of their original weight. After being transferred into a 1 mg/l chloride solution, the egg masses originally incubated at the lowest chloride concentration (1 mg/l) lost 2 percent of their original weight. Those that were originally incubated at the intermediate concentration gained 15 percent of their original weight, while those originally incubated at the highest chloride concentration lost another 10 percent of their original weight. The results of this experiment suggest that exposure to high enough concentrations of chloride salts and salinity can cause permanent damage to amphibian eggs through disruption of their osmoregulatory ability. When egg masses are exposed to relatively low concentrations of chloride, they remain able to take up water when the solution they are in becomes more dilute. In nature, it means that when spring rains lower chloride concentrations in ponds, water losses from egg masses caused by salinity may be reversed. But there is a limit to this ability. When the concentration of chloride becomes too high, the egg masses lose this capacity and spring rains may not be adequate to reverse the water loss caused by elevated chloride concentration.

Elevated salinity and concentrations of chloride salts can also induce production of deformed embryos in developing amphibian eggs. The mechanisms are similar to those that produce deformities in fish embryos—higher salinity causes shrinkage of the perivitelline space in the egg decreasing the volume in which the embryo has to develop leading to the induction of deformities[304] (see the section on effects on fish). Several types of deformities have been reported. Wood frog (*Lithobates sylvatica*) eggs and larvae exposed to sodium chloride concentrations between 0 mg/l and 750 mg/l until they underwent metamorphosis at 100 days showed deformities at higher salt concentrations.[305] These deformities included bent tails, scoliosis, missing forelimbs, elongated rear limbs, and missing portions of their lower jaws. Swelling from abdominal edema was common at higher concentrations. This edema indicates that the frogs

---

[303] *Karraker and Gibbs 2011* Environmental Pollution, op. cit.

[304] *A.D. Padhye and H.J. Ghate, "Sodium Chloride and Potassium Chloride Tolerance of Different Stages of the Frog,* Microhyla ornate,*'* Herpetological Journal*, 2:18-23, 1992.*

[305] *M.L. Harless,* Effects of Chemical Deicers on Amphibian Communities*, Ph.D. Dissertation, Michigan Technological University, Houghton, Michigan, 2012.*

were undergoing kidney failure. The percentage of rough-skinned newts (*Taricha granulosa*) embryos hatching with deformities increased with increasing concentration of chloride in the medium in which they were incubated.[306] In control solutions, about 6 percent of embryos showed deformities. This percentage rose to about 74 percent at a chloride concentration of 2,000 mg/l with sodium chloride and 61 percent at the same chloride concentration with magnesium chloride. In these experiments, higher salt concentration led to more severe deformities. Increases in deformities at relatively high values of specific conductance have also been reported in green frogs (*Rana clamitans*).[307]

Impacts of Chloride Salts and Salinity on Amphibian Stress Hormones

High levels of exposure to sodium chloride can increase levels of amphibian stress hormones such as corticosterone.[308] This can lower the ability of amphibians to produce a normal immune response and can reduce larval growth by increasing the development rate.[309] Hormone changes can also alter the behavior of larval amphibians.[310]

---

[306] *G.R. Hopkins, S.S. French, and E.M. Brodie, Jr., "Increased Frequency and Severity of Developmental Deformities in Rough-Skinned Newt (*Taricha granulosa*) Embryos Exposed to Road Deicing Salts (NaCl & $MgCl_2$),"* Environmental Pollution*, 173:264-269, 2013.*

[307] *N.E. Karraker, "Are Embryonic and Larval Green Frogs (*Rana clamitans*) Insensitive to Road Deicing Salts?"* Herpetological Conservation and Biology*, 2:35-41, 2007.*

[308] *M.F. Bennett and A.O. Johnson, "Osmotic Stress ACTH and the White Blood Cell Picture in Newts,* Notophthalamus viridescens*,"* Journal of Comparative Physiology*, 82:333-338, 1973.*

[309] *A. Tournefier, "Corticosteroid Action of Lymphocyte Subpopulations and Humoral Immune Responses of Axolotl (Urodele Amphibian),"* Immunology*, 45:155-162,1982; B.E. LaFonte and P.T.J. Johnson, "Experimental Infection Dynamics: Using Immunosuppression and In Vivo Parasite Tracking to Understand Host Resistance in an Amphibian-Trematode System,"* Journal of Experimental Biology*, 216: 3,700-3,708, 2013.*

[310] *M.E. Fraker, F. Hu, V. Chuddapah, S.A. McCollum, R.A. Relyea, J. Hempel, and R.J. Denver, "Characterization of an Alarm Pheromone Secreted by Amphibian Tadpoles that Induces Behavioral Inhibition and Suppression of the Neuroendocrine Stress Axis,"* Hormones and Behavior*, 55:520-529, 2009.*

Amphibian Ability to Adapt to Higher Salinity

It should be noted that some amphibians have demonstrated adaptation to increased salinity.[311] Examples of this come mostly from coastal amphibian populations and probably developed over many generations.[312]

Reptiles

Turtles are the only group of reptiles in Wisconsin that live in aquatic habitats. Unlike other reptiles, turtles have shells that are fused to their backbones and ribs. Turtles are long-lived and slow to mature. Blanding's turtle (*Emydoidea blandingii*), for example, may live for over 80 years and females can take 17 to 20 years to reach sexual maturity.[313] Turtles also have long generation times, low survival of eggs and hatchlings, high adult survival, and low mobility.[314]

Eleven species of turtles live in Wisconsin. Four of these species are shown in Figure 3.10. The habitats they use vary among species, although most species are aquatic. Two species, Blanding's turtle and wood turtle (*Clemmys insculpta*) are semi-terrestrial, meaning they move between land and water. One species, the ornate box turtle (*Terrapene ornata*) is fully terrestrial. Regardless of the habitat used by adults, all turtles lay their eggs on land. Most turtle species do this in self-excavated nests, though common musk turtles (*Sternotherus odoratus*) will sometimes lay their eggs on bare ground or in shallow depressions under logs or rocks.

Turtles perform several roles in aquatic and terrestrial communities. Many species are omnivorous, eating plants as well as living and freshly dead animals. The diets of some turtle species change as they age. For example, juvenile painted turtles (*Chrysemys picta*)[315] feed heavily on insects. As painted turtles mature,

---

[311] *T.J.C. Beebee, "Salt Tolerance of Natterjack Toad (*Bufo calamita*) Eggs and Larvae from Coastal and Inland Populations in Britain,"* Herpetological Journal*, 1:14-16, 1985; I. Gomez-Mestre, and M. Tejedo, "Contrasting Patterns of Quantitative and Neutral Variation in Locally Adapted Populations of the Natterjack Toad,* Bufo calamita*,"* Evolution*, 58:2,343-2,352, 2004.*

[312] *Karraker 2008,* op. cit.

[313] *R. Christoffel, R. Hays, and M. Monroe,* Turtles & Lizards of Wisconsin, *Wisconsin Department of Natural Resources PUB-ER-104 2002, 2002.*

[314] *J.P. Vanek and G.A. Glowacki, "Assessing the Impacts of Urbanization on Sex Ratios of Painted Turtles (*Chrysemys picta*),"* Diversity, *11:72, doi: 10.3390/d11050072, 2019.*

[315] *Wisconsin is home to two subspecies of painted turtles: the western painted turtle (*Chrysemys picta belli*) and the midland painted turtle (*Chrysemys picta marginata*). Both subspecies are found throughout the State.*

their diet shifts to herbivory.[316] Where turtles feed is based on the habitats that they use. Aquatic species feed only in water, though common snapping turtles (*Chelydra serpentina*) may occasionally pull some prey into water edge. Terrestrial turtle species feed only on land while semi-terrestrial species feed in both water and on land.

Turtle eggs, hatchlings, and juveniles serve as prey for other animals. These include birds such as crows, ravens, and herons; fish such as bass and pike; and mammals such as mink, raccoons, skunks, and coyotes. Turtles are also preyed upon by free-ranging house cats. Eggs and hatchlings are heavily preyed upon. Only about five to 10 percent of turtle eggs survive to hatch and only one to three percent of hatchlings survive to adulthood.[317]

Factors Affecting Turtle Sensitivity to Chloride Salts and Salinity

Some general features of the biology of turtles can make them sensitive to impacts from chloride salts and salinity in general. Some species dwell primarily at the bottom of waterbodies. In addition, all Wisconsin turtles except the ornate box turtle spend winter under water. In some species, individuals bury themselves in the substrate while individuals in other species lie on top of the substrate during winter. Because of the effects of salt on water's density (see Chapter 2 of this report), these behaviors may expose turtles to levels of chloride and salinity higher than the average in the water column. Turtles that excavate nests in soils near roads may also expose their eggs to higher concentrations of salinity and chloride.

Impacts of Chloride Salts and Salinity on Freshwater Turtles

The impacts of chloride salts and salinity on freshwater turtles have been poorly studied. The available studies examine only a few species. In addition, the emphasis in most research has been on turtle species living in marine coastal areas that use estuaries as habitats or that may be impacted by sea level rise due to climate change. These studies have often examined the impacts of chloride concentrations and salinities typical of brackish water systems and seawater, which can be even higher than those found in contaminated inland waters. Less research has examined impacts on inland species or salinity and chloride concentrations more typical of contaminated inland waters.

[316] *Christoffel et al. 2002,* op. cit.

[317] Ibid.

High salinity has been found to produce physiological effects in turtles. Many species lose body mass when exposed to saline conditions, presumably from water loss.[318] At salinities over 17,500 mg/l, this may be accompanied by rapid water loss, decreased muscle moisture, and increased levels of plasma electrolytes.[319] Extended exposure to high salinity can lead to dehydration.[320] Net water loss in turtles due to salinity is inversely proportional to body size.[321] This provides larger turtles greater tolerance to salinity than smaller turtles.

Salinity can also affect reproduction in turtles. One experiment incubated eggs of broad-shelled snake-necked turtles (*Chelodina expansa*) in media consisting of one-to-one mixtures of vermiculite and water.[322] Treatments consisted of differences in the salinity of the water. Eggs incubated in mixtures with higher salinities had 39 percent less survival than those incubated in freshwater. In addition, hatchlings emerging from eggs in saline treatments were smaller and had higher plasma concentrations of sodium, chloride, and urea than those incubated in freshwater. The size difference is important because larger hatchlings survive longer, grow faster, and move more quickly than smaller ones. At hatching the residual yolk sac was larger in hatchlings incubated at higher salinities. This indicated that less yolk was used during growth and may reflect lower absorption of water by the eggs during incubation.[323]

Water exchange between turtle eggs and the environment during incubation is a major factor determining hatchling characteristics. Water exchange is influenced by the salinity of the soil surrounding the nest, with

---

[318] *P.J. Bentley, W.L. Bretz, and K. Schmidt-Nielsen, "Osmoregulation in the Diamondback Terrapin* Malaclemys terrapin centrata*,"* Journal of Experimental Biology*, 46:161-1678, 1967; W.A. Dunson and M.E. Seidel, "Salinity Tolerance of Estuarine and Insular Emydid Turtles (*Pseudemys nelson *and* Trachemys decussata*),"* Journal of Herpetology*, 20:237-245, 1986.*

[319] *M. Agha, J.R. Ennen, D.S. Bower, A.J. Nowakowski, S.C. Sweat, and B.D. Todd, "Salinity Tolerances and Use of Saline Environments by Freshwater Turtles: Implications of Sea Level Rise,"* Biological Reviews*, 93:1,634-1,648, 2018.*

[320] *D.S. Bower, C.E. Death, and A. Georges, "Ecological and Physiological Impacts of Salinization on Freshwater Turtles of the Lower Murray River,"* Wildlife Research*, 39:705-710, 2012.*

[321] *W.A. Dunson, "Estuarine Populations of the Snapping Turtle* (Chelydra*) as a Model for the Evolution of Marine Adaptations in Reptiles,"* Copeia*, 1986:741-756, 1986.*

[322] *D.S. Bower, K.M. Hodges, and A. Georges, "Salinity of Incubation Media Influence Embryonic Development of a Freshwater Turtle,"* Journal of Comparative Physiology B*, 183:235-241, 2013.*

[323] *D. Booth, "Incubation of Rigid-Shelled Turtle Eggs: Do Hydric Conditions Matter?"* Journal of Comparative Physiology B*, 172:627-633, 2002.*

more saline soil leading to less exchange. The amount of exchange can affect several turtle egg characteristics including the amount of time the eggs take to hatch,[324] the sex of the hatchlings,[325] and the size of the hatchlings.[326]

Mechanisms That Allow Freshwater Turtles to Cope with Chloride Salts and Salinity

Some freshwater turtle species have means for coping with increased concentrations of chloride salts and salinity. These include both physiological and behavioral mechanisms. Physiologically, many freshwater turtles regulate osmotic pressure from salinity by increasing the concentration of urea in their plasma.[327] This decreases the rate at which these turtles lose water to the environment.[328] In addition, some turtles can accumulate high concentrations of salts in their bladder fluids and excrete these salts with urea.[329]

Flexible behavior also allows some turtle species to temporarily occupy environments with high salinity, including brackish water environments.[330] At least three types of behaviors are seen that confer some tolerance to salinity. First, some turtle species may move frequently between saline and freshwater areas allowing them to use the saline environments. For example, common snapping turtles (*Chelydra serpentina*) cannot survive prolonged exposure to salinities of about 14,000 mg/l but are able to persist in estuarine

---

[324] *M. Charnier, "Action de la Temperature sur la Sex-Ration Chez L'embryon d'*Agama agama *(Agamidae, Lacertilien),"* Societe de Biologie de l'Ouest Africain, *160:620-622, 1966 cited in* Bower *et al. 2013*, op. cit.

[325] *C.L. Yntema, "Times for Eggs of the Turtle* Cheldra Serpentina *(Testundines: Chelydridae) at Various Temperatures,"* Herpetologica*, 34:227-274, 1978.*

[326] *M.S. Finkler, "Influence of Water Availability During Incubation on Hatchling Size, Body Composition, Desiccation Tolerance, and Terrestrial Locomotor Performance in the Snapping Turtle* Chelydra serpentina*,"* Physiological and Biochemical Zoology*, 72714-722, 1999.*

[327] *M. Gilles-Baillien, "Urea and Osmoregulation in the Diamondback Terrapin* Malaclemys centrata centrata*, (Latreille),"* Journal of Experimental Biology*, 52:691-697, 1970.*

[328] *S.M.L. Lee, W.P. Wong, A.M. Loong, KC. Hiong, F.C. Shit, and K.I Yuen, "Postprandial Increases in Nitrogen Excretion and Urea Synthesis in the Chinese Soft-Shelled Turtle,* Pelodiscus sinensis*,"* Journal of Comparative Physiology B, *177:19-29, 2006.*

[329] *M. Gilles-Baillien, "Hibernation and Osmoregulation in the Diamondback Terrapin* Malaclemys centrata centrata*, (Latreille),"* Journal of Experimental Biology*, 59:45-51, 1973.*

[330] *R. Greenberg and J.E. Maldonado, "Diversity and Endemism in Tidal-Marsh Vertebrates,"* Studies in Avian Biology*, 32-53, 2006.*

habitats through periodic access to freshwater during low tide.[331] This behavior requires that there be freshwater refuges available for these turtles to use. Second, turtles can reduce feeding and drinking that result in the ingestion of high salinity water.[332] This reduction can cause large increases urea in internal fluids, which cannot be maintained indefinitely without access to fresh water.[333] Third, some turtles will drink fresh water that is floating on top of more saline water in the environment.[334]

Effects on Terrestrial Organisms

The presence of chloride salts in the environment can also impact terrestrial organisms. In general, this has not been as well-studied as the impacts on aquatic organisms. While considerable information is available on impacts of chloride salts on terrestrial plants, less information is available on other terrestrial organisms. This section reviews the impacts of chloride salts on terrestrial plants and vertebrates such as birds and mammals.

Plants

Terrestrial plants include both mosses and vascular plants that live on land. These form integral parts of terrestrial food webs, converting carbon dioxide and inorganic nutrients present in the air and soil into organic compounds that are directly available as food for other organisms. This process, known as photosynthesis, uses energy from sunlight to create carbohydrates and releases oxygen required by other organisms. Plants serve as the base of the food web, providing food for animals. They also provide habitat for other organisms. In addition, plants provide food, fibers, and building materials for humans.

<u>Roles of Chloride Salts in Plant Nutrition</u>

Chloride is a necessary micronutrient for plants. Adequate tissue concentrations are about 100 mg of chloride per kilogram of plant dry mass (mg/kg).[335] The amount of chloride needed for plant and crop

---

[331] *J.J. Kinneary, "Salinity Relations of* Chelydra serpentina *in a Long Island Estuary,"* Journal of Herpetology*, 27:441-446, 1993.*

[332] *Bower et al. 2012,* op. cit.

[333] *J. Davenport and J.F Ward, "The Effects of Salinity and Temperature on Appetite in the Diamondback Terrapin* Malaclemys terrapin *(Latreille),"* The Herpetological Journal*, 3:95-98, 1993.*

[334] *W.A. Dunson, "Effect of Water Salinity and Food Salt Content on Growth and Sodium Efflux of Hatchling Diamondback Terrapins (*Malaclemys*),"* Physiological Zoology*, 58:736-747, 1985.*

[335] *N.P. Cain, B. Hale, E. Berkalaar, and D. Morin,* Review of Effects of Road Salt on Terrestrial Vegetation in Canada, *Environment Canada, July 2000.*

growth can generally be supplied by rainfall.[336] Chloride deficient plants are rarely seen in nature or agriculture. Chloride is a major osmotically active solute in the vacuoles of plant cells.[337] It is involved in turgor regulation and osmoregulation. Chloride regulates the activities of some enzymes in the cytoplasm of plant cells. It is also involved in the expansion of leaves and elongation of leaf, shoot, and root cells, processes that are important in plant growth.[338] While plants require small amount of chloride, excessive levels can produce toxic effects and have other impacts.

Some of the cations that are associated with chloride in salts are also plant nutrients. Plants require calcium as a structural component of cell walls and cell membranes and as a messenger within cells. An adequate tissue concentration of calcium is about 5,000 mg/kg.[339] Magnesium is a component of chlorophyll and is involved in protein synthesis and the activation of some enzymes. Adequate tissue concentrations of magnesium range between about 1,500 mg/kg and 3,500 mg/kg.[340] Excesses of calcium and magnesium can result in plants experiencing deficiencies of other nutrients.[341] Potassium regulates the opening and closing of stomates, is important in activating some enzymes, including enzymes related to energy production, and facilitates the synthesis of proteins and starches. Adequate plant tissue concentrations of potassium range between about 3,100 mg/kg and 3,900 mg/kg.[342] Sodium is not required for plant growth, development, or reproduction but is commonly found in plant tissues.

<u>Features of Plant Biology that Can Affect Sensitivity to Chloride Salts</u>

Some features of the biology of plants can make them sensitive to impacts from chloride salts and salinity. Most terrestrial plants are anchored to the substrate by roots or rhizoids. They are unable to escape to

---

[336] *P.J. White and M.R. Broadley, "Chloride in Soils and Its Uptake and Movement Within the Plant,* Annals of Botany*, 88:967-988, 2001.*

[337] Ibid.

[338] *J. Colmenero-Flores, J.D. Franco-Navarro, P. Cubero-Font, P. Peinado-Torrubia, and M.A Rosales, "Chloride as a Beneficial Macronutrient in Higher Plants: New Roles and Regulation,"* International Journal of Molecular Sciences*, 20:4578, doi: 10.3390/ijms20194686, 2019.*

[339] *K. Thor," Calcium—Nutrient and Messenger,"* Frontiers in Plant Science, *10:440, doi: 10.3389/fpls.2019.00440, 2019.*

[340] *W. Guo, H. Nazim, Z. Liang, and D. Yang, "Magnesium Deficiency in Plants: An Urgent Problem,"* The Crop Journal*, 4:83-91, 2016.*

[341] *Evans and Frick 2001,* op. cit.

[342] *A. Rodriguez-Navarro, "Potassium Transport in Fungi and Plants,"* Biochimica et Biophysica Acta*, 1469:1-30, 2000.*

another location when conditions become unfavorable. Much of the physiology of terrestrial plants depends on the movement of water from the soil into roots and through shoots into leaves. This is driven by transpiration, which consists of water evaporating from leaves into the atmosphere. This water uptake is also important for the ability of plants to maintain turgor, the rigidity of plant cells and ultimately tissues. Changes in soil conditions, including salinity, can affect the efficiency of the transpiration process. This uptake of water also brings ions from the soil into the plant. While some plants have mechanisms for preventing some ions from entering roots, plants have no means of excreting ions. As a result, ions such as sodium and chloride can build up in plant tissues.

The growth stage a plant is in can affect its sensitivity to chloride salts. For example, seedlings of grasses and herbaceous plants tend to be more sensitive to impacts from road salt than adult plants.[343] Similarly, young trees may be more susceptible to damage from salts than older ones.[344]

Cell division and growth in plants occurs at specific locations called meristems. The meristems responsible for longitudinal growth are typically found at the tips of shoots and roots, although grasses also have them at the base of leaves. Meristems are also found within buds. Salt on foliage tends to be more rapidly transported into these young, rapidly growing tissues.[345] These meristem tissues can be highly sensitive to impacts from salts.

Trees growing in urban environments may be particularly vulnerable to impacts from salts because they are already under stress from other elements of the urban environment.[346] Urban trees often have a small volume of substrate in which to grow. The presence of building foundations, streets, sidewalks, and other underground infrastructure restricts the space available for root growth which reduces the amount of water and nutrients available to the tree. In addition, soil in urban areas is often compacted during development.

---

[343] *A.S.N. Liem, A. Hedricks, H. Kraal, and M. Loenen, "Effects of De-Icing Salts on Roadside Grasses and Herbs,"* Plant and Soil, *84:299-310, 1985.*

[344] *R.E. Hanes C.W. Zelazney, and R.E. Blaser,* Effects of Deicing Salts on Water Quality and Biota—Literature Review and Recommended Research, *Highway Research Board Report No. 91, 1970.*

[345] Ibid.

[346] *M. Sieghardt, E. Mursch-Radlgruber, E. Paoletti, E.A.M. Couenberg, A. Dimitrakopoulus, F. Rego, A. Hatzisthathis, and T.B. Randrup, "The Abiotic Urban Environment: Impact of Urban Growing Conditions on Urban Vegetation," Pages 281-23 in: C.C. Konijnendijk, K. Nilsson, T.B. Randrup, and J. Schipperijn (Editors),* Urban Forests and Trees: A Reference Book, *Springer Verlag, Berlin, 2005.*

Compacted soils are more difficult for roots to penetrate and hold less water and oxygen than uncompacted soils. Finally, urban areas often have dense layers of turf grass. This can result in competition between grasses and trees for water and nutrients, reducing the amount available to the trees.

Environmental Factors that Affect Impacts of Chloride Salts on Vegetation

Several environmental factors also affect the impacts that chloride salts and salinity have on vegetation.[347] Many of these factors work through their influence on water relations in plants. Higher temperatures can lead to greater transpiration which can result in greater uptake and absorption of ions, including chloride salts. Higher temperatures can also increase dehydration of plants. Greater exposure to direct sunlight can also lead to more transpiration and increase dehydration. Low humidity and higher wind speeds can also increase transpiration and lead to dehydration. The salinity of the soil serving as a plant's substrate also has an effect. As the salinity of soil water rises, its availability to the plant decreases. Finally, the amount and timing of precipitation and the texture and drainage of the soil affect the availability of water to the plant.

How Plants Are Exposed to Chloride Salts

Plants can be exposed to chloride salts in two different ways. Salts can be deposited on the above ground portions of the plant through physical mechanisms such as aerial deposition caused by splashing and spraying of salt from impervious surfaces, contact with runoff containing salt, or placement of snow containing salt upon the plants. These forms of deposition were discussed in Chapter 2 of this report. This type of direct contact can result in some types of plants experiencing different levels of exposure to chloride. Herbaceous plants, for example, are not usually exposed to salt spray, aerial deposition, or direct contact during the winter as their above ground parts are dead and may be absent during this season.[348] Plants can also be exposed to chloride salts through uptake of ions from the soil and soil water. Introduction of chloride salts to soil and movement of chloride salts through soils is also discussed in Chapter 2 of this Report.

Exposure to chloride salts has been reported to cause several types of injuries to terrestrial plants. Individual plant species can differ in the injuries that they experience depending on the route of exposure and details of plant biology. In particular, different types of injuries have been observed in herbaceous plants versus woody plants, and deciduous woody plants versus evergreen woody plants.

---

[347] *Levelton Consultants, Ltd.,* Guidelines for the Selection of Snow and Ice Control Materials to Mitigate Environmental Impacts, *National Cooperative Highway Research Program Report No. 577, Transportation Research Board, 2007.*

[348] *Cain et al. 2000,* op. cit.

Plant Injuries Caused by Elevated Sodium Chloride Concentration in Soil

Several general symptoms have been reported following exposure of plants to elevated concentrations of sodium chloride in soil.[349] These include reductions in plant growth, damages to leaves, wilting of plants in hot, dry weather, tissue death, reduced or delayed germination of seeds, and nutrient deficiencies. Examples of these impacts are discussed in the next three paragraphs.

Growth in soil with elevated concentrations of sodium chloride can lead to reduced growth and stunting of plants. For example, a greenhouse study found that the dry weight of shoots of two grasses, red fescue (*Fescuta rubra*) and perennial ryegrass (*Lolium perenne*) and two herbs, narrow-leaf plantain (*Plantago lanceolata*) and white clover (*Trifolium repens*), decreased with higher concentration of sodium chloride in soil.[350] Another study reported that the dry weight of leaves and stems and the water content of leaves in common reed (*Phragmites australis*) lessened with increasing soil sodium chloride concentration.[351] This study also found that more sodium accumulated in reed stem tissues at higher soil sodium chloride concentrations. Elevated levels of sodium chloride in soil also led to a decrease in root proliferation and length of shoots in turf grasses.[352] Calcium chloride was reported to cause similar effects on turf grasses.[353]

Plants grown in soil treated with sodium chloride showed damage to leaves.[354] Foliar symptoms included wilting leaves, chlorosis or yellowing, and necrosis or tissue death. Examples of these are shown in Figure 3.11. In deciduous trees and herbaceous plants, this typically began along the margins of the leaves, and progressed to the leaf interior. An example of this is shown in Figure 3.12. In evergreens, foliar symptoms began at the tip of the needle and progressed toward the needle base. In trees, these symptoms typically started near the base of the tree and progressed toward the top. Severely damaged leaves and

---

[349] *Environment Canada*, Priority Substances List Assessment Report: Road Salts*, 2001*.

[350] *K.F. Akbar, A.D. Headley, W.H.G. Hale, and M. Athar, "A Comparative Study of De-Icing Salts (Sodium Chloride and Calcium Magnesium Acetate) on the Growth of Some Roadside Plants of England,"* Journal of Applied Sciences and Environmental Management*, 10:67-81, 2006*.

[351] *M. Gorai, M. Ennejeh, H. Dhemira, and M. Neff, " Influence of NaCl-Salinity on Growth, Photosynthesis, Water Relations and Solute Accumulation in* Phragmites australis*,"* Acta Physiologiae Plantarum*, 33:963-971, 2011*.

[352] *J.L. Eggens, "De-icing Salt Injury to Turfgrasses,"* Landscape Ontario*, 8:17-19, 1980*.

[353] *W.E. Cordukes and A.J. MacLean, "Tolerance of Some Turfgrass Species to Different Concentrations of Salt in Soils,"* Canadian Journal of Plant Science*, 53:69-73, 1973*.

[354] *See, for example, M.A. Dirr, "Tolerance of Seven Woody Ornamentals to Soil-Applied Sodium Chloride,"* Journal of Arboriculture*, 4:162-165, 1978*.

needles may fall off the plants prematurely and the amount of leaf loss can be substantial. One study found that sumacs (*Rhus typhina*) exposed to sodium chloride in roadside soils were essentially defoliated, with most having lost about 90 percent of their leaves.[355] Leaf damage is often associated with the accumulation of sodium ions in leaf tissues. For example, a study that examined browning of needles in roadside pines (*Pinus* spp.) found that trees showing symptoms of foliar damage had tissue concentrations of sodium 75 times higher than those found in unimpacted trees.[356]

Finally, high concentrations of sodium in soils can displace other cations such as calcium, magnesium, and potassium and cause these cations to be lost from the soil. The mechanisms through which this displacement occurs were discussed in Chapter 2 of this report. This cation loss can lead to nutritional deficiencies in plants.[357]

<u>Plant Injuries Caused by Physical Deposition of Chloride Salts</u>

Physical deposition of salts on plants can also cause injury. This deposition may occur through four mechanisms. Chloride in dust or aerosols in the atmosphere may settle on plants. Chloride may also be deposited on plants in rainfall. Snow that contains chloride may be plowed or placed on plants as part of road, parking lot or driveway clearing. Finally, chloride may be deposited by chloride contaminated snow or water splashing onto plants.

Such transport typically occurs through young shoots, buds, needles, and leaves. These tissues may show evidence of injuries from the salt. By contrast, direct injuries to mature bark by chloride salts has not been reported.[358] Most of the literature examining physical deposition of salt addresses aerial deposition.

Several general symptoms have been reported following physical deposition of chloride salts on plants. These include diebacks of buds and twigs, delays in bud opening, failure to flower, reduced fruit yields, reduced stem and leaf growth, and damage to leaves. On large plants like trees, these impacts are often

---

[355] *G.E. Bryson and A.V. Barker, "Sodium Accumulation in Soils and Plants along Massachusetts Roadsides,"* Communications in Soil Science and Plant Analysis*, 33:67-78, 2002.*

[356] Ibid.

[357] *F.W. Holmes, "Salt Injuries to Trees,"* Phytopathology*, 51:712-718, 1961.*

[358] *Cain et al. 2000,* op. cit.

heavier on the sides of the plant facing the salt source and/or to the height reached by salt spray.[359] An example of this is shown in Figure 3.13. In this example snow plowed from a driveway was piled next to and upon the bottom branches of the trees. The impacts of physical deposition of chloride salts on plants are further discussed in the next seven paragraphs.

Physical deposition of salt on plants can lead to dieback of buds and twigs.[360] This can lead to mishappen plants. For example, death of stems and buds on first year shoots may result in multiple new shoots sprouting below the dead branch tip. This can result in the plant developing a brush-like morphology. In addition, bud and twig dieback can lead to thinning of tree crowns and death of large branches causing damage to tree crowns.[361] A study examining the impact of spraying roads with magnesium chloride for dust suppression found that about 60 percent of lodgepole pines (*Pinus contorta*) near the roads had damage to their crowns.[362] The amount of damage to individual pine trees correlated with the concentration of chloride in their needles. The amount of crown damage was not related to the concentrations of chloride or magnesium in the soil.

Flower buds are especially sensitive to the effects of salt spray.[363] Common observations supporting this is that the sides of trees exposed to road salt spray may have no flowers, and the shrubs adjacent to highways may have flowers only below the snowline. A progression of injuries related to flowering with exposure to increasing levels of deicing salt has been reported for apple trees near highways.[364] At lower levels of salt deposited on twigs, the apple trees showed a slight reduction in flowering. Greater exposure led to a marked reduction in flowering. At even higher levels of exposure to chloride salt spray, flowering shoots, that are

---

[359] *E-.L. Viskari and L. Kärenlampi, "Roadside Scots Pine as an Indicator of Deicing Salt Use—A Comparative Study from Two Consecutive Winters,"* Water, Air, and Soil Pollution*, 122:405-419, 2000.*

[360] Ibid.

[361] *L. Bäckman, and L. Folkerson,* The Influence of Deicing Salt on Vegetation, Groundwater, and Soil along Highways E20 and 48 in Skaraborg County During 1994*, Swedish National Road and Transport Research Institute Report No. 775A, 1995.*

[362] *B.A. Goodrich, R.D. Koski, and W.R. Jacobi, "Conditions of Soils and Vegetation along Roads Treated with Magnesium Chloride for Dust Suppression,"* Water, Air, and Soil Pollution*, 198:165-188, 2009.*

[363] *G. Hofstra, R. Hall, and G.P. Lumis, "Studies of Salt-Induced Damage to Roadside Plants in Ontario,"* Journal of Arboriculture*, 5:25-31, 1979.*

[364] *G. Hofstra and G.P. Lumis, "Levels of Deicing Salt Producing Injury on Apple Trees,"* Canadian Journal of Plant Science*, 55:113-115, 1975.*

spurs off vegetative shoots began to die. Still higher exposure led to a marked dieback of flowering shoots on the apple trees.

The impacts of physical deposition of chloride salts on flowering can lead to reduced fruit yields. One study examined the impacts of aerial deposition of road salt on lowbush blueberries (*Vaccinium angustifolium*) in two commercial fields adjacent to a major highway in Nova Scotia.[365] Examination of plants showed that the concentrations of road salt on exposed plant stems were highest on plants that were next to the highway and decreased with longer distances from the road. The number of blossoms on and fruit yields from the blueberry plants were lowest near the road and rose with increasing distance from the road. The number of live flower buds and blossoms per plant and the yield of fruit per plant was inversely proportional to the concentration of road salt found on the stems. Placing plastic shields over blueberry plants near the road protected them from salt deposition from spray. Plants protected in this manner had lower concentrations of salt on their stems than unprotected plants located the same distance from the road. The shields increased the number of live buds and blossoms on the plants and the yields of fruit. Aerial deposition of road salt has also been shown to lower fruit yields from peach trees.[366]

Physical deposition of chloride salts can lead to reduced growth in plants. New growth in plants impacted by salts is often less than that in unimpacted plants.[367] For example, spruces at locations impacted by road salt were shorter and had smaller diameters at breast height than those at unimpacted sites.[368] This may be related to the growth of impacted plants resuming later in the growing season than uninjured plants. One study found that salt deposition on deciduous trees can delay resumption of spring growth by as much as three weeks.[369]

---

[365] *L.J. Eaton, J. Hoyle, and A. King, "Effects of Deicing Salt on Lowbush Blueberry Flowering and Yield,"* Canadian Journal of Plant Science*, 79:125-128, 1999.*

[366] *J. Northover, "NaCl Injury to Dormant Roadside Peach Trees and its Effect on the Incidence of Infections by* Leucostoma *spp.,"* Phytopathology*, 77:835-840, 1987.*

[367] *Hofstra et al. 1979,* op. cit.

[368] *M. Kayama, A.M. Quoreshi, S. Kitaoka, Y.K. Tahashi, Y. Sakamoto, Y. Maruyama, M. Kitao, and T. Korke, "Effects of Deicing Salts on the Vitality and Health of Two Spruce Species,* Picea abies *Karst., and* Picea glehnii *Masters Planted Along Roadsides in Northern Japan,"* Environmental Pollution*, 124:127-137, 2003.*

[369] *E. Sucoff,* Effect of Deicing Salts on Woody Vegetation Along Minnesota Roads*, Minnesota Agricultural Experiment Station Technical Bulletin No. 303, 1975.*

Aerial deposition of chloride salts can also damage leaves. For example, a greenhouse study examined the effects of spraying water containing different concentrations of sodium chloride on two grasses, red fuscue (*Fescuta rubra*) and perennial ryegrass (*Lolium perenne*) and two herbs, narrow-leaf plantain (*Plantago lanceolata*) and white clover (*Trifolium repens*).[370] The study found that the amount of damage to leaves rose with increasing sodium chloride concentration in the spray. It also found that the grasses tested were more tolerant of sodium chloride in the spray than the herbs.

Aerial deposition of chloride salts can also damage needles on evergreen trees. This damage tends to have distinct symptoms. Initially, the tips of mature needles exposed to salt turn yellow or brown. This discoloration moves toward the base of the needle, ultimately resulting in complete yellowing. The damaged needles then fall off the tree. An example of salt damage to pine needles is shown in Figure 3.14.

Damage from salt shortens the lives of conifer needles. For example, one study in Japan found that Norway spruce (*Picea abies*) and Sakhalin spruce *(Picea glehnii*) impacted by road salt lost their needles more quickly than unimpacted spruces.[371] The amount of needle shedding increased as the concentrations of chloride and sodium in the needles increased. As a result, the crowns of trees examined at locations impacted by aerial deposition of road salt had lower densities of needles than those at unimpacted sites. Examination of needles using scanning electron microscopy showed that the stomates of needles from salt-impacted sites were covered with large amounts of dust, which likely interfered with gas exchange and transpiration. This dust was absent from stomates of needles from unimpacted sites. These injuries affected the photosynthetic capabilities of the needles. Two-year-old needles from spruces growing at locations impacted by aerial deposition of road salt showed light-saturated ranges of photosynthesis that were less than half of those observed in needles from unimpacted sites.[372]

<u>Effects of Injuries on Plant Vigor</u>

Repeated injury from chloride salts can reduce the vigor of perennial plants and make the plants more sensitive to other hazards such as disease, insects, drought, or winter injury.[373] An examination of Scots pine (*Pinus sylvestris*) found that needles on trees growing near a heavily salted highway were common infected

---

[370] *K.F. Akbar et al. 2006,* op. cit.

[371] *M. Kayama, et al. 2003,* op. cit.

[372] Ibid.

[373] *Sucoff 1975,* op. cit.

with pine needle cast fungus and aphid eggs. These plant pests were not observed on needle growth on trees at a lightly salted site.

Spatial and Temporal Patterns of Injury to Terrestrial Plants from Road Deicing

Certain patterns of plant injury have been associated with using chloride salts for road deicing.[374] Spatially, injury occurs in a linear pattern along roads or in areas where road runoff collects. Injury tends to be more severe on the side of the plant facing the road and injury decreases with distance from the road. For instance, one study examining damage from road salt to roadside trees and shrubs found that the levels of visible injury to woody plants fell to background levels between 130 and 330 feet from the edge of the pavement.[375] Injury is often worse downwind of the road. Parts of woody plants that are covered by snow or sheltered from spray lack injury symptoms. Portions of trees that extend above the salt spray zone are not injured or are less injured than those in the salt spray zone. Also, salt spray injury extends only a short distance into plants with dense foliage.

Temporal patterns of injury to plants have also been reported.[376] Injury to evergreen trees and shrubs becomes evident in late winter and extends into the growing season. The appearance of injury can be quite sudden, occurring with the onset of temperatures that are above freezing.[377] For evergreens and shrubs this is when transpiration begins and may be related to the obstruction of stomates which was discussed previously in the section on plant injuries due to aerial deposition of chloride salts. Injury to deciduous trees and shrubs become evident in spring when growth resumes and extends into the growing season.

Mechanisms Underlying Chloride Salt Impacts on Terrestrial Plants

Several mechanisms likely underlie the effects of chlorides and chloride salts on terrestrial plants. First, accumulation of sodium in soil can cause deterioration of soil structure which was discussed in Chapter 2 of this Report. Such deterioration can reduce soil permeability. This reduction makes soil less suitable as a substrate for plant growth by reducing the movement of air and water into and through soil. As also

---

[374] *G.P. Lumis, G. Hofstra, R. Hall, "Sensitivity of Roadside Trees and Shrubs to Aerial Drift of Deicing Salt,"* Canadian Journal of Plant Science*, 8:475-477, 1973; M.A. Dirr, "Selection of Trees for Tolerance to Salt Injury,"* Journal of Arboriculture*, 2:209-216, 1976.*

[375] *Bäckman, and Folkerson 1995,* op. cit.

[376] *Lumis et al. 1973,* op. cit.*; Dirr 1976,* op. cit.

[377] *Hofstra et al. 1979,* op. cit.

discussed in Chapter 2, accumulation of sodium affects cation exchange processes in soil, which can lead to nutrient imbalances in plants. These effects can negatively impact seedling emergence and root growth.

Chloride salts damage plants through osmotic effects. Salt deposited on the leaves causes water to move out of plant cells.[378] Enough water loss leads to the membrane in a plant cell being pulled away from its cell wall, which can cause collapse of plant tissue.[379] Osmotic effects from chloride salts in soil can reduce the ability of plants to take up water and nutrients. For example, one study found that chloride salts in the soil reduced the uptake of nitrate and its accumulation in crop plants.[380] Reductions in water and nutrient uptake from chloride salts can lead to reduced plant growth, nutrient deficiencies, and the appearance of drought-like symptoms. In essence, salt can act as a non-selective herbicide by creating osmotic stresses that lead to water loss from affected plant tissues.

Chloride salts in soils can also injure plants by disrupting the microscopic organisms in the rhizosphere. This is a narrow region of soil surrounding roots that is affected by secretions from the roots (see Figure 3.15). This zone contains bacteria, fungi, and small animals like nematode worms that are associated with the plant. Interactions between the plant and these organisms are important in maintaining plant health.[381] Maintenance of rhizosphere biota is important in maintaining the plant abilities to take up water and nutrients. Fungi and bacteria in the rhizosphere can protect plants against pathogens through ecological processes such as competition.

Accumulation of chloride salts in soil can reduce the diversity of organisms in the rhizosphere. In one study, a solution containing deicing salt was applied to soil surrounding the ornamental shrub Japanese spindle (*Euonymus japonica*) for 27 days.[382] As a result of salt application, the diversity of bacteria species in the top six inches of soil decreased. More importantly, the diversity and evenness of fungal species in the top

---

[378] *E.M. Smith, "Tree Stress from Salts and Herbicides,"* Journal of Arboriculture*, 1:201-205, 1975.*

[379] *W.E. Barrick and H. Davidson, "Deicing Salt Spray Injury in Norway Maple as Influenced by Temperature and Humidity Treatments,"* HortScience*, 15:203-205, 1980.*

[380] *R. Munns and A. Termaat, "Whole-Plant Responses to Salinity,"* Australian Journal of Plant Physiology*, 13:143-160, 1986.*

[381] *L. Moulin, A. Munive, B. Dreyfus, and C. Boivin Masson, "Nodulation of Legumes by Members of the ß-Subclass of Proteobacteria,"* Nature*, 411:948-950, 2001.*

[382] *C. Ke, Z. Li, Y. Liang, W. Tao, and M. Du, "Impacts of Chloride De-Icing Salt on Bulk Soils, Fungi, and Bacterial Populations Surrounding the Plant Rhizosphere,"* Applied Soil Ecology*, 72:69-78, 2013.*

18 inches of soil decreased. The decrease in evenness suggests that the fungal community was dominated by a few salt-tolerant species by the end of the experiment.

Chloride salts may cause some plant injuries through interference with assimilation and incorporation of inorganic nitrogen into plant tissue. In one experiment, young barley plants grown in pots were watered with a sodium chloride solution and radioactively labelled inorganic ammonium nitrate.[383] Less nitrogen was incorporated into amino acids and protein in treated plants than in controls. In addition, inorganic nitrogen compounds accumulated in the tissues of treated plants. These chloride salt effects were more pronounced in plant shoots than in roots.

Accumulation of sodium chloride in plant cells can interfere with cellular process. This accumulation can inhibit the activity of some enzymes.[384] In addition, accumulation of sodium chloride in plant cells can damage cell membranes. Because of this, plant cells will sometimes compartmentalize sodium chloride by directing it to the central cell vacuole; however, there are limits to the extent that plants are able to do this.

Photosynthesis is another cellular process that sodium chloride may impact. Exposure to sodium chloride can lead to a loss of plant photosynthetic capacity which leads to slower or reduced growth.[385] This happens through at least two different mechanisms. First, higher sodium chloride in soils or plant tissue can cause reductions in the concentration of chlorophyll in leaves.[386] This reduces the ability of the plant to capture light, leading to lower photosynthetic capacity. Second, higher soil salinity can lead to a decrease in carbon dioxide assimilation which was observed in the common reed *Phragmites australis*.[387] Lower carbon dioxide assimilation may be related to conductance of gases and water vapor through stomates on leaves. This

---

[383] *H.M. Helal and K. Mengel, "Nitrogen Metabolism of Young Barley Plants as Affected by NaCl—Salinity and Potassium,"* Plant and Soil*, 51:457-462, 1979.*

[384] *K.M. Volkmar, Y. Hu, and H. Steppuhn, "Physiological Responses of Plants to Salinity: A Review,"* Canadian Journal of Plant Science, *78:1-27, 1998; R. Munns, "Comparative Physiology of Salt and Water Stress,"* Plant, Cell & Environment*, 25:239-250, 2002.*

[385] *Cain et al. 2000,* op. cit.

[386] *J.P. Syversten, J. Lloyd, and P.E. Kriedmann, "Salinity and Drought Stress Effects on Foliar Ion Concentrations, Water Relations, and Photosynthetic Characteristics of Orchard Citrus,"* Australian Journal of Agricultural Research*, 39:619-627, 1988.*

[387] *M. Gorai, M. Ennajeh, H. Khemira, and M. Neffati, "Combined Effect of NaCl-Salinity and Hypoxia on Growth, Photosynthesis, Water Relations, and Solute Accumulation in* Phragmites australis *Plants,"* Flora*, 205:462-470, 2010.*

suggests that when confronted with higher concentrations of sodium chloride in soil or on leaves, plants may close their stomates to reduce water loss. The opening and closing of stomates is illustrated in Figure 3.16.

Three findings from additional research provide support for this stomate closure hypothesis.[388] First, the rate at which photosynthesis occurs in common reed is correlated with the rate of gas conductance through stomates. Second, the rate of transpiration decreases with increasing concentration of sodium chloride in soil. Third, the rate of photosynthesis decreases with increasing soil sodium chloride concentration.

Plant Ability to Compensate for or Tolerate Chloride Salts

Plant species differ in their sensitivity to injury from chloride salts. This is partially related to plant growth forms and life histories. Herbaceous and annually seeded plants may not be exposed to salt spray or dust during winter and spring, thus avoiding some injury to stems and leaves. Similarly, during winter, above ground growth from previous growing seasons for many perennial, biennial, and overwintering annual plants die back. The overwintering parts of these plants are underground and protected from exposure to salt from dust and spray.[389] By contrast, the above ground parts of woody trees, shrubs, and vines persist through the winter and are subject to direct contact from salts. Nevertheless, all these plants may be exposed to chloride salts in soils.

Some patterns have been observed in plant sensitivity to chloride salts. Shrubs and grasses are generally more tolerant to sodium chloride than trees.[390] Conifers are generally more sensitive to injury than deciduous trees.[391] Differences in sensitivity among conifer species are related to the amount of foliar uptake.[392] While injury to needles tends to occur at about the same internal chloride concentration, differences in uptake have been observed that are related to at least two structural differences among the needles of different species. First, needles that are flatter have a higher surface-to-volume ratio and take up salt more rapidly than rounder needles. Second, needles with a thicker cuticle take up salt less readily. Both these qualities vary among conifer species.

---

[388] *M. Gorai et al. 2011,* op. cit.

[389] *Cain et al. 2000,* op. cit.

[390] *Sucoff 1975.* op. cit.

[391] *Hanes et al. 1970,* op. cit.

[392] *Hofstra et al. 1979,* op. cit.

Botanists and horticulturalists have classified plants by relative tolerance to sodium chloride. Appendix C summarizes this classification.

Some plants have mechanisms that allow them to compensate for some impacts from chloride salts. Plants may partially compensate for osmotic stress by increasing the concentrations of solutes in their cytoplasm. For example, cellular concentrations of sugars and the amino acid proline in *Phragmites australis* cells increased with higher exposure to sodium chloride.[393] The presence of these solutes draw water into tissues[394] and this water movement balances osmotic potential and protects enzymes. There are limits, however, to the ability of a plant to do this.

Finally, some plants are able to exclude sodium and/or chloride ions from entering their roots or can immobilize these ions in root and stem tissue.[395] This ability is not perfect and with continued exposure, sodium and chloride ions will accumulate in leaves over time. In addition, woody plants appear to control entry of sodium ions more efficiently than they control entry of chloride ions.

Vertebrates

Terrestrial vertebrates are animals with backbones that live predominantly or entirely on land. They vary in size and weight. For example, the ruby-throated hummingbird (*Archilochus colubris*) is a small bird found in Wisconsin. An adult may be as small as 2.8 inches long and weigh as little as 0.12 ounce. By contrast, adult male black bears (*Ursus americanus*) may be four to six feet long and weigh over 300 pounds. There are three groups of terrestrial vertebrates: reptiles, birds, and mammals.

Terrestrial vertebrates perform several roles in biological communities. They occupy all consumer categories in food webs. This includes detritivores; herbivores that feed on plant material; and predators that feed on invertebrates or other vertebrates. Some are generalists or omnivores that feed on an array of food items. Many also serve as prey to other organisms.

Some terrestrial vertebrates also have an important role in outdoor recreation in Wisconsin. People in the State commonly hunt mammals such as deer, bear, and small mammals and birds such as ducks, geese, and

---

[393] *Gorai et al. 2010,* op. cit.

[394] *M. Ashaf and M.R Foolad, "Role of Glycine Betaine, and Proline in Improving Plant Abiotic Stress Resistance,"* Environmental and Experimental Botany, *59:206-216, 2007.*

[395] *Munns and Tester 2008,* op. cit.

turkey. In addition, people trap furbearers such as mink, fox, and muskrat. Hunting has a large economic impact on Wisconsin. A survey by the U.S. Census Bureau estimated that hunting generated about $2.5 billion in expenditures in Wisconsin in 2011, the most recent year for which data were available.[396] The same survey estimated that wildlife watching, including observing, photographing, and feeding wildlife, generated about $1.5 billion in expenditures.

Limited data are available on the effects of chloride salts on terrestrial vertebrates. With the exception of turtles, which were discussed in the section on the effects of chloride on aquatic organisms, a search of the literature found no studies examining impacts on reptiles. Most of the studies that examine effects on birds address passerine or perching birds, especially seed-eating finches. Many of the studies that examine the effects on mammals address large ungulates such as moose or deer.

Features of Vertebrate Biology that Can Affect Sensitivity to Chloride Salts

Some features of vertebrate biology may affect the sensitivity of these animals to impacts from chloride salts. All vertebrates require sodium as an essential nutrient, generally in amounts of milligrams per gram of food consumed. Depending on their diet, obtaining sufficient sodium to meet nutritional requirements may be difficult for some species. Plant material often contains insufficient sodium to meet the needs of herbivores, and animals that feed mostly on plant material may need to find supplemental sources. For example, herbivorous and granivorous birds will seek out salt due to the sodium-deficient nature of their diet.[397] Similarly, mammalian herbivores, including ungulates like deer, will seek out sources of salt such as mineral licks to obtain sodium.[398] The need for sodium in some species can be driven by seasonal processes. For example, seasonal loss of sodium from the body due to antler development and gestation may compel deer to seek out sodium.[399] Because of these needs, the presence of road salt may be one of the factors inducing white-tailed deer, for example, to forage along roads.[400]

---

[396] *U.S. Census Bureau,* 2011 National Survey of Fishing, Hunting, and Wildlife-Associated Recreation: Wisconsin*, FHW/11-WI (RV), Revised October 2018.*

[397] *J. Schulkin,* Sodium Hunger: The Search for a Salty Taste, *Cambridge University Press, Cambridge, Massachusetts, 1991.*

[398] *H.P. Weeks, Jr., and C.M. Kirkpatrick, "Adaptations of White-Tailed Deer to Naturally Occurring Sodium Deficiencies,"* Journal of Wildlife Management*, 40:610-625, 1976.*

[399] Ibid.

[400] *G. Fieldhammer, J. Gates, D. Harman, A. Loranger, and K. Dixon, "Effects of Interstate Highway Fencing on White-Tailed Deer Activity,"* Journal of Wildlife Management*, 50:497-503, 1986.*

Birds may also ingest salt for use as grit. Birds use grit to improve the mechanical grinding of food in their gizzards.[401] The amount of grit that they ingest depends on how long grit is retained in their gizzards. When birds have free access to grit, they may consume and excrete a considerable amount daily.[402] House sparrows (*Passer domesticus*), for example, tend to choose grit particles with characteristics similar to those of road salt.[403] They prefer angular and oblong particles over rounded and spherical ones,[404] yellow or white colored grit over black and blue grit,[405] and grit in the size range of 0.1 to 2.4 millimeters (mm).[406] It should be noted that the sizes of road salt particles often overlap the sparrow's preferred size range for grit. The size range of road salt particles coming from a mine in Ontario were reported as ranging between 0.6 and 9.5 mm. Once it is applied to a road, salt undergoes gradual size reduction as it enters solution. Thus, at some time after application, salt particles will be at the preferred grit sizes for many bird species.[407] This suggests that many bird species, in addition to house sparrows, may use road salt particles as grit. For some species, this could be a major route of exposure to chloride.

Toxicity Effects of Chloride Salts to Vertebrates

Consumption of chloride salts can produce toxic effects in birds and mammals. Birds and small mammals are generally more susceptible to toxic effects from sodium chloride.[408] By contrast, the chloride tolerance of large mammals is usually quite high, as long as adequate drinking water is available. Water availability is

---

[401] *J.P. Gionfriddo, and L.B. Best, "Grit Use by House Sparrows: Effects of Diet and Grit Size,"* The Condor, *97:57-67, 1995.*

[402] *J.C. Alonso, "Grit in the Gizzard of Spanish Sparrows (*Passer Hispaniolensis*),"* Vogelwarte*, 33:135-143, 1985.*

[403] *T.K. Bollinger, P. Mineau, and M.L. Wickstrom, "Toxicity of Sodium Chloride to House Sparrows (*Passer domesticus*),"* Journal of Wildlife Diseases*, 41:363-370, 2005.*

[404] *L.B. Best and J.P. Gionfriddo, "Effects of Surface Texture and Shape on Grit Selection by House Sparrows and Northern Bobwhite,"* Wilson Bulletin*, 106:589-695, 1994.*

[405] *J.P. Gionfriddo, and L.B. Best, "Grit Color Selection by House Sparrows and Northern Bobwhites,"* Journal of Wildlife Management*, 60:836-842, 1996.*

[406] *Gionfriddo and Best 1995,* op. cit.

[407] *Environment Canada 2001,* op. cit.

[408] *P.H. Jones, B.A. Jeffrey, P.K. Watler, and H. Hutchon, "Environmental Impact of Road Salting," Chapter 1 pages 1-116 in F.M. D'Itri (editor),* Chemical Deicers and the Environment, *Lewis Publishers, Boca Raton, Florida, 1992.*

an important factor in toxicity of sodium chloride to vertebrates. In general, the effects of salt consumption increase when water is in short supply, such as during winter.[409]

A literature review found 12 published reports of bird kills associated with road salts.[410] At least two of the reports involved over 1,000 birds. These bird kills involved several species such as red crossbill (*Loxia curvirostra*), white-winged crossbill (*Loxia leucoptera*), pine siskin (*Spinus pinus*), evening grosbeak (*Hesperiphona vespertina*), bobwhite quail (*Colinus virginianus*), ring-necked pheasant (*Phasianus colchicus*), and domestic pigeon (*Columba livia domsitica*). The authors of the review argue that the number of reports likely underestimate the occurrence of bird kills due to road salt because of difficulties in finding avian carcasses, scavenging by other animals at kill sites , and the generally low rates of reporting of wildlife mortality incidents. They also argue that the number of anecdotal reports of bird kills they were able to find implies that such events are more common than the literature would suggest.

Among songbirds, highway mortality events are most commonly reported for some groups of seed-eating finches.[411] The cause of this mortality is unclear. These birds may be attracted to salted roadways due to a need for dietary sodium.[412] Alternatively, they may be ingesting salt for use as grit.[413]

The effects of sodium chloride on the house sparrow (*Passer domesticus*) have been studied in detail.[414] Although they have considerable dietary flexibility, house sparrows typically feed on seeds of grains and weeds.[415] Seeds generally make up about 90 percent of their diet. The LD50 for house sparrows for sodium

---

409 *P. Mineau and L.J. Brownlee, "Road Salts and Birds: An Assessment of the Risk with Particular Emphasis on Winter Finch Mortality,"* Wildlife Society Bulletin*, 33:835-841, 2005.*

410 Ibid.

411 *R. Tozer, "Red Crossbills Feeding at Mineral Sources,"* Ontario Birds*, 12:102-108, 1994.*

412 *D. Frasier, "Mammals, Birds and Butterflies at Sodium Sources in Northern Ontario Forests,"* Canadian Field Naturalist*, 99:365-367, 1985.*

413 *T.K. Bollinger, P. Mineau, and M.L. Wickstrom, "Toxicity of Sodium Chloride to House Sparrows (*Passer domesticus*),"* Journal of Wildlife Diseases*, 41:363-370, 2005.*

414 Ibid.

415 *T.R. Anderson,* Biology of the Ubiquitous House Sparrow: From Genes to Populations*, Oxford University Press, Oxford, United Kingdom, 2006.*

chloride is 3,108 mg per kg body weight.[416,417] Given that the average weight of a house sparrow is slightly less than one ounce, this is equivalent to a dose of about 90 mg, or about five 2.4-millimeter-long salt granules. This represents a tiny fraction of the number of grit particles typically found in house sparrow gizzards[418].

Exposure to sodium chloride also causes sublethal effects in house sparrows.[419] Edema or swelling of the gizzard occurs about one hour after birds receive a dose of 500 mg per kg body weight or about 14 mg for the average size sparrow. Clinical signs of effects appeared at doses of 1,500 mg per kg body weight or about 42 mg for the average size sparrow. These included reduced activity, reduced response to visual and auditory stimuli, loss of coordination and balance, and inability to fly or perch. In instances where sodium chloride doses did not result in the birds dying, it took them five to eight hours to recover from the effects.

Aberrant behavior in birds exposed to road salt has been reported in several studies.[420] Reported behavior include the birds appearing fearless and being more easily approached. These reports also indicated that the birds appeared weak, and exhibited slow movement, tremors, and partial paralysis. These symptoms are similar to those seen in laboratory studies examining the toxicity of road salt to birds.

Toxicity of road salt has been less studied in small mammals than in birds. One report of a bird kill also reported a kill of cottontail rabbits (*Sylvilagus floridanus*) associated with toxicity from road salt.[421]

---

[416] *Bollinger et al. 2005,* op. cit.

[417] *For organisms ingesting a toxin, acute toxicity is often expressed as the LD50. This is the dose of the toxin at which 50 percent of the organisms die over the duration of the test. A higher LD50 indicates lower toxicity to the organism, while a lower LD 50 indicates greater sensitivity to the toxin.*

[418] *Gionfriddo and Best 1995,* op. cit.

[419] *Bollinger et al. 2005,* op. cit.

[420] *These are reviewed in: D.L. Kelting and C.L. Laxson,* Review of Effects and Costs of Road De-Icing with Recommendations for Winter Road Management in the Adirondack Park, *Adirondack Watershed Institute Report No. AWI 2010-01, 2010.*

[421] *D.O. Trainer and L. Karstad, "Salt Poisoning in Wisconsin Wildlife,"* Journal of the American Veterinary Medicine Association*, 13:614-617, 1960.*

Role of Road Salt in Wildlife-Vehicle Collisions

The presence of salt along roads and in ponds and pools near roads may contribute to wildlife-vehicle collisions. Birds, for example, are commonly struck by vehicles as they consume salt off of roadways.[422] These collisions are often attributed to the attraction of the salt, either as a dietary supplement or for grit, and the inability of birds to recognize the threat posed by an approaching motor vehicle.[423] It has also been suggested that the disorientation caused by salt consumption may lead birds to have increased susceptibility to collisions with vehicles.[424]

The presence of salt on and adjacent to roads may also be a factor in collisions involving mammals. While this appears to not have been formally studied, small mammals such as woodchucks (*Marmota monax*), porcupines (*Erethizon dorsatum*), and showshoe hares (*Lepus americanus*) have been reported as being frequently observed feeding on roadside salt.[425]

Much of the research on the effect of road salt on wildlife-vehicle collisions has focused on large ungulates, especially moose (*Alces alces*). This reflects the threat to public safety posed by collisions between vehicles and deer and moose. For example, an annual average of about 2,900 deer-vehicle collisions occurred in southeastern Wisconsin in the years 2017 through 2020.[426]

Several observations support the idea that road salt may contribute to attracting these large mammals to roads and increase the likelihood of collisions with vehicles. Radio-collared moose in New Hampshire were found to extend their ranges to include roadside pools contaminated with road salt.[427] A study in Ontario found that peak incidence of moose-vehicle collisions occurred during periods when moose had the highest

---

[422] *Mineau and Brownlee 2005,* op. cit.

[423] *K.D. Baker, "An Observation of Bird Mortality on Highways,"* The Blue Jay, *25:79-80, 1965; D. Leatherman, "Crossbill Attraction to Salt: A Colorado Episode,"* Colorado Field Ornithologist Journal*, 23:102, 1989.*

[424] *T. Topfer, "Suspected Road Salt Poisoning in Bohemian Waxwings* Bobycilla garrulous *(Aves: Passeriformes: Bombycillidae),"* Vertebrate Zoology, *60:174-174, 2010.*

[425] *A.H. Hubbs and R. Boostra,* Study Design to Assess the Effects of Highway Median Barriers on Wildlife*, Ontario Ministry of Transportation, MAT-94-03, 1995.*

[426] *Wisconsin Department of Natural Resources,* Deer Vehicle Collisions*, dnr.wi.gov/wideermetrics/DeerStts.aspx?R=collisions, accessed January 23, 2023.*

[427] *B.K. Miller and J.A. Litvaitis, "Use of Roadside Salt Licks by Moose,* Alces alces, *in Northern New Hampshire,"* Canadian Field Naturalist*, 106:112-117, 1992.*

sodium needs and not during periods of the highest vehicular traffic.[428] Sightings of moose were also highest and about half of all collisions occurred at or near roadside pools containing salt. The roadside pools used by moose tended to have high concentrations of sodium and chloride. This last observation was similar to results from a study in Quebec that found that moose visits to roadside pools were greatest at pools with high concentrations of sodium and chloride.[429] Removal of salt deposits and pools from roadsides at a site in Quebec reduced the number of moose-vehicle collisions and the number of moose road crossings during spring and summer.[430] Finally, it has been observed that deer and moose drinking salty water tend to lose their fear of humans and vehicles.[431] The makes them prone to bolt, sometimes into the path of a vehicle instead of moving away as they normally would.

Summary of Effects on Organisms

Increased levels of chloride salts in the environment can result in a variety of impacts on organisms. These effects can vary depending on the type of organism. Chloride impacts can also vary depending on how the organism is exposed.

Many of the impacts of chloride salts on organisms result from the toxicity of chloride or the cations associated with chloride to organisms. The threshold at which toxic effects occur varies among groups of organisms, as well as within groups. One way to compare the sensitivity of different organisms to the most severe effects from chloride is to determine their LC50s, the exposure concentration at which half the test organisms die over the course of the test. As measured by 96-hour LC50s, groups of organisms vary widely in their sensitivity to chloride salts. For instance, zooplankton are relatively sensitive to chloride toxicity with 96-hour LC50s ranging between about 1,000 mg/l and 1,600 mg/l. Freshwater fish are much less sensitive with 96-hour LC50s for adults ranging between 3,000 mg/l and 13,000 mg/l. Different taxa within larger groups also vary in their sensitivity to chloride. While 96-hour LC50s for aquatic insects range between about 400 mg/l and 8,100 mg/l, some insect groups show more sensitivity than others. Mayflies are quite

---

[428] *D. Frasier and E.R. Thomas, "Moose-Vehicle Accidents in Ontario: Relation to Highway Salt,"* Wildlife Society Bulletin*, 10:261-265, 1982.*

[429] *Kelting and Laxson 2010,* op. cit.

[430] *P.D. Grosman, J.A.G. Jaeger, P.M. Biron, C. Dussault, and J.-P. Ouellet, "Reducing Moose-Vehicle Collisions through Salt Pool Removal and Displacement: An Agent-Based Modeling Approach,"* Ecology and Society*, 14:17, www.ecologyandsociety.org/vol14/iss2/art17, 2009.*

[431] *P.H. Jones, B.A. Jeffrey, P.K. Watler, and H. Hutchon,* Environmental Impact of Road Salting—State of the Art*, Ontario Ministry of Transport and Communication, MTC No. RR237, 1986.*

sensitive to chloride with 96-hour LC50s ranging between 425 mg/l and 2,800 mg/l. Caddisflies are less sensitive with 96-hour LC50s ranging between 2,100 mg/l and 8,100 mg/l.

Many other factors can affect the toxicity of chloride salts to organisms. These include factors related to level of exposure the organism experiences such as the dose or concentration of chloride and length of time that the organism is exposed. Environmental factors such as temperature, water hardness, the presence of other chemicals, and the presence of food can affect the toxicity of chloride. Similarly, elements of the biology of a species, such as the developmental stage the organism is in, affect its sensitivity to chloride toxicity. Finally, chemicals associated with chloride such as the cation in a chloride salt or additives can also be toxic.

Organisms also suffer sublethal effects from chloride salts. Many of these impacts result from chronic exposure. These impacts can also be influenced by the same factors discussed above that affect lethal toxicity of chloride salts.

Exposure to chloride salts has been shown to cause several types of sublethal impacts to plants. The most visible impacts are injuries to plant tissues. Depending on the type of plant and route of exposure, these can include wilting or browning of leaves and shoots, killing of buds, loss of leaves and needles, and dieback of tissues. Exposure to chloride salts can lead to reductions in growth resulting in plants of smaller size or biomass. Exposure to chloride can also impact plant reproduction through reducing flower production and seed germination. Finally, exposure to chloride salts can have adverse effects on plant physiological processes, such as causing nutritional deficiencies or reductions in photosynthesis.

Chloride salts can also cause sublethal impacts to animals. Exposure to chloride salts can induce changes in the metabolism rates of some animals that can increase energy requirements or lead to weight loss. Chloride exposure can affect animal growth and development. Slower growth, reduced growth, lengthened development times, altered sex ratios, and more developmental deformities have all been linked to exposure to high concentrations of chloride salts. Exposure to chloride salts can also affect animal reproduction. Reported impacts include reduced fertilization of eggs, increased age at first reproduction, reduced numbers of offspring produced, and inhibition of hatching of eggs. Exposure to chloride salts can also lead to altered behavior in animals. These alterations include changes to mobility such as slower swimming speeds, changes in feeding, and changes in habitats use. Finally, exposure to chloride salts can increase the susceptibility of some animals to parasites.

3.3 IMPACTS OF CHLORIDE ON BIOLOGICAL COMMUNITIES

Background on Biological Communities

Biological communities consist of associations of species that occupy the same geographical area at the same time. For example, a pond community may consist of a variety of organisms including:

- Plants, bacteria, fungi, protists, and animals on the water surface
- Phytoplankton, zooplankton, bacteria, fungi, protists, invertebrates, and fish in the water column
- Plants, algae, bacteria, invertebrates, fungi, and protists on the sediment surface
- Bacteria, protists, fungi, and animals within the sediment

Any given community may be composed of a large number of species. This creates practical problems in studying communities. Because of this, many studies of communities examine portions of a community. These portions can be defined in different ways. Some consist of taxonomically related species, such as "the fish community." Others consist of species living in a single habitat type within a community, such as "the benthic community" or "the plankton community." Still other definitions consist of species that perform similar functions, such as "the herbivore community." Finally, another way to define a community is by simplified subsets of a community, such as interactions between a small number of species.

Community Measures

Several measures are used to describe and examine communities. Some of these measures describe the structure of the community. Species richness describes the number of species present in a community. In studies where organisms are not identified to the species level, this may also be presented as genus richness or taxon richness. Evenness describes how the relative numbers of individuals of each species compare to one another. High evenness is present when the relative abundances of all species are similar. Diversity measures the complexity of a community, and it takes both species richness and evenness into account. Communities with a larger number of species and more even abundances have higher diversity. Descriptions can also be made using biomass, which consists of the mass or weight of organisms in the community.

Communities can also be measured through community functions. Examples of this include primary production, secondary production, and processing of organic material. Primary production is defined as the formation of biomass resulting from photosynthesis. Secondary production consists of the formation of biomass by consumers. Processing of organic material refers to the breakdown of material produced by living organisms.

Community Structure

Community structure is a description of what species of organisms are present in a community, what their relative abundances are, and what relationships and interactions occur among these species. Several factors can influence community structure. Features of the abiotic environment such as climate, geography, and geology can exert strong effects on community structure. These environmental features partially determine which species can survive at a location. Their predictability may also influence the ability of a species to occupy an area. The heterogeneity of the environment determines the number of potential habitats that are present. More potential habitats create opportunities for species to be present within a community. Interactions among organisms also influence community structure. Adverse effects due to ecological processes such as interspecific competition, predation, and parasitism, as well as beneficial effects due to ecological processes such as mutualism, can influence the presence and abundance of species. Finally, random events, such as disturbances or colonization by other species, can affect community structure.

Factors that influence community structure may act by directly impacting some constituents of the community. For example, competition between zooplankton species for phytoplankton as a food resource may act to exclude some zooplankton species from the community. This may occur even though the excluded species could survive in the environment in the absence of its competitors. Other factors may influence community structure indirectly through mediation by other components of the community. For example, predation on large-bodied zooplankton by zooplanktivorous fish may allow phytoplankton populations to bloom, reducing the amount of light that reaches the lakebed, thereby reducing the abundance and biomass of macrophytes. In this example, the effect of the fish on macrophytes is indirect because it is mediated through the impacts of zooplankton on phytoplankton abundance and phytoplankton on light levels.

Some species may have stronger roles than others in creating or maintaining the structure of a community. Some species may act as habitat for other species. If they are not present, the species dependent on them will be unable to persist in the community. Aquatic macrophytes are an example of this. By creating physical structure in streams, ponds, and lakes macrophytes create habitat usable by some fish and macroinvertebrate species. Other species may maintain community structure through strong effects on other species. Piscivorous fish may act in this manner. By reducing the abundance of zooplanktivorous fish, they may allow the abundance of large zooplankton to increase, which acts to reduce the abundance of phytoplankton through grazing.

Food Webs

Food webs are one way to summarize and examine the structure of communities. They show links between different species of organisms. These linkages are based on trophic relationships—who eats whom and who eats what. Food webs may also incorporate some features of the abiotic environment such as nutrients or organic material that are required for some organisms to grow. Food webs present a conceptual approach to understanding species interactions and energy flow in ecological communities.

Presentations of food webs are often simplified relative to the number of species in a community. This simplification is partly a practical matter. It can be very difficult and require substantial effort to identify all the species present in a community and all the interactions among those species. At the same time, though, aggregating species into functional groups, such as species that use similar resources, can improve the ability to make generalizations regarding processes in a community.

Figure 3.17 shows an example of a food web in a pond. The figure shows individual food chains embedded within a network of feeding relationships. Producers such as algae and aquatic plants grow through photosynthesis based on the availability of light and nutrients. They are fed upon by a variety of consumers, ranging from zooplankton such as the water flea *Daphnia*, to snails, tadpoles, insects, and muskrat. Most of these are fed upon, in turn, by larger consumers such as fish. Large predators, such as herons, may feed on the fish. Excretions by these organisms and breakdown of dead organisms by scavengers such as crayfish and decomposers such as bacteria return nutrients to the system. While the figure is simplified, it presents a useful framework for examining this type of community.

Aquatic food webs are often connected to terrestrial food webs. For example, leaves shed by trees growing in riparian areas constitute a major source of energy and nutrients to food webs in small headwater streams. Bacteria and fungi grow on these leaves and serve as a source of food to many macroinvertebrates. This input of leaves ultimately supports higher trophic levels through food web interactions.

Effects of Chloride Tolerance Differences among Species in Communities

As discussed in the section on the effects of chloride salts on organisms in this Chapter, some species of organisms are more tolerant of chloride and chloride salts than other species. Among macroinvertebrates, for example, mayflies, stoneflies, and pulmonate snails are especially sensitive to salinity while beetles, dragonflies, damselflies, and some flies and crustaceans are more tolerant.[432] As the concentration of chloride salts and salinity increase, these differences in sensitivity to chloride can lead to changes in community composition and structure. At high enough chloride concentrations, sensitive species are likely to be lost from the community. While this could simply lead to increases in the abundance of tolerant species already present in the community, it could also lead to changes in the intensity of ecological processes such as competition or predation among the more tolerant species remaining in the community.[433] As a result, the abundance of members of some tolerant species could increase, while the abundance of others could decline.

Differences in tolerance and changes in the intensity of ecological processes could also allow other tolerant species to invade and establish in the community. In some communities this could enhance colonization by alien exotic species while preventing the establishment of more sensitive native species.[434] Three examples illustrate this outcome. In the first example field surveys showed that the presence of exotic macroinvertebrate species was significantly associated with the highest salinity reaches of the Meurthe River in France.[435] In the second example, salinization of the River Weser in Germany led to several exotic crustacean species colonizing the river.[436] As salinity in the river increased, colonization proceeded upstream from the estuary. In the third example, a salt storage pile was operated adjacent to a bog in Indiana for 10 years.[437] The mean concentrations of chloride in water in the affected portion of the bog pile was 1,215 mg/l. The plant community in this area of the bog came to be dominated by invasive narrow-leaf cattail (*Typha angustifolia*). This cattail species can tolerate brackish water conditions, which may have contributed

---

[432] *J.E. Dunlop, N. Horrigan, G. McGregor, B.J. Kefford, S. Choy, and R. Prasad, "Effect of Spatial Variation on Salinity Tolerance of Macroinvertebrates in Eastern Australia and Implications for Ecosystem Protection Trigger Values,"* Environmental Pollution*, 151:621-630, 2008.*

[433] *L. Blaustein and J.M. Chase, "Interactions Between Mosquito Larvae and Species that Share the Same Trophic Level,"* Annual Review of Entomology*, 52:489-507, 2007.*

[434] *U. Braukmann and D. Böhme, "Salt Pollution of the Middle and Lower Sections of the River Werra (Germany) and Its Impact on Benthic Macroinvertebrates,"* Limnologica*, 41:113-124, 2011.*

[435] *C. Piscart, J.-C. Moreteau, and J.-N. Beisel, "Biodiversity and Structure of Macroinvertebrate Communities Along a Small Permanent Salinity Gradient (Meurthe River, France),"* Hydrobiologia*, 551:227-236, 2005.*

[436] *J. Bäthe, "Decreasing Salinity in Werra and Weser (Germany): Reactions of the Phytoplankton and Macrozoobenthos,"* Limnologica*, 27:111-119, 1997.*

[437] *Wilcox 1986,* op. cit.

to its ability to invade this bog. In contrast, nearly all the endemic native plant species were absent from the affected portion of the bog.

Effects of Chloride Salts and Salinity on Community Composition

Several studies have examined the effect of chloride salts and salinity on the composition of communities. Most of these studies have examined impacts on aquatic communities. In addition, most have focused on only a portion of the community present at the study sites, such as plankton assemblages, periphyton assemblages, or macroinvertebrate assemblages. These studies also differ in the methodologies used. Some field studies have compared the composition of communities in waterbodies located in the same landscape that differed from one another in chloride concentration, salinity, or specific conductance. Other field studies have observed changes in community composition in a single waterbody as chloride concentrations increased. Experimental studies have taken a mesocosm approach and examined changes in community composition in response to manipulation of chloride concentration or salinity. Some of these experimental studies have reported thresholds of chloride concentration at which major changes in community composition were observed.

Effects of Chloride Salts on the Composition of Plankton Assemblages

Plankton communities consist of organisms that live in the water column that are unable to propel themselves against a current. Planktonic organisms are generally small. Many are microscopic and larger ones rarely exceed lengths of about 0.08 inch. Plankton communities are a resource base which supports higher trophic levels and provides ecosystem services such as primary production, decomposition, and nutrient cycling. Plankton in freshwater systems are composed of diverse assemblages of algal phytoplankton, zooplankton, bacteria, fungi, and protists such as ciliates and flagellates.

Several studies have found that increases in salinity can reduce the number of phytoplankton present.[438] For example, a field study compared the phytoplankton assemblages in the forebays and pond areas of two stormwater ponds in Ontario.[439] In Harding Pond, specific conductance in the forebay was 1,150 µS/cm,

---

[438] *See for example: Batterton and Baalen 1971,* op. cit.*; L.M. Kipriyanova, N.I. Yermolaeva, D.M. Bezmaternykh, S.Y. Dvurechenskaya, and E.Y. Mitrofanova, "Changes in the Biota of Chany Lake Along a Salinity Gradient,"* Hydrobiologia*, 576:83-93, 2007; and E. Coring and J. Bäthe, "Effects of Reduced Salt Concentrations on Plant Communities in the River Werra (Germany),"* Limnologica*, 41:134-142, 2011.*

[439] *D.D. Olding, "Algal Communities as a Biological Indicator of Stormwater Management Pond Performance and Function,"* Water Quality Research Journal of Canada*, 35:489-503, 2000.*

while specific conductance was only 520 µS/cm in the pond. The phytoplankton assemblage in the forebay consisted of 10 genera, but a species of *Euglena*, a euglenoid flagellate, accounted for about 89 percent of the phytoplankton biovolume, a measure of phytoplankton biomass. The phytoplankton assemblage in the pond consisted of 16 genera including green algae, cryptomonad flagellates, diatoms, and euglenoid flagellates. *Euglena* accounted for only 56 percent of the biovolume of phytoplankton. The concentration of phytoplankton biovolume in the pond was about 25 percent of that found in the forebay. This study also looked at a second stormwater pond. In Rouge Pond, specific conductance in the forebay was 2,950 µS/cm, while specific conductance was 1,900 µS/cm in the pond. While similar differences in specific conductance were observed in the forebay and the pond, no differences were observed between the forebay and pond phytoplankton assemblages. The different response observed in the phytoplankton assemblage in Rouge Pond may be the result of the much higher levels of specific conductance in this pond.

A study examining plankton assemblages in four urban lakes exposed to road salt in Quebec City, Canada showed that differences in the taxonomic composition of both bacterial and eukaryotic plankton was explained by differences in the concentrations of chloride and total nitrogen under both ice-covered and open water conditions.[440] The plankton assemblages in lakes with the lowest levels of salinization consisted mostly of chrysophyte flagellates and ciliates, while more salinized lakes had higher abundance of dinoflagellates, cryptomonad flagellates, and haptophyte flagellates. Differences were also seen among the bacterial assemblages that were present based on the degree of salinization.

A mesocosm experiment examined the effects of chloride concentrations on the plankton assemblage from a lake with low chloride concentrations.[441] Ambient chloride concentrations in Lake Croche in Quebec, Canada are normally about 0.27 mg/l. Plankton from the lake was placed into 21 mesocosms to which sodium chloride was added at chloride concentrations ranging between 0.27 mg/l and 1,400 mg/l. These mesocosms were incubated within the lake for six weeks. Several changes in community composition occurred over the chloride gradient. While total phytoplankton biomass did not change with chloride concentration, a major shift in phytoplankton composition was observed. At chloride concentrations below 185 mg/l, green algae and cryptomonad flagellates were the dominant groups of phytoplankton present. At chloride concentrations equal to and above 185 mg/l, the assemblage shifted to one dominated by

---

[440] *I.B. Fournier, C. Lovejoy, and W.F. Vincent, "Changes in Community Structure of Under-Ice and Open-Water Microbiomes in Urban Lakes Exposed to Road Salts,"* Frontiers in Microbiology, *12:660719, 2021.*

[441] *L. Astorg, J.-C. Gagnon, C.S. Lazar, and A.M. Derry, "Effects of Freshwater Salinization on a Salt-Naïve Planktonic Eukaryote Community,"* Limnology and Oceanography Letters*, 8:38-47, 2023.*

chrysophyte flagellates with some diatoms and xanthrophyte algae. This change reduced the value of the phytoplankton assemblage as a food resource for zooplankton.[442] At chloride concentrations above 640 mg/l, only phytoplankton were present in the mesocosms.

Changes in other components of the plankton also occurred in the Lake Croche mesocosms with increasing chloride concentration.[443] Zooplankton, including cladocerans, copepod, and rotifers disappeared at chloride concentrations above about 40 mg/l. Ciliates were common at chloride concentrations below 185 mg/l but decreased in abundance above this chloride level. At chloride concentrations between 185 mg/l and 640 mg/l fungi dominated the plankton but they were not present at concentrations above 640 mg/l.

Effects of Chloride Salts on the Composition of Periphyton Assemblages

Periphyton consists of organisms growing on submerged surfaces, such as rock and plants. It is composed of a complex mixture of organisms and their secretions. Organisms making up the periphyton include bacteria, cyanobacteria, eukaryotic algae, microinvertebrates, and protozoa such as ciliates, flagellates, and amoeboid protists. Major algal components often include diatoms and green algae. Periphyton can serve as an important food resource for other organisms including some species of macroinvertebrates, tadpoles, and fish.

A field study compared the periphyton assemblages in the forebays and pond areas of two stormwater ponds in Ontario.[444] In Harding Pond, specific conductance in the forebay was 1,150 µS/cm, while specific conductance was only 520 µS/cm in the pond. The periphyton assemblage in the forebay consisted of nine genera, mostly diatoms and a species of the green alga *Oedogonium*. The periphyton assemblage in the pond consisted of eight genera consisting mostly of green algae, including the species *Protococcus viride*. In Rouge Pond, specific conductance in the forebay was 2,950 µS/cm, while specific conductance was 1,900 µS/cm in the pond. The periphyton assemblage in the forebay consisted of five genera, with diatoms accounting for about 95 percent of the biovolume. The periphyton assemblage in the pond consisted of 17 genera, mostly green algae and cyanobacteria species. The concentration of biovolume in the pond was almost nine times that of the forebay. The study noted that the two ponds treated stormwater from drainage

---

[442] *S. Taipale, U. Strandberg, E. Peltomaa, A.W.E. Galloway, A. Ojala, and M.T. Brett, "Fatty Acid Composition as Biomarkers in Freshwater Microalgae: Analysis of 37 Strains of Microalgae in 22 Genera and in Seven Classes,"* Aquatic Microbial Ecology, *71:165-178, 2013.*

[443] *Astorg et al. 2023,* op. cit.

[444] *Olding 2000,* op. cit.

areas with different land uses. The area draining to Harding Pond consisted mostly of residential developments. Stormwater entering this pond contained high concentrations of nutrients, which might explain why periphyton species richness was similar between the forebay and pond area. The area draining to Rouge Pond consisted of industrial development. Stormwater entering this pond contained much lower nutrient concentrations.

A field study sampled the periphyton of 41 streams across a chloride gradient in south-central Ontario and examined the composition of the periphytic diatom assemblages in the streams.[445] Chloride concentrations in the streams ranged between 5 mg/l and 502 mg/l. While the study found a strong association between species composition and specific conductance, diversity measures did not correlate with chloride concentration. This suggests that as chloride concentration increased across the streams, sensitive species were replaced by more tolerant species, resulting in little change in the diversity measures. This is supported by the fact that several species disappeared entirely as the concentration of chloride increased, and the abundances of several species were correlated with chloride concentration. Taxonomic indicator analysis showed that the greatest change in the structure of the diatom assemblages occurred over chloride concentrations ranging between 15 mg/l and 35 mg/l. Above 35 mg/l the diatom assemblage changes were more gradual.

Effects of Chloride Salts on the Composition of Macroinvertebrate Assemblages

Macroinvertebrates were previously described in the section on effects of chloride salts on freshwater organisms. As noted in that section, some groups of these animals are more sensitive to impacts from chloride salts than others. These differences in sensitivity can lead to variations in the composition of macroinvertebrate assemblages in aquatic environments with different chloride concentrations.

A field study southeastern Ontario that examined 20 groundwater fed streams with average chloride concentrations ranging between 8 mg/l and 1,149 mg/l found differences in the composition of macroinvertebrate assemblages that were related to chloride concentration.[446] Fly species such as crane flies and biting midges were found at sites with higher chloride concentrations. Amphipods, especially the

---

[445] *Porter-Goff et al. 2013,* op. cit.

[446] *D.D. Williams, N.E. Williams, and Y. Cao, "Spatial Differences in Macroinvertebrate Community Structure in Springs in Southeastern Ontario in Relation to Their Chemical and Physical Environment,"* Canadian Journal of Zoology, *75:1,404-1,414, 1997.*

species *Gammarus pseudolimneaus*, and flatworms were found only in streams with low chloride concentrations.

Another field study surveyed macroinvertebrates assemblages at 107 sampling sites on rivers in eastern Spain and found differences in the assemblages that were related to salinity and specific conductance.[447] As salinity and specific conductance increased along the salinity gradient, the percentage of mayflies, stoneflies, and caddisflies in the assemblage decreased. At the same time, the percentages of beetles, true bugs, dragonflies, and damselflies in the assemblage increased. As previously noted, mayflies and stoneflies are relatively sensitive to effects from chloride. Macroinvertebrate assemblages at salinized sites were dominated by species with short life spans, especially fly species such as midges, biting midges, black flies, and shore flies.

Another study of the effects of salinity on stream macroinvertebrate assemblages found shifts in assemblage composition related to salinity.[448] Shifts in assemblage membership from sensitive taxa to tolerant taxa were observed to occur at salinities between 544 mg/l and 680 mg/l. This corresponded to a specific conductance range of 800 μS/cm to 1,000 μS/cm. In riffle habitats, shifts were seen to occur at a salinity of 440 mg/l which corresponded to a specific conductance of 300 μS/cm.

Effects of Chloride Salts and Salinity on Biodiversity

Biodiversity describes the variety of living organisms. There are several different ways of measuring it, each of which captures an important component contributing to total biodiversity. Taxonomic diversity, for example, examines the different types of organisms that are present. It can be evaluated at the species level, the genus level, or some other level of biological organization. As another example, trait diversity examines the different types of traits present in organisms. Traits may include mode of feeding, where or how reproduction occurs, and how respiration occurs. Studies have examined the effects of chloride salts and salinity on both taxonomic and trait biodiversity.

---

[447] *C. Gutiérrez-Cánovas, D. Sánchex-Fernández, M. Cañedo-Argülles, A. Millán, J. Velasco, R. Acosta, P. Fortuño, N. Otero, A. Soler, and N. Bonada, "Do All Roads Lead To Rome? Exploring Community Trajectories in Response to Anthropogenic Salinization and Dilution of Rivers,"* Philosophical Transactions of the Royal Society Series B, *374:20180009, 2018.*

[448] *N. Horrigan, S. Choy, J. Marshall, and F. Recknagel, "Response of Stream Macroinvertebrates to Changes in Salinity and the Development of a Salinity Index,"* Marine and Freshwater Research*, 56:825-833, 2005.*

Effects of Chloride Salts and Salinity on Taxonomic Diversity

Several studies have examined the effect of chloride salts and salinity on taxonomic diversity in aquatic communities. In combination, these studies address a range of organisms. Many studies analyze their results using diversity indices and measures of species richness. Interpretation of these indices requires some caution. If the loss of sensitive species from a community due to increased chloride concentration is accompanied by the community gaining more tolerant species, aggregate metrics such as species richness or diversity indices might not change.[449]

A paleolimological study examined the impact of increasing salinity on planktonic and periphytic diatoms following construction of a salt storage area adjacent to Fonda Lake near Brighton, Michigan.[450] The diversity and evenness of the diatom assemblage decreased following construction of the storage facility. Over the same time period the abundance of salt-loving diatom species increased. These trends peaked at about the time that the storage area was modified to reduce salt loading to the lake. Following modifications to the storage area, the levels of diatom species diversity and evenness increased, and the abundance of salt-loving species decreased. A second study that examined the same Fonda Lake sediment core found that changes occurred in the assemblage of scaled chrysophyte flagellates that mirrored those seen in the diatom assemblage.[451]

A field experiment found that continuous exposure to elevated concentrations of sodium chloride for periods as short as one week can result in significant changes in periphyton assemblages.[452] Sodium chloride was added to water at four locations along Heyworth Stream in Heyworth, Quebec to maintain instream chloride concentrations at 1,000 mg/l along a reach of the stream for 10 weeks. Periphyton was sampled weekly using artificial substrate samplers. The diversity of algae on the samplers was consistently lower at sites with high chloride concentration than it was at control sites. Some of reduction of diversity with higher chloride concentration may have been related to some diatom species forming auxospores, a

---

[449] *A.M. Wallace and R.G. Biastoch, "Detecting Changes in the Benthic Invertebrate Community in Response to Increasing Chloride in Streams in Toronto, Canada,"* Freshwater Science, *35:353-363, 2016.*

[450] *M.L Tuchman, E.F. Stoermer, and H.J. Carney, "Effects of Increased Salinity on the Diatom Assemblage of Fonda Lake, Michigan,"* Hydrobiologia*, 109:179-188, 1984.*

[451] *B.A. Zeeb and J.P. Smol, "Paleolimnological Investigation or the Effects of Road Salt Seepage on Scaled Chrysophyte Flagellates in Fonda Lake, Michigan,"* Journal of Paleolimnology, *5:263-266, 1991.*

[452] *M.D. Dickman and M.B. Gochnauer, "Impact of Sodium Chloride on the Microbiota of a Small Stream,"* Environmental Pollution*, 17:109-126, 1978.*

resting stage whose formation is generally triggered by environmental stress. The standing crop of algae on the samplers was also lower at sites with high chloride concentrations. High salt concentrations also affected other groups of periphytic organisms. Bacterial diversity was higher at sites with high chloride concentration than at controls. This was attributed to reductions in grazing pressure on bacteria due to reduced numbers of flagellates, ciliates, and other protozoa that graze on bacteria. Finally, the incidence of parasitism on diatoms by fungi was lower at sites with high chloride concentrations. This was likely due to elevated concentrations of chloride inhibiting fungal growth. Other studies have noted decreases in the incidence of fungal infections at elevated chloride concentrations,[453] although some others have noted that brief exposures to high salinity may increase the rate of infection of embryonic amphibians by lethal water mold.[454]

Chloride has also been associated with reductions in zooplankton diversity. A mesocosm study observed that reductions in zooplankton abundance and species richness occurred at chloride concentrations of 250 mg/l.[455] The study also found that in the assemblage from one lake, salt additions drove zooplankton species composition toward dominance by cladoceran species normally found in the littoral area.

Increased chloride concentrations and salinity have been associated with changes in stream macroinvertebrate diversity. One study that noted a dramatic increase in chloride concentration downstream of a heavily salted highway observed a significant decrease in the diversity of aquatic insects inhabiting artificial substrate samplers placed downstream of the highway.[456] Several other field studies

---

[453] *See, for example: L.A. Kszos, J.D. Winter, and T.A. Storch, "Toxicity of Chautauqua Lake Bridge Runoff to Young-of-the-Year Sunfish (*Lepomis macrochirus*),"* Bulletin of Environmental Contamination and Toxicology*, 45:923-930, 1990; J. Rantamaki, L. Cerenius, and K. Soderhall, "Prevention of Transmission of the Crayfish Plague Fungus (*Aphanomyces astaci*) to the Freshwater Crayfish* Astacus astacus *by Treatment with* $MgCl_2$*,"* Aquaculture*, 104:11-18, 1992.*

[454] *N.E. Karraker and G.R. Ruthig, "Effect of Road Deicing Salt on the Susceptibility of Amphibian Embryos to Infection by Water Molds,"* Environmental Research*, 109:40-45, 2009.*

[455] *J.S. Sinclair and S.E. Arnott, "Local Context and Connectivity Determine the Response of Zooplankton Communities to Salt Contamination,"* Freshwater Biology*, 63:1,273-1,286, 2018.*

[456] *C. Demers and R. Sage 1990,* op. cit.

have observed that macroinvertebrate species richness decreased with higher specific conductance.[457] One of these studies observed significant decreases in macroinvertebrate species richness occurring at specific conductance above 1,500 µS/cm.[458] This study also noted that smaller increases in salinity sometimes resulted in increases in overall macroinvertebrate species richness; however, species richness of mayflies, stoneflies, and caddisflies (EPT species richness), decreased with increasing salinity. Since high EPT species richness is usually interpreted to indicate better water quality, this result suggests a community response reflecting a decline in water quality. A flow-through mesocosm study found that short-term exposures to chloride can have large effects on stream macroinvertebrate assemblages.[459] Benthic macroinvertebrate assemblages in these mesocosms were exposed to pulses of sodium chloride lasting 24 to 72 hours, with different microcosms being exposed to different sodium chloride concentrations. At higher chloride concentrations, macroinvertebrate diversity, as measured by the Shannon diversity index, decreased after 24 hours of exposure and taxon richness and EPT richness decreased after 72 hours of exposure. The most sensitive species were lost from the assemblages at higher chloride levels. The authors suggested a specific conductance threshold of 5,000 µS/cm at which short-term exposure to salt has a significant effect on macroinvertebrate community structure.

Chloride concentration can also affect the diversity of vertebrate assemblages. A field study found that amphibian species richness decreased with chloride concentration in a survey of 26 ponds in Nova Scotia.[460] Ponds that were near roads had chloride concentrations of around 400 mg/l. These ponds typically contained only one or two amphibian species. Ponds in a wood lot away from roads had chloride concentrations below 50 mg/l and they typically contained three to six amphibian species. Chloride can also affect the diversity of fish assemblages. Examination of field data from the State of Maryland's biological

---

[457] *A.M. Pinder, S.A. Halse, J.M. McRae, and R.J. Shiel, "Occurrence of Aquatic Invertebrates of the Wheatbelt Region of Western Australia in Relation to Salinity,"* Hydrobiologia*, 543:1-24, 2005; D. Böhme, "Evaluation of Brine Discharge to Rivers and Streams: Methodology of Rapid Impact Assessment,"* Limnologica*, 41:80-89, 2011; B.J. Kefford, R. Marchant, R.B. Schäfer, L. Metzeling, J.E. Dunlop, S.C. Choy, and P. Goonan, " The Definition of Species Richness Used by Species Sensitivity Distributions Approximates Observed Effects of Salinity on Stream Macroinvertebrates,"* Environmental Pollution*, 159:302-310, 2011.*

[458] *Kefford et al. 2011,* op. cit.

[459] *Cañedo-Argüelles et al. 2012,* op. cit.

[460] *Collins and Russell 2009,* op. cit.

stream surveys showed that fish assemblage diversity can be reduced at chloride concentrations between 33 mg/l and 108 mg/l.[461]

Finally, chloride concentrations can affect the diversity of plants in wetlands. A study of an acid peat-rich fen that received inputs of deicing salts from the Massachusetts turnpike found that plant species richness and total plant cover were lower in pots where soil pore water chloride concentrations were greater than 54 mg/l.[462]

Effects on of Chloride Salts and Salinity on Trait Diversity

Increases in concentration of chloride salts and salinity have also been associated with changes in the diversity of traits present in a community or assemblage. This has been studied mostly in macroinvertebrate assemblages. A three-year field study that examined biological traits in macroinvertebrates at 15 sites along the River Werra in Germany found the dominant traits at sites with high specific conductance were different from those at sites with low specific conductance.[463] This study found that macroinvertebrates at sites with higher specific conductance tended to incubate their eggs within their bodies, exchange gases through gills, and reproduce several times a year, while those at sites with lower specific conductance tended to lay their eggs in clutches, exchange gases through their body walls, and reproduce once a year. In a second study, surveys of macroinvertebrates at 107 sites along streams and rivers in eastern Spain found that macroinvertebrate assemblages at salinized sites were dominated by species that had short life spans, incubated their eggs within their bodies or laid their eggs outside of water, conducted gas exchange using air through a variety of means, and reproduced several times a year.[464] Other macroinvertebrate traits

---

[461] *R.P. Morgan, K.M.L. Kline, M.J. Kline, S.F. Cushman, M.T. Sell, R.E. Weitzell, Jr., and J.B. Churchill, "Stream Conductivity: Relationships to Land Use, Chloride, and Fishes in Maryland Streams,"* North American Journal of Fisheries Management, *32:941-952, 2012.*

[462] *J.A. Richburg, W.A. Patterson, and F. Lowenstein, "Effects of Road Salt and* Phragmites australis *Invasion on the Vegetation of a Western Massachusetts Calcareous Lake Basin Fen,"* Wetlands*, 21:247-255, 2001.*

[463] *Gutiérrez-Cánovas et al. 2018,* op. cit.

[464] *Scozs et al. 2014,* op. cit.

associated with higher salinity include species having limited dispersal abilities and feeding through predation, deposit feeding, or filter feeding as opposed to grazing on periphyton or shredding leaves.[465]

Effects of Chloride Salts and Salinity on Interspecific Competition

Interspecific competition is an interaction that can occur between two or more species that require a resource that is in limited supply. Over time one species will obtain more of the resource and be able to grow more quickly, either through obtaining the resource more efficiently or interfering with the ability of the other species to obtain the resource. The competitive abilities of species are based on their traits and physiologies and how these are affected by their environment. Over time, a superior competitor can exclude a poorer competitor from an area; however, disturbance to and short-term changes in the environment may alter the relative competitive abilities of species, allowing them to coexist. In addition, coexistence may also occur when species compete for more than one resource that could potentially limit their growth.[466]

Increases in concentrations of chloride salts and salinity may change competitive relationships between species through favoring more physiologically tolerant species.[467] In particular, differences in how the energetic costs of osmoregulation for each species changes with increasing salinity could alter species relative competitive abilities.[468] A few experiments have examined the effects of chloride levels and salinity on interspecific competition.

Increased salinity changed the competitive outcome between two planktonic green algal species in 96-hour competition assays.[469] In single species tests at low salinity, *Raphidocelis subcapitata* grew more quickly than

---

[465] *N.A. Marshall, and P.C.E. Bailey, "Impact of Secondary Salinization on Freshwater Ecosystems: Effects of Contrasting, Experimental, Short-Term Releases of Saline Wastewater on Macroinvertebrates in a Lowland Stream,"* Marine and Freshwater Research, *55:509-523, 2004; Piscart et al. 2006;* op. cit.*; B.J. Kefford, R.B. Schäfer, and L. Metzeling, "Risk Assessment of Salinity and Turbidity in Victoria (Australia) to Stream Insects' Community Structure Does Not Always Protect Functional Traits,"* Science of the Total Environment*, 415:61-68, 2012.*

[466] *D. Tilman,* Resource Competition and Community Structure*, Princeton University Press, Princeton, New Jersey, 1982.*

[467] *Busse et al. 1999,* op. cit.; *S.S.S. Sarma, B. Elguea-Sánchez, and S. Nandini, "Effect of Salinity on Competition Between the Rotifers* Brachionus rotundiformis *Tschugunoff and* Hexarthra jenkinae *(De Beauchamp) (Rotifera),"* Hydrobiolgia*, 474:183-188, 2002.*

[468] *Coring and Bäthe 2011,* op. cit.

[469] *C. Venâncio, E. Anselmo, A.M.V.M. Soares, and I. Lopes, "Does Increased Salinity Influence the Competitive Outcome of Two Producer Species?"* Environmental Science and Pollution Research, *24:5,888-5,897, 2017.*

Chlorella vulgaris. As salinity increased, *R. subcapitata's* growth rate decreased more rapidly than *C. vulgaris'*. At higher salinity, *C. vulgaris* outcompeted *R. subcapitata* in combined tests.

Similar results were observed in competition experiments with two zooplankton species.[470] At sodium chloride concentrations below 750 mg/l, the water flea *Daphnia galeata* outcompeted the water flea *Simocephalus vetolus* in competition assays. At higher sodium chloride concentrations, *D. galeata's* growth rate dropped below that of *S. vetolus*. Over the course of the experiment at these concentrations, *S. vetolus* was able to outcompete *D. galeata*.

The effects of chloride salts on competition can be complex, especially when several species are potentially competing for the same resources or when some of the competing species could potentially prey on other competing species. A series of mesocosm experiments examined the effects of chloride salts on both competitive and predatory relationships between large zooplankton, aquatic insect larvae, and tadpoles.[471] In this experiment, the researchers set up mesocosms with different concentrations of road salts. The salts used consisted mostly of sodium chloride. The mesocosms also contained large zooplankton and tadpoles. They were open to the air, allowing aquatic insects such as mosquitoes, midges, and shore flies to lay their eggs in the mesocosm.

For most of the invertebrate taxa in the mesocosms, the absolute number of individuals present decreased with salt concentration; however, the relative abundance of some taxa increased over some ranges of salt concentration. At low salt concentrations, large zooplankton consisting mostly of cladocerans dominated the invertebrate assemblages, accounting for 83 to 97 percent of the invertebrates present. This changed at higher salt concentrations. Mosquito larvae dominated the invertebrate assemblage at salt concentrations between 1,000 mg/l and 4,000 mg/l, representing over 80 percent of the invertebrates present. Midge larvae dominated at salt concentrations between 4,000 mg/l and 6,000 mg/l. Shore flies were the only invertebrates present at salt concentrations above 6,000 mg/l.

Additional experiments in this study showed that egg laying by mosquitoes was not affected by the concentration of salt in the mesocosms. It was affected by the presence of tadpoles or cladocerans, potential

---

[470] *C. Loureiro, J.L. Pereira, M.A. Pedrosa, F. Gonçalves, and B.B. Castro, "Competitive Outcome of* Daphnia-Simocephalus *Experimental Microcosms: Salinity Versus Priority Effects,"* PLoS One*, 8:e70572, 2013.*

[471] *J.W. Petranka and E.J. Doyle, "Effects of Road Salts on the Composition of Seasonal Pond Communities: Can the Use of Road Salts Enhance Mosquito Recruitment?"* Aquatic Ecology*, 44:155-166, 2010.*

competitors for food with mosquito larvae. Mosquitoes laid fewer clutches of eggs in mesocosms containing cladoceran zooplankton than in those containing no invertebrates. Also, clutches laid in mesocosms containing tadpoles contained fewer eggs than those laid in mesocosms containing no invertebrates. This suggests that adult mosquitoes chose egg laying sites based on the presence or absence of other species that are potential competitors with their larvae for resources.

The authors of the study concluded that the differences in taxa present in mesocosms at different salt concentrations occurred due to a combination of salt intolerance, competition, and predation. The increase in the relative abundance of mosquito larvae occurred at the same salt concentration at which cladoceran zooplankton declined. Both mosquito larvae and cladocerans feed on phytoplankton. At low salt concentrations, zooplankton were able to outcompete mosquitoes for this food and mosquitos were rare in these mesocosms. The greater sensitivity of zooplankton to salt altered the competitive balance between them and the mosquitoes.

Similarly, the increase in the relative abundance of midge larvae occurred at the same salt concentration at which tadpole survival and growth rates are reduced. Both tadpoles and midges feed on detritus, but tadpoles are also able to feed on midge larvae. Depression of tadpole performance at higher salt concentrations released the midges from the stresses of both competition and predation, allowing them to dominate the mesocosms at higher salt concentrations.

Effects of Chloride Salts and Salinity on Food Web Interactions

As previously described, food webs show trophic linkages between different species in a community. These linkages can influence the abundance of different species in the community. Some influences move upward through the food web from lower levels such as from producers or primary consumers (bottom-up effects). In order to persist in a community, a consumer species needs an adequate supply of food. This may be provided either by the presence of a large standing crop or a high production rate of food organisms or nutrients. Increases in either the standing crop, the rate of production of the food organisms, or nutrients can result in increases in the abundance or biomass of the consumer species in the community. The converse is true as well, as decreases in the standing crop, the production rate of the food organisms, or nutrients can result in lower abundance or biomass of the consumer species.

Other influences move downward through the food web from higher levels such as top predators (top-down effects). An increase in the abundance of a top predator may reduce the abundance of its food species. For example, an increase in the abundance of piscivorous fish could reduce the abundance of zooplanktivorous fish. This could reduce feeding pressure exerted by the second species on its food leading to an increase in the abundance of the third species. In this example the result might be an increase in the abundance of zooplankton.[472]

Increases in the concentration of chloride salts and salinity may change the structure of food webs by having a greater effect on some species more than others. The direct effects of chloride or salinity on some species may lead to indirect effects on other species through changes in the strength of food web linkages. An example of food web linkage is illustrated in the complete food chain shown in Figure 3.17. As noted earlier in this Chapter, zooplankton such as *Daphnia* are generally more sensitive to toxicity from chloride salts than fish or algae. Higher chloride salt concentrations could result in a decrease in *Daphnia* abundance and biomass. This decrease could reduce the amount of grazing on planktonic algae, resulting in an increase in phytoplankton abundance and biomass. Lower *Daphnia* abundance and biomass also reduces the food resources for small, zooplanktivorous fish like gizzard shad, causing a decrease in the abundance and biomass of these fish. Similarly, a reduction in the abundance and biomass of smaller fish reduces the food resources available to larger, piscivorous fish like largemouth bass. While the effect of chloride on *Daphnia* in this example is direct, the effect on other species is indirect and mediated through reductions in the availability of *Daphnia* biomass. An example of this food web effect was seen in Lake Michigan where a reduction in the abundance and diversity of zooplankton led to a reduction in fish recruitment and growth.[473]

One study described a full set of potential effects that might occur in the food webs of lakes in response to contamination by road salt.[474] This study suggested that several food web linkages could be indirectly affected by increasing concentrations of chloride salts. Potential effects include:

1. Road salt contamination reduces the abundance and diversity of zooplankton

2. Reduced grazing by zooplankton leads to algal blooms which are exacerbated by the release of phosphorus from sediment in the lakebed (see No. 5 below)

---

[472] *J.M. Dettmers, M.J. Raffenberg, and A.K. Weis, "Exploring Zooplankton Changes in Southern Lake Michigan: Implications for Yellow Perch Recruitment,"* Journal of Great Lakes Research*, 29:35-364, 2003.*

[473] Ibid.

[474] *Hintz and Relyea 2019,* op. cit.

3. Shading of the lakebed by algal blooms combines with chloride toxicity effects to reduce primary production by benthic algae and macrophytes

4. Road salt causes a density gradient which inhibits lake mixing and causes oxygen depletion in deep water

5. Oxygen depletion in deep water leads to the release of phosphorus from the sediment

6. Reduced benthic primary production leads to reductions in macroinvertebrate production

7. Reduced abundance and biomass of zooplankton and macroinvertebrates leads to reduced fish recruitment

8. Reduced fish recruitment leads to reductions in the abundance and biomass of piscivorous fish

9. Shading due to phytoplankton blooms reduce the foraging success of visual predators such as fish

Several studies have reported food web effects with increases in concentrations of chloride or salinity . These include both field and experimental studies. Most of these studies examined only a small number of linkages within a food web.

Several field studies show food web effects in response to changes in the concentration of chloride salts or salinity. A study on solar evaporation ponds in the Mojave Desert found that increased salinity favored a predatory water boatman (*Trichocorixa reticulata*) that feeds on algae-eating brine shrimp (*Artemia franciscana*).[475] The reductions in brine shrimp abundance due to increased feeding by the water boatman led to an increase in algal biomass in the ponds. Similarly, a survey of eight stormwater ponds near Baltimore, Maryland over spring and early summer showed differences in the sizes of phytoplankton and zooplankton assemblages that were related to specific conductance.[476] Ponds with low and moderate levels of specific conductance had higher abundance of zooplankton and lower biomass of phytoplankton than

---

[475] *D. Herbst, "Salinity Controls on Trophic Interactions among Invertebrates and Algae of Solar Evaporation Ponds in the Mojave Desert and Relation to Shorebird Foraging and Selenium Risk,"* Wetlands*, 26:475-485, 2006.*

[476] *R.J. Van Meter, C.M. Swan, and J.W. Snodgrass, "Salinization Alters Ecosystem Structure in Urban Stormwater Detentio Ponds,"* Urban Ecosystems*, doi: 10.1007/s1 1252-011-0180-9, 2011.*

ponds with higher levels of specific conductance. The authors suggested that the negative effects of salt on zooplankton reduced grazing pressure on the phytoplankton. Studies on Third Sister Lake in Ann Arbor, Michigan found that the abundance of large-bodied zooplankton decreased as the concentration of chloride in the lake increased.[477] This reduced the amount of grazing on phytoplankton led to increases in phytoplankton abundance.

Experimental studies have also shown food web responses to changes in the concentration of chloride salts. A six-week mesocosm experiment exposed the plankton community from Convict Lake, an oligotrophic lake in California, to chloride concentrations ranging between one and 2,900 mg/l.[478] Chloride concentrations in this lake are normally less than two mg/l, but calcium concentrations are relatively high. Zooplankton biomass initially increased with chloride concentration; however, at chloride concentrations above 481 mg/l, zooplankton biomass decreased with increasing chloride concentration. The initial increase in biomass may have been due to the animals requiring a small amount of sodium in their diet. Alternatively, the presence of high calcium concentrations may have mitigated the toxic effects of sodium chloride at lower chloride concentrations. Zooplankton species richness and average body size decreased with rising chloride concentration. This partially reflects shifts in the zooplankton species that were present. Ostracods became more common at higher chloride concentrations and were the only zooplankton in the mesocosms at chloride concentrations above 1,200 mg/l. The phytoplankton assemblage showed the opposite response to chloride concentration. At chloride concentrations below 500 mg/l, phytoplankton biomass decreased with increasing chloride concentration. Phytoplankton biomass remained stable at chloride concentrations between 500 mg/l and 900 mg/l. At concentrations above 900 mg/l, phytoplankton biomass increased with increasing chloride concentration. This experiment shows strong food web effects. The inverse response of zooplankton and phytoplankton suggests that the main effect of chloride salts on phytoplankton occurred through changes in grazing pressure by zooplankton.

---

[477] *T.B. Bridgeman, C.D. Wallace, G.S. Carter, R. Carvajal, L.C. Schiesari, S. Aslam, E. Cloyd, D. Elder, A. Field, K.L. Yurista, and G.W. Kling, "A Limnological Survey of Third Sister Lake, Michigan with Historical Comparisons,"* Lake and Reservoir Management*, 16:253-267, 2000; K.E. Judd, H.E. Adams, N.S. Bosch, J.M. Kostrzewski, C.E. Scott, B.M. Schultz, D.H. Wang, and G.W. Kling, "A Case History: Effects of Mixing Regime on Nutrient Dynamics and Community Structure in Third Sister Lake, Michigan During Late Winter and Early Spring 2003,"* Lake and Reservoir Management*, 21: 316-329, 2005.*

[478] *E.R. Moffett, H.K. Baker, C.C. Bonadonna, J.B. Shurin, and C.S. Symons, "Cascading Effects of Freshwater Salinization on Plankton Communities in the Sierra Nevada,"* Limnology and Oceanography Letters*, 8:30-37, 2023.*

A 78-day mesocosm experiment examined the effect of chloride on lake organisms including phytoplankton, periphyton, filamentous algae, zooplankton, and macroinvertebrates.[479] The abundance of zooplankton and macroinvertebrates decreased as chloride concentration increased. At the same time, biomass of phytoplankton and periphyton increased due to the reduced grazing by herbivores.

Effects of Interactions Between Chloride Salts and Other Factors

The structure of biological communities can be influenced by numerous factors. These factors often operate simultaneously which can make it difficult to evaluate the importance of impacts from a single factor, such as increases in the concentration of chloride salts or salinity. In addition, there may be interactions between factors leading to different or more severe outcomes than would result from the effects of a single factor. Experimental studies have examined the interactions between the concentration of chloride salts and two other factors—nutrient concentrations and the presence of predators.

Interactions Between Chloride Salts and Nutrients

A six-week experiment exposed the plankton community taken from Long Lake in Ontario, Canada to different combinations of chloride and phosphorus concentrations.[480] Plankton from the lake were placed into mesocosms with chloride concentrations ranging between 0.41 mg/l, the ambient concentration found in the lake, and 1,500 mg/l. Two nutrient treatments were established in the mesocosms. In one treatment, phosphorus was added to bring the concentration to 0.031 mg/l. Nitrogen compounds were also added to this treatment in order to maintain a constant ratio of nitrogen atoms to phosphorus atoms. In the other treatment, phosphorus concentrations were left at 0.014 mg/l, the ambient concentration in the lake. Zooplankton and phytoplankton responded differently to these treatments. Zooplankton abundance, biomass, and taxonomic richness decreased with increasing chloride concentration. Zooplankton levels were not affected by the addition of nutrients. The response of phytoplankton to increasing chloride concentration depended on the nutrient concentration present. At the lower nutrient level, the total abundance and biomass of phytoplankton increased with increasing chloride concentration. Phytoplankton taxonomic richness increased up to a chloride concentration of 350 mg/l, and then decreased after that. At high nutrient levels, no relationship was observed between chloride concentration and phytoplankton abundance or biomass, but phytoplankton taxon richness declined with increasing chloride concentration. The combination of high nutrient levels and high chloride concentration resulted in the phytoplankton

---

[479] *K.D. Delaune, D. Nesich, J.M. Goos, and R.A. Relyea, "Impacts of Salinization on Aquatic Communities: Abrupt vs. Gradual Exposures,"* Environmental Pollution, *285:117636, 2021.*

[480] *Greco et al. 2022,* op. cit.

assemblage being dominated by groups such as cyanobacteria that are difficult for zooplankton to consume.[481] Cyanobacteria lack sterols which are components of zooplankton cell membranes. Dietary deficiencies of these sterols can make zooplankton more sensitive to chloride by increasing the permeability of their cell membranes.[482] Many cyanobacteria species also produce toxins that can limit zooplankton abilities to utilize them as food.[483]

A second mesocosm experiment found that the combination of high salt concentrations and high nutrient concentrations created a very eutrophied system. This included reduced macrophyte coverage of the bottom, higher levels of primary production in the water column, and lowered abundance of consumers at higher trophic levels.[484]

Interactions Between Chloride Salts and the Presence of a Predator

An 83-day mesocosm experiment examined the effects of chloride concentration on an aquatic community in the presence and absence of zooplanktivorous fish.[485] Phytoplankton biomass increased with increasing chloride concentration. The impact was much greater when fish were present. This was due in part to a synergistic effect between the presence of fish and high salt concentrations on zooplankton abundance and biomass. The impacts experienced in these treatments were greater than would be suggested by the effects of either high salinity or fish presence alone.

Are Current Water Quality Standards for Chloride Protective of Ecological Communities?

As noted earlier in this Chapter, Wisconsin's water quality standards include criteria for the protection of aquatic life from chloride. These consist of an acute criterion in which the surface water daily maximum chloride concentration is not to exceed 757 mg/l more than once every three years and a chronic criterion in which the four-day average of daily maximum chloride concentration is not to exceed 395 mg/l more than once every three years. The U.S. Environmental Protection Agency (USEPA) has also issued aquatic life

---

[481] *H.W. Paerl and T.G. Otten, "Harmful Cyanobacterial Blooms: Causes, Consequences, and Controls,"* Microbial Ecology, *65:995-1,010, 2013.*

[482] *J. Istant-Navarro, S.E. Arnott, T. Klauschies, and D. Martin-Creuzburg, "Dietary Lipid Quality Mediates Salt Tolerance of a Freshwater Keystone Herbivore,"* Science of the Total Environment*, 769:144657, 2021.*

[483] *J.J. Gilbert, "Susceptibility of Planktonic Rotifers to a Toxic Strain of* Anabaena flos-aquae*,"* Limnology and Oceanography*, 39:1,286-1,297, 1994.*

[484] *Lind et al. 2018,* op. cit.

[485] *Hintz et al. 2017,* op. cit.

criteria for chloride. These serve as recommendations to states and tribes for setting their water quality criteria. Under the USEPA criterion, the acute criterion is that the one-hour average of chloride concentration should not exceed 860 mg/l. Similarly, the USEPA chronic criterion is that the four-day average chloride concentration should not exceed 230 mg/l.

Both the Wisconsin chloride criteria and the USEPA recommended chloride criteria were developed using data from laboratory toxicity studies for individual species.[486] The effects of chloride and chloride salts on biological communities were not considered in developing these standards. Development and application of these criteria assume that if the criteria are generally protective for the organisms that were tested, they will be protective for biological communities in which these organisms reside. Results from the literature reviewed in this Chapter suggest that this assumption may not be valid.

The studies reviewed in this Chapter document many effects of chloride and chloride salts on organisms and biological communities. These results occur over a wide range of chloride concentrations. A few of the studies present thresholds at which effects appear. These thresholds are summarized in Table 3.17. Impacts for which thresholds have been reported include decreases in organism abundance, reductions in diversity, changes in community composition, changes in organism physiological processes, and changes in organism behavior related to the use of habitats.

Most of the thresholds presented in Table 3.17 are lower than Wisconsin's chronic water quality criterion for chloride and many are lower than the USEPA recommended criterion continuous concentration. This suggests that these water quality criteria may be too high to be fully protective of aquatic communities. It should be noted that these thresholds derive from a small number of studies and may not fully characterize the range of responses aquatic communities might show to chloride enrichment.

---

[486] *For Wisconsin criteria, see: Chapter NR 105, Surface Water Quality Criteria and Secondary Values for Toxic Substances,* Wisconsin Administrative Code*. For USEPA criteria, see: U.S. Environmental Protection Agency,* Ambient Water Quality Criteria for Chloride—1988*," EPA 440/5-88-01, February 1988.*

A recent study presents stronger evidence that current water quality criteria may not be fully protective of aquatic communities.[487] This study established an experimental network of mesocosm experiments at 16 sites across North America and Europe. Experiments at these sites used standardized methods to examine the effects of chloride on zooplankton and phytoplankton from natural lake habitats. Each experiment incubated zooplankton and phytoplankton from nearby lakes in 20-32 mesocosms at chloride concentrations ranging between 2 mg/l and 1,500 mg/l. These mesocosms were incubated for 41-51 days. The study examined changes in the abundance of zooplankton species from four groups and phytoplankton biomass over the course of the experiment.

At each lake site, the study assessed the concentration of chloride that reduced the abundance of zooplankton in each group by 50 percent. At most sites, this concentration was lower than 230 mg/l, the USEPA chronic criterion (see Table 3.18). The study also assessed the magnitude of reductions seen in each of the zooplankton groups at a chloride concentration of 230 mg/l. While there was considerable variation among sites, for all zooplankton groups reductions greater than 80 percent occurred (see Table 3.18). Food web effects were also observed at some sites, with phytoplankton biomass increasing at 47 percent of the lake sites.

Based on these results, the authors concluded that the current chronic criterion does not protect lake food webs from chloride salt impacts. Based on a similar analysis, they also concluded that the Canadian chronic toxicity standard of 120 mg/l fails to protect lake food webs. The study authors recommended that these criteria be reassessed.

3.4 IMPACTS OF CHLORIDE ON ECOSYSTEMS

Ecosystem Functions and Structures

An ecosystem consists of the organisms within an area and the physical environment with which those organisms interact. A stream ecosystem, for example, includes the plants, animals, and microorganisms in

---

[487] *W.D. Hintz, S.E. Arnott, C.C. Symons, D.A. Greco, A.McClymont, J.A. Brentrup, M. Canedo-Arguelles, A.M. Derry, A.L. Downing, D.K. Gray, S.J. Melles, R.A. Relyea, J.A. Rusak, C.L. Searle, L. Astorg, H.K. Baker, B.E. Beisner, K.L. Cottingham, Z. Ersoy, C. Espinosa, J. Franceschini, A.T. Giorgio, N. Gobeler, E. Hassal, M.-P. Hebert, M. Huynh, S. Hylander, K.L. Jonasen, A.E. Kirkwood, S. Langenheder, O. Langvall, H. Laudon, L. Lind, M. Lundgren, L. Proia, M.S. Schuler, J.B. Shurin, C.F. Steiner, M. Striebel , S. Thibodeau P Urrutia-Cordero, L. Vendrell-Puigmitja, and G.A. Weyhenmeyer, "Current Water Quality Guidelines Across North America and Europe Do Not Protect Lakes from Salinization,"* Proceedings of the National Academy of Sciences*, 119:e2115033119, 2022.*

the stream as well as the water of the stream, the sediment and rock making up the streambed, and the soil making up the stream bank. Ecosystems may be linked to one another through movement or exchange of matter or energy.

Ecosystems provide numerous services to humans. These include the provision of fresh water through the hydrologic cycle, development and maintenance of soils, decomposition of wastes, regulation of the climate, and food.

An ecosystem is described through the movement and transformation of materials and energy through various compartments in the environment. Figure 3.18 shows a simplified depiction of phosphorus movement through a pond ecosystem. Organic phosphorus compounds from the land surface are carried into the pond as detritus in runoff. This detritus settles onto the pond bed and is incorporated into sediment. Runoff also carries inorganic phosphorus compounds into the pond. Algae and plants in the pond take up inorganic phosphorus and incorporate it into their tissue. Herbivorous animals consume algae and plants and incorporate the phosphorus contained in this food into their own tissue. Similar incorporation of phosphorus into predator tissue occurs when other animals consume the herbivores. When organisms die, their bodies sink to the pond bed and are incorporated into sediment. Excretory products from animals also sink and are incorporated into sediment. Bacteria within the sediment decompose this organic material, converting organic phosphorus compounds to inorganic phosphorus compounds. Inorganic phosphorus in the sediment can be released back into the water column. Over time, some inorganic phosphorus in sediment may be incorporated into rock as sediments lithify. Similarly, degradation of rock may release phosphorus to the sediment or water.

The flow of material through ecosystems is often cyclic. This is often examined as cycles of chemical elements. An example of this is the nitrogen cycle which was described in Chapter 2 of this Report (see Figure 2.17). Cycling of material can involve changes in the location of the element in the environment. For example, a change in solubility may result in an element moving from sediment into the water column. These changes may involve movement between biotic and abiotic compartments of the environment. The chemical form of an element may also change through biogeochemical transformations that occur during cycling. Some of these transformations occur through purely chemical mechanisms. For example, when a carbon dioxide (CO_2) molecule dissolves in water it combines with a water molecule to form carbonic acid (H_2CO_3) which then dissociates to form a bicarbonate ion (HCO_3^-). Other transformations are biologically mediated. Nitrogen fixation, nitrification, and denitrification, which were discussed in Chapter 2 of this

Report, are examples of biologically mediated transformations. Each of these steps in the nitrogen cycle is conducted by a specific species of bacteria.

Cycling of nutrients through food webs is an example of an ecosystem process. Other examples of processes include primary production through photosynthesis or chemosynthesis, community respiration, and decomposition of organic matter. Increasing concentrations of chloride salts and salinity may have impacts on these processes in terrestrial and freshwater ecosystems. This may in turn affect the ability of these ecosystems to provide ecosystem services.

Chloride salts and salinity can have impacts on ecosystem processes. Impacts of chloride on several nutrient cycles were previously discussed in the section on impacts on wetlands in Chapter 2 of this Report. This section describes the impacts of chloride salts and salinity on energy flow.

Energy Flow Through Ecosystems

Energy flows through ecosystems. Organic carbon compounds represent the basic energy for ecosystems. While these compounds are all ultimately formed through primary production, mostly via photosynthesis, they are provided within ecosystems both through primary production and organic matter decomposition.[488]

The relative importance of the processes of primary production and decomposition to energy flow varies depending on the specific ecological system. In streams the relative importance of these processes as energy sources depends on the size or order of the stream. In general, photosynthesis becomes relatively more important in higher order, downstream sections of the stream network.[489] For example, a study of the Little Tennessee River in Georgia and North Carolina assigned about 81 percent of available organic carbon of the entire stream network to gross primary production.[490] Most of this organic carbon was in the lower reaches.

---

[488] *R.B. Schäfer, M. Bondschuh, D.A. Rouch, E. Szöcs, P.C. van der Ohe, V. Pettigrove, R.Schulz, D. Negrgoda, and B.J. Kefford, "Effects of Pesticide Toxicity, Salinity, and Other Environmental Variables on Selected Ecosystem Functions and the Relevance for Ecosystem Services,"* Science of the Total Environment*, 415:69-78, 2012.*

[489] *R. L. Vannote, W.G. Minshall, K.W. Cummins, J.R. Sedell, and C.E. Cushing, "The River Continuum Concept,"* Canadian Journal of Fisheries and Aquatic Sciences*, 37:130-137, 1980.*

[490] *J.R. Webster, "Spiraling Down the River Continuum: Stream Ecology and the U-Shaped Curve,"* Journal of the North American Benthological Society*, 26:375-389, 2007.*

The size of a lake partially determines the relative importance of primary production and decomposition as energy sources. Most lakes are relatively small, and the littoral area can represent a large portion of their surface area. Breakdown of terrestrial organic material originating in the watershed can represent a large portion of the energy process in the lake. Photosynthesis may be more important in larger lakes; however, much of the organic material produced through primary production in these lakes will ultimately end up being decomposed.

The relative importance of primary production and decomposition as energy sources is dynamic. As indicated in the Little Tennessee River example, it can vary by location within an ecosystem. It can also vary seasonally with climatic changes or loading of organic material from upstream or terrestrial sources.

Freshwater aquatic food webs are dependent on inputs of organic material from surrounding terrestrial landscapes for energy and nutrients.[491] While some of this material enters aquatic systems as dissolved organic matter, much enters as detritus such as leaves, wood, dead organisms, and other forms of particulate organic material. Leaf litter in particular is a major organic carbon source that sustains biomass at higher trophic levels in temperate forest and low order streams.[492] Microbial organisms such as bacteria and fungi colonize this litter, decomposing it. These microbes may either assimilate carbon from the litter to create more biomass or respire it. The underlying mechanisms determining the balance between these two physiological processes is not well understood. Colonization of the litter by microbes also mineralizes nutrients from the litter, making them available to support primary production and the growth of primary producers.[493]

Microbial conditioned litter is a major energy source for many aquatic organisms. While many macroinvertebrates feed on leaf litter, they are unable to digest the leaves. The bacteria, fungi, and periphyton growing on the litter provide them with energy and nutrients. This allows the energy in the litter to enter the aquatic food web and be passed to higher trophic levels. By processing microbially conditioned

---

[491] *A.B. Stoler, W.D. Hintz, D.K. Jones, L. Lind, B.M. Mattes, M.S. Schuler, and R.A. Relyea, "Leaf Litter Mediates the Negative Effect of Road Salt on Forested Wetland Communities,"* Freshwater Science*, 36:415-426, 2017.*

[492] *E. Berger, O. Frör, and R.B. Schäfer, "Salinity Impacts on River Ecosystem Processes: A Mini-Review,"* Philosophical Transactions of the Royal Society Series B*, 374:20180010, 2018.*

[493] *M.J. Rubbo, J.J. Cole, and J.M. Kiesecker, "Terrestrial Subsidies of Organic Carbon Support Net Ecosystem Production in Temporary Forest Ponds: Evidence from an Ecosystem Experiment,"* Ecosystems*, 9:1,170-1,176, 2006.*

litter, macroinvertebrates provide a link between terrestrial flora and aquatic food webs.[494] In fact, the amount and type of terrestrial plant litter available may limit energy flow in some aquatic ecosystems.[495]

There can be a spatial dimension to energy flow in some systems. Water flow in stream systems can transport material including both dissolved and particulate organic matter downstream. As a result, primary production and decomposition in a stream provide energy to both local food webs and food webs downstream.[496]

Toxicity and other effects of chloride salts and salinity on organisms could potentially disrupt linkages between trophic levels in some environments. These linkages constitute pathways for energy flow in environments. These sorts of impacts on energy flow are likely to vary among systems depending on how much redundancy in functional roles there is among the species present.[497] If each role is performed by multiple species and if species performing a given role differ in their sensitivity to chloride salts, the effects of chloride might have little impact on energy flow. Impacts on energy flow are more likely to occur when either a small number of species perform a critical role or when species performing similar roles have similar sensitivity to chloride.

Impacts of Chloride Salts and Salinity on Organic Matter Decomposition

In general, organic matter decomposition in streams decreases with increasing salinity.[498] For example, one field study found that the breakdown rate of leaf packs placed in streams decreased with increasing salinity over a range of specific conductance between 50 and 3,500 μS/cm.[499] The biomass of fungi on and in the

---

[494] *M.A. Palmer, A.P. Covich, S. Lake, P. Biro, J.J. Brooks, J. Cole, C. Dahm, J. Gibert, W. Goedkoop, K. Martens, J. Verhoeven, and W.J. van de Bund, "Linkages Between Aquatic Sediment Biota and Life Above Sediments as Potential Drivers of Biodiversity and Ecological Processes,"* BioScience*, 50:1,062-1,075, 2000.*

[495] *J.B. Wallacd, S.L. Eggert, J.L. Meyer, and J.R Webster, "Effects of Resource Limitation on a Detrital-Based Ecosystem,"* Ecological Monographs*, 69:409-433, 1999.*

[496] *J.D. Allan and M.M. Castillo,* Stream Ecology: Structure and Function of Running Waters*, Springer-Verlag, Dordrecht, Netherlands, 2007.*

[497] *D. Tilman, J. Knops, D. Wedin, and P. Reich, "Experimental and Observational Studies of Diversity, Productivity, and Stability," pages 42-70 in: A.P Kinzig, S.W. Pacala, and D. Tilman,* The Functional Consequences of Biodiversity: Empirical Progress and Theoretical Extensions*, Princeton University Press, Princeton, New Jersey, 2002.*

[498] *Berger et al 2018*, op. cit.

[499] *Schäfer et al 2012*, op. cit.

leaves also decreased with rising salinity. The same study reported similar results for the breakdown of cotton strips that were placed in a stream. A second field study reported that the breakdown rates of birch wood sticks placed in streams decreased with increasing salinity.[500] This study found that fungal biomass and microbial activity both were reduced with increasing salinity.

Similar results have been reported from mesocosm studies. One mesocosm study found that the mass loss of decomposing leaves decreased with increasing salinity.[501] In this study, fungal biomass in the leaves rose with increasing salinity at low levels of salinity, and then decreased with increasing salinity at higher levels of salinity. A second mesocosm study, which also found decreasing mass loss from decomposing leaves with increasing salinity. found that fungal respiration, an indicator for fungal activity, fell with increasing salinity.[502] A third study did not detect any effect of salinity on litter breakdown rates.[503]

Some studies have tied reductions in organic material decomposition rates to chloride concentrations. A 16-day study found that a chloride concentration of 5,000 mg/l reduced the decomposition of beech tree litter incubated in laboratory chambers by 44 percent.[504] A 75-day study found that a chloride concentration of 645 mg/l reduced the breakdown rate of oak leaf litter in pond mesocosms at by almost 10 percent.[505]

The mechanisms through which chloride salts reduce the rates of decomposition of organic matter are not well understood. It is likely that toxicity effects on fungi and bacteria play a role. It has been suggested that elevated concentrations of chloride salts may impair the activities of enzymes that microbial decomposers

---

[500] *R. Gómez, A.D. Asencio, J.M. Picón, R. del Campo, M.I. Arce, M. del Mar Sánchez-Montoya, M.W. Suárez, and M.R. Vidal-Abarca, "The Effect of Water Salinity on Wood Breakdown in Semiarid Mediterranean Streams,"* Science of the Total Environment*, 541:491-501, 2016.*

[501] *M. Cañedo-Argüelles, M. Bundschuh, C. Gutiérrez-Cánovas, B.J. Kefford, N. Prat, R. Trobajo, and R.B Schäfer, "Effects of Repeated Salt Pulses on Ecosystem Structure and Functions in a Stream Mesocosm,"* Science of the Total Environment*, 476-477:634-642, 2014.*

[502] *C. Canhoto, S. Simões, A.C. Gonçalves, L. Guihermino, and F. Bärlocher, "Stream Salinization and Fungal-Mediated Leaf Decomposition: A Microcosm Study,"* Science of the Total Environment*, 599-600:1,638-1,645, 2017.*

[503] *Stoler et al 2017*, op. cit.

[504] *C.M. Swan and C.A. DePalma, "Elevated Chloride and Consumer Presence Independently Influence Processing of Stream Detritus,"* Urban Ecosystems*, 15:625-635, 2012.*

[505] *R.J. Van Meter, C.M. Swan, and C.A. Trossen, "Effects of Road Deicer (NaCl) and Amphibian Grazers on Detritus Processing in Pond Mesocosms,"* Environmental Toxicology and Chemistry*, 31:2,306-2,310, 2012.*

use to process litter.[506] Another suggestion is that elevated salt levels may reduce the functional capacities of decomposers.[507] Effects of salinity on macroinvertebrates may also play a role, as the physical breakdown of litter by macroinvertebrates can be important in the colonization of leaf litter by microbes.[508]

Reductions in the breakdown of organic matter could lead to less biomass in an aquatic ecosystem. This depends on whether the primary production through photosynthesis within the system is sufficient to maintain overall levels of production.[509] In watersheds with sparser riparian vegetation, instream or in-lake autotrophic organisms such as phytoplankton and aquatic plants are the most important sources of organic carbon. If these sources can sustain biomass decomposition in the presence of elevated concentrations of chloride salts, reduced decomposition of litter will have little effect on production at higher trophic levels. Where autotrophic sources cannot sustain biomass, reduced decomposition due to elevated chloride salt concentrations could have several effects. The amount of biomass at higher trophic levels might be reduced. If the magnitude of the reduction in biomass due to reduced decomposition is great enough, this could include a reduction in the number of trophic levels the system could support. Reduced decomposition due to increased concentrations of chloride salt could also result in an accumulation of litter on the bed and in the sediment of the stream or lake. Over time, this would lead to changes in habitat. Finally, in streams the reduced decomposition of litter could result in transport of organic material downstream. This could have indirect impacts affecting production in downstream reaches through reducing light penetration, altering energy flow from upstream, or changing habitat conditions. It should be noted that this first impact could potentially lead to reductions in primary production in downstream reaches while the other two impacts might affect production at higher trophic levels.

Impacts of chloride salts and salinity on the decomposition of organic matter may also affect biogeochemical transformations of other elements, potentially disrupting other biogeochemical cycles. Biogeochemical cycles of biologically active elements are linked, especially through physical and chemical

---

[506] *M.C. Roache, P.C. Bailey, and P.I. Boon, "Effects of Salinity on the Decay of the Freshwater Macrophyte,* Triglochin procerum*,"* Aquatic Botany*, 84:45-52, 2006.*

[507] *Van Meter et al 2012,* op. cit.

[508] *M. Santonja, L. Pellan, and C. Piscart, "Macroinvertebrate Identity Mediates the Effects of Litter Quality and Microbial Conditioning on Leaf Litter Recycling in Temperate Streams,"* Ecology and Evolution*, 8:2,542-2,553, 2018.*

[509] *K.M. Fritz, S. Fulton, B.R. Johnson, C.D. Barton, J.D. Jack, D.A. Word, and R.A. Burke, "Structural and Functional Characteristics of Natural and Constructed Channels Draining a Reclaimed Mountaintop Removal and Valley Fill Coal Mine,"* Journal of the North American Benthological Society*, 29:673-689, 2010.*

changes that occur in sediment.[510] Many biotransformations of other elements are dependent on the availability of organic carbon to provide the energy source for the bacteria that mediate the transformations.[511] Examples of these include the nitrification and denitrification reactions discussed in Chapter 2 of this Report.

Impacts of Chloride Salts and Salinity on Primary Production

The impact of increased concentrations of chloride salts and salinity on primary production is uncertain. Results from some studies suggest that salinity increases may reduce primary production in freshwater aquatic systems, while results from other studies suggest that this may not be the case. Several studies found that vascular plant communities show reduced primary production in response to increasing salinity.[512] A different study found that primary production in diatoms assemblages increased with increasing salinity.[513] Still other studies suggest that at low salinities primary production may rise with increasing salinity, while at higher salinity primary production may decrease with increasing salinity.[514]

Some results from studies examining the effects of chloride salts and salinity on freshwater organisms suggest that increases in the concentrations of chloride could result in reductions in primary production in aquatic systems. As discussed in the section on effects on algae in this Chapter, some studies have reported that increases in concentrations of chloride salts can lead to reductions in algal concentrations in the water column, reduced photosynthetic pigment content in algal cells, and reduced algal photosynthetic efficiency. Some similar effects have been reported for some other photosynthesizing organisms. As discussed in the section on the effects on aquatic organisms in this Chapter, higher chloride salt concentrations have been

---

[510] *J.J. Middelburg and L.A. Levin, "Coastal Hypoxia and Sediment Biogeochemistry,"* Biogeosciences*, 6:1,273-1,293, 2009.*

[511] *T.A. Newcomer, S.S. Kaushal, P.M. Mayer, A.R. Shields, E.A. Canuel, P.M. Groffman, and A.J. Gold, "Influence of Natural & Novel Organic Carbon Sources on Denitrification in Forested, Degraded-Urban, and Restored Streams,"* Ecological Monographs*, 82:449-466, 2012; S-.W. Dawn, and S.S. Kaushal, "Warming Increased Carbon and Nutrient Fluxes from Sediments in Streams Across Land Use,"* Biogeosciences*, 10:1,193-1,207, 2013.*

[512] *See, for example, W.E. Odum, "Comparative Ecology of Tidal Freshwater and Salt Marshes,"* Annual Review of Ecology and Systematics*, 19:147-176, 1988; D.L. Nielsen, M.A. Brock, N. Rees, and D.S. Baldwin, "Effects of Increasing Salinity on Freshwater Ecosystems in Australia,"* Australian Journal of Botany*, 51:655-665, 2003.*

[513] *M.G. Ros, J.P. Marín-Murcia, and M. Aboal, "Biodiversity of Diatom Assemblages in a Mediterranean Semiarid Stream: Implications for Conservation,"* Marine and Freshwater Research*, 60:14-24, 2009.*

[514] *Silva and Davies 1999,* op. cit.*; E.I.L. Silva, A. Shimizu, and H. Matsunami, "Salt Pollution in a Japanese Stream and Its Effects on Water Chemistry and Epilithic Algal Chlorophyll-a,"* Hydrobiologia*, 437:139-148, 2000.*

reported to induce growth reductions, reductions in biomass, and reduced carbon fixation in aquatic macrophytes.

Finally, the ability of water to hold gases in solution decreases with increasing salinity.[515] Increases in chloride salt concentration could reduce the amount of carbon dioxide available to aquatic plants and algae for photosynthesis.

Impacts of Chloride Salts and Salinity on Ecosystem Services

Increased concentrations of chloride salts and salinity could potentially affect the ability of some systems to provide ecosystem services. Ecosystem processes such as organic matter breakdown and primary production provide the basis for many potential ecosystem services.[516] This section discusses a few examples in which reductions in energy flow could potentially lead to impacts on ecosystem services.

A reduction in the decomposition of organic matter due to higher concentrations of chloride salts could lead to less biomass in a stream or lake system.[517] Given that fish generally occupy higher trophic levels in these systems, such a reduction could potentially reduce the sizes and quality of fish populations in a waterbody. This could reduce the ability of the waterbody to provide food to humans. It could also reduce the recreational opportunities provided by a healthy and diverse fish assemblage.

As previously discussed, decomposition of organic material provides the energy for many biogeochemical transformations. These transformations are important in cycling of elements, such as in the nitrogen cycle. Reductions in organic matter breakdown due to higher concentrations of chloride salts could lower the amount of material processed in nitrogen cycle steps such as denitrification. This could cause increases in the ambient concentrations of nitrogen compounds in aquatic ecosystems because the ability of the system to convert nitrate into nitrogen gas would be reduced under such conditions. This change would represent a reduction in the ability of the ecosystem to provide water purification services.

A study of two rivers in Poland found that increases in salinity reduced the ability of these ecosystems to provide another water purification service. While the use of sulfonamide antibiotics to treat human diseases

---

[515] *R.F. Weiss, "Carbon Dioxide in Water and Seawater: The Solubility of a Non-Ideal Gas,"* Marine Chemistry*, 2:203-215, 1974.*

[516] *Berger et al 2018,* op. cit.

[517] *Fritz et al 2010,* op. cit.

has decreased in recent decades, these drugs are commonly used in veterinary applications. The study found that degradation rates of four sulfonamides in river water decreased with increasing salinity.[518]

3.5 SUMMARY

Review of the scientific and technical literature indicates that increased concentrations of chloride salts can cause adverse impacts on biological systems. These impacts can occur at several biological levels affecting individual organisms, species, communities and ecosystems. While limited research has been conducted on some groups of organisms, the available studies suggest that chloride salts can produce several different types of impacts.

- Many biological impacts are caused by toxicity of chloride, the cations associated with chloride, or salinity to aquatic organisms
    - Organisms differ from one another in their sensitivity to toxic effects from chloride salts
    - The toxicity of chloride salts can be influenced by many factors including:
        - The level, frequency, duration, and manner of exposure
        - Environmental factors such as temperature and water hardness
        - The biology and developmental stage of the organism
    - Chloride salt toxicity can occur through acute or chronic exposure
    - Chloride salts can produce lethal and sublethal effects
- While different impacts have been reported in different organisms, numerous types of sublethal effects are associated with exposure to chloride salts including:

---

[518] *E. Adamek, W. Baran, and A. Sobczak, "Assessment of the Biodegradability of Selected Sulfa Drugs in Two Polluted Rivers in Poland: Effects of Seasonal Variation, Accidental Contamination, Turbidity, and Salinity,"* Journal of Hazardous Materials*, 313:147-158, 2016.*

- Lower population growth rates
- Reduced organismal growth and slower development
- Smaller organism size
- Physical and cellular damage
- Reduced activity including less feeding, mobility, and antipredator and antiparasite behavior
- Less abundance
- Reduced longevity
- Reductions in photosynthesis due to reduced leaf production, reduced photosynthetic pigment content, and physiological effects
- Interference with reproduction through several mechanisms
- Reduced assimilation of food and nutrients
- Induction of developmental deformities
- Increased energy requirements
- Behavioral alterations

- Some factors may act to mitigate an organism's sensitivity to impacts of chloride salts including greater food availability, higher water hardness, and specific biological traits
- Increases in concentration of chloride salts can cause changes in composition and structure of ecological communities including:
  - Changes in which species are present

- Reductions in taxonomic richness, evenness, and diversity
- Reductions in abundance and biomass of organisms

- Increased concentrations of chloride salts may alter outcomes of ecological processes such as competition and predation

- Changes in community composition and structure may occur at chloride concentrations lower than the Wisconsin chronic chloride criterion of 395 mg/l, suggesting that current water quality standards may not be protective of ecological communities

- Increases in the concentrations of chloride salts in the environment have impacts to the energy flow through an ecosystem due to
  - Decreases in primary production
  - Decreases in organic matter breakdown

- Chloride induced changes in energy flow could reduce the ability of some ecosystems to provide certain services to humans (e.g., recreational fishing)

SEWRPC Technical Report No. 62

IMPACTS OF CHLORIDE ON THE NATURAL AND BUILT ENVIRONMENT

Chapter 3

IMPACTS OF CHLORIDE ON BIOLOGICAL SYSTEMS

TABLES

#264831 – TR-62 (Chloride Study Impacts) Table 3.1
200-1100
JEB/LKH/mid
9/14/21, 8/7/23, 12/4/23

Table 3.1
Waterbodies Listed as Impaired Due to Chloride in Southeastern Wisconsin: 2022

Name	WBIC[a]	County	Extent (River mile)[b]	Impairment: Acute Toxicity	Impairment: Chronic Toxicity	Listing Date
Beaver Creek	20000	Milwaukee	0.00-2.65	--	X	2020
Brown Deer Creek	19700	Milwaukee	0.00-2.30	X	X	2018
Burnham Canal	3000042	Milwaukee	0.00-1.05	--	X	2018
Butler Ditch	18100	Waukesha	0.00-2.85	--	X	2020
Crestwood Creek	19450	Milwaukee	0.00-1.35	X	X	2020
Dousman Ditch	17100	Waukesha	0.00-2.50	X	X	2022
Fish Creek	44700	Ozaukee, Milwaukee	0.00-3.38	--	X	2018
Honey Creek	16300	Milwaukee	0.00-8.96	X	X	2018
Indian Creek	19600	Milwaukee	0.00-2.63	X	X	2018
Kilbourn Road Ditch	736900	Racine	0.0-14.3	--	X	2022
Kinnickinnic River	15100	Milwaukee	5.49-9.93	X	X	2018
Kinnickinnic River	15100	Milwaukee	3.16-5.49	X	X	2014
Kinnickinnic River	15100	Milwaukee	0.00-3.16	X	X	2022
Lilly Creek	18400	Waukesha	0.00-4.70	--	X	2016
Lincoln Creek	19400	Milwaukee	0.0-9.7	X	X	2014
Little Menomonee River	17600	Ozaukee, Milwaukee	0.0-9.0	X	X	2016
Meadow Brook Creek	772300	Waukesha	0.00-3.14	--	X	2018
Menomonee River	16000	Washington, Waukesha, Milwaukee	0.00-24.81	X	X	2018
Mitchell Field Drainage Ditch	14800	Milwaukee	0.0-2.3	X	X	2020
North Branch Oak Creek	14900	Milwaukee	0.0-5.7	X	X	2018
North Branch Pike River	1900	Racine, Kenosha	5.23-7.87	--	X	2018
Nor-X-Way Channel	18450	Ozaukee, Washington, Waukesha	0.0-4.9	--	X	2020
Noyes Creek	17700	Milwaukee	0.00-3.54	X	X	2020
Oak Creek	14500	Milwaukee	0.00-13.32	X	X	2014
Pewaukee River above Pewaukee Lake	771800	Waukesha	0.00-.45	--	X	2020
Pike Creek	1200	Kenosha	0.00-3.69	X	X	2016
Pike River	1300	Kenosha	1.45-9.50	X	X	2016
Pike River	1300	Kenosha	0.00-1.45	--	X	2016
Root River	2900	Waukesha, Milwaukee	25.80-43.69	X	X	2014
Root River	2900	Milwaukee, Racine	5.82-20.48	--	X	2022
South 43rd Street Ditch	15900	Milwaukee	0.00-1.16	X	X	2022
Southbranch Creek	3000073	Milwaukee	0.00-2.36	X	X	2018
South Branch of Underwood Creek	16800	Waukesha, Milwaukee	0.00-1.11	X	X	2018
Ulao Creek	21200	Ozaukee	0.0-8.6	X	X	2016
Underwood Creek	16700	Waukesha, Milwaukee	0.00-8.54	X	X	2018
Unnamed Tributary to North Branch Pike River	2450	Racine	0.00-0.58	--	X	2016

Table continued on next page.

Table 3.1 (Continued)

Name	WBIC[a]	County	Extent (River mile)[b]	Impairment		Listing Date
				Acute Toxicity	Chronic Toxicity	
Wilson Park Creek	15200	Milwaukee	0.0-3.5	X	X	2018
Zablocki Park Creek	5036633	Milwaukee	0.0-0.9	X	X	2020

[a] *The WBIC is a unique identification number for a waterbody assigned and used by the Wisconsin Department of Natural Resources.*

[b] *River mile is measured upstream from the confluence with whatever the waterbody drains into.*

Source: Wisconsin Department of Natural Resources

Table 3.2
LC50 Ranges Reported for Chloride for Zooplankton Species at Different Exposure Durations[a]

Species	LC50 (mg chloride/l)			
	24-hour	48-hour	72-hour	96-hour
Cladocerans				
Ceriodaphnia dubia	300-2,050	275-1,836	--	1,400-1,596
Ceriodaphnia sylvestrii	--	971	--	--
Daphnia ambigua	--	1,213	--	--
Daphnia hyalina	--	5,303	--	--
Daphnia carinata	--	--	--	1,062-1,400
Daphnia longispina	--	1,504-1,759	--	--
Daphnia magna	132-4,704	84-4,004	56-485	14-4,071
Daphnia pulex	1,652	1,099-1,239	--	1,470
Daphnia similis	--	328-566	--	--
Pseudosida ramosa	--	9-838	--	--
Copepods				
Cyclops abyssorum prealiinus	--	12,395	--	--
Eudiaptomus padanus padanus	--	7,092	--	--
Nitocra sinipes	--	406	--	--
Ostracods				
Cypris subglobasa	--	611-1,365	--	--
Rotifers				
Brachionus calyciflorus	804-2,220	--	--	--

Note: References for sources are given in Appendix-B.

[a] *Ranges include toxicities of chloride salts commonly used for deicing, water softening, and agriculture including sodium chloride (NaCl), calcium chloride ($CaCl_2$), potassium chloride (KCl), and magnesium chloride ($MgCl_2$).*

Source: SEWRPC

Table 3.3
LC50 Ranges Reported for Chloride for Macroinvertebrate Species at Different Exposure Durations[a]

Species	LC50 (mg chloride/l)			
	24-hour	48-hour	72-hour	96-hour
Annelida				
Lumbriculus variegatus (California blackworm)	--	--	--	3,100
Tubifex tubifex (Sludge worm)	1,441-1,928	1,077-1,567	--	778-6,008
Crustacea-Amphipoda				
Gammarus pseudolimnaeus (Scud)	--	--	--	4,670
Gammarus sobaegensis (Scud)	--	2,171-2,766	--	--
Hyalella azteca (Mexican Scud)	--	--	--	1,382-3,947
Crustacea-Branchiopoda				
Streptocephalus probocideus (Sudanese fairy shrimp)	889-3,961	--	--	--
Crustacea-Isopoda				
Lirceus fontinalis (Pillbug)	--	--	--	2,970
Insecta-Diptera				
Chironomus attenuatus (Midge)	--	--	--	4,026
Chironomus dilutus (Midge)	--	--	--	5,867
Cricotopus trifascia (Midge)	--	--	--	3,149
Culex sp. (Mosquito)	6,369	6,222	--	--
Gluptotendipes tokunagai (Midge)	--	2,355-2,690	--	--
Insecta-Ephemeroptera				
Ameletus sp. (Brown dun mayfly)	>4,853	4,222	3,118	2,505
Baetis tricaudatus (Blue-winged olive mayfly)	--	3,233-3,300	--	--
Callibaetis coloradensis (Gray quill mayfly)	--	--	--	425
Caridina dentriculata denticulata (Sawtooth caridina mayfly)	--	9,801-11,580	--	--
Centroptilum triangulifer[b] (Triangle small minnow mayfly)	--	400-931	--	1,062
Cloeon dipterum (Common wetland mayfly)	--	3,073-3,766	--	--
Ecdyonurus levis (Western ginger quill mayfly)	--	3,876-3,943	--	--
Hexagenia limbata (Giant mayfly)	--	--	--	1,456-2,822
Isonychia bicolor (Mahogany dun mayfly)	--	--	--	1,880
Neocloeon triganulifer (Triangle small minnow mayfly)	--	--	--	1,062
Stenonema rubrum (Flatheaded mayfly)	--	1,517	--	--
Insecta-Trichoptera				
Anabolia nervosa (Brown sedge caddisfly)	--	--	4,255	--
Hydropsyche betteni (Spotted sedge caddisfly)	--	--	--	8,073
Hydropsyche sp. (Caddisfly)	--	--	--	5,459
Hydroptila angusta (Varicolored microcaddisfly)	--	--	--	3,352
Lepidostoma sp. (Little brown sedge caddisfly)	--	--	--	3,640
Limnephilus stigma (Summer flier sedge caddisfly)	--	--	4,255	--
Pycnopsyche guttifer (Great autumn brown sedge caddisfly)	--	--	--	2,140
Pycnopsyche lepida (Great autumn brown sedge caddisfly)	--	--	--	2,140
Mollusca-Basommatophora				
Gyraulus parvus (Planorbid snail)	--	--	--	3,009-3,078
Lymnaea sp. (Pond snail)	923-2,865	709-2,055	484-2,113	523-1,644
Melanoides tuberculate (Red-rimmed melania snail)	--	333	--	--
Physa gyrina (Tadpole physa snail)	--	--	--	2,480
Physa heterostropha (European physa snail)	3,354	3,112	2,966	447-2,863
Mollusca-Bivalvia				
Anodonta anatina (Duck mussel)	2,505	--	--	--

Table continued on next page.

Table 3.3 (Continued)

Species	LC50 (mg chloride/l)			
	24-hour	48-hour	72-hour	96-hour
Mollusca-Bivalvia (continued)				
Corbicula fluminea (Asian clam)	--	--	--	2,162->22,581
Dreissena polymorpha (Zebra mussel)	49-66	70-71	--	--
Elliptio complanata (Eastern elliptio mussel)	1,620	1,353	--	--
Epioblasma torulosa (Northern riffle shell mussel)	244	--	--	--
Lampsilis cardium (Plain pocketbook mussel)	817	--	--	--
Lampsilis fascicola (Wavy-rayed lampmussel)	113-1,559	1,055	--	2,414
Lampsilis siliquoidea (Fat mucket mussel)	168-1,430	340-2,166	--	1,595-2,766
Ligumia recta (Black sandshell mussel)	764	2,275	--	1,523
Musculium transversum (Long fingernail clam)	--	--	--	1,930
Obliquaria reflexa (Threehorned wartyback mussel)	--	>972	--	--
Ptychobranchus fasciolaris (Kidneyshell mussel)	3,416	--	--	--
Sphaerium simile (Fingernail clam)	--	--	--	740-1,100
Villosa constricta (Notched rainbow mussel)	1,674	1,574	--	--
Villosa delumbis (Eastern creekshell mussel)	2,008	2,202	--	3,173
Nematoda				
Caenorhabditis elegans (Round worm)	28,367	--	--	--

Note: References for sources are given in Appendix-B.

[a] *Ranges include toxicities of chloride salts commonly used for deicing, water softening, and agriculture including sodium chloride (NaCl), calcium chloride ($CaCl_2$), potassium chloride (KCl), and magnesium chloride ($MgCl_2$).*

[b] *This taxon is also referred to as* Neocloeon triangulifer *in older literature.*

Source: SEWRPC

Table 3.4
LC50 Ranges Reported for Chloride for Fish Species at Different Exposure Durations[a]

Species	LC50 (mg chloride/l)			
	24-hour	48-hour	72-hour	96-hour
Anguilla rostrata (American eel)	--	--	--	10,900-13,085
Carassius auratus (Goldfish)	8,341	--	--	4,453
Catla catla (Major carp)	4,550	--	--	3,021
Cirrhinius mrigalo (Mrigal carp)	4,550	--	--	3,021
Gambusia affins (Mosquito fish)	4,745-13,931	1,993-13,189	--	437-12,260
Ictalurus punctatus (Channel catfish)	3,489	342	--	--
Labeo rohita (Rohu carp)	163-4,550	38-6,524	27-6,448	19-6,370
Lepomis macrochirus (Bluegill)	2,615-8,568	--	--	956-7,864
Molliensia latipinna (Sailfin mollie)	--	10,067	--	--
Notemigonus crysolucas (Golden shiners)	388	--	--	--
Oncorhynchus mykiss (Rainbow trout)	566-3,334	766	--	6,030-12,371
Pimephales promelas (Fathead minnow)	452-5,023	433-4,665	4,640	418-6,570
Stizostedion canadense (Sanger)	238	--	--	--
Stizostedion vitreum (Walleye)	344	--	--	--

Note: References for sources are given in Appendix-B.

[a] *Ranges include toxicities of chloride salts commonly used for deicing, water softening, and agriculture including sodium chloride (NaCl), calcium chloride ($CaCl_2$), potassium chloride (KCl), and magnesium chloride ($MgCl_2$).*

Source: SEWRPC

Table 3.5
LC50 Ranges Reported for Chloride for Amphibian Species at Different Exposure Durations[a]

	LC50 (mg chloride/l)[b]			
Species	24-hour	48-hour	72-hour	96-hour
Ambystoma maculatum (Spotted salamander)	--	--	--	1,178
Bufo americanus (American toad)	--	--	--	3,928
Bufo boreas (Boreal toad)	3,271	3,271	--	483
Eurycea bislineata (Northern two-lined salamander)	--	--	--	5,505
Lithobates sylvatica (Wood frog)[b,c]	248-5,532	147-5,421	112-5,392	86-5,295
Microhyla ornata (Ornate narrow-mouthed frog)	2,378-3,932	--	--	672-4,203
Pseudacris crucifer (Spring peeper)	--	--	--	2,830
Rana clamitans (Green frog)	--	--	--	2,421-3,109

Note: References for sources are given in Appendix-B.

[a] *Ranges include toxicities of chloride salts commonly used for deicing, water softening, and agriculture including sodium chloride (NaCl), calcium chloride ($CaCl_2$), potassium chloride (KCl), and magnesium chloride ($MgCl_2$).*

[b] *The wide range of some LC50s may reflect studies using different life stages of the species or tests conducted under different conditions.*

[c] *This species is also referred to as* Rana sylvatica *in some older literature.*

Source: SEWRPC

Table 3.6
LC50s for Four Mayfly Species Exposed to Sodium Chloride for 96 hours at Different Water Temperatures

Temperature (°F)	Triangle Small Minnow Mayfly (*Neocloeon triangulifer*) (mg Cl^-/l)	Fragile Small Minnow Mayfly (*Procloeon fragile*) (mg Cl^-/l)	Cream Cahill Mayfly (*Maccaffertium modestum*) (mg Cl^-/l)	Early Brown Spinner Mayfly (*Leptophlebia cupida*) (mg Cl^-/l)
41.0	9,655	--	10,086	--
45.5	10,462	--	10,152	7,236
50.0	6,719	6,874	10,439	5,429
54.5	5,101	4,115	11,908	7,792
59.0	2,573	3,239	8,368	7,808
68.0	2,755	767	4,588	2,760
77.0	364	766	3,216	1,656

Source: J.K. Jackson and D.H. Funk, "Temperature Affects Acute Mayfly Responses to Elevated Salinity: Implication for Toxicity of Road De-icing Salts," Philosophical Transactions of the Royal Society, Series B, *374:20150081, doi: 10.1098/rstb.2018.0081, 2018*

Table 3.7
Acute Toxicity of Sodium Chloride (NaCl) to the Water Flea *Ceriodaphnia dubia* Exposed for 48 Hours at Different Levels of Water Hardness

Average Hardness (mg/l as $CaCO_3$)	Hardness Range (mg/l as $CaCO_3$)	Average Calcium Concentration (mg/l)	Average Magnesium Concentration (mg/l)	Average LC50 (mg/l Chloride)
28	25-30	5.3	2.3	977
47	44-49	10.8	4.8	861
96	95-96	20.9	9.3	1,249
187	180-194	42.0	18.7	1,402
388	375-400	81.8	36.9	1,589
565	560-570	123.0	56.1	1,779
796	792-800	170.5	77.3	1,836

Source: D.J. Soucek, T.K. Linton, C.D. Tarr, A Dickinson, N. Wickramanayake, C.G. Delos, and, L.A. Cruz, "Influence of Water Hardness and Sulfate on the Acute Toxicity of Chloride to Sensitive Freshwater Invertebrates," Environmental Toxicology and Chemistry, *30:930-938, 2011*

#264748 – TR-62 (Chloride Study Impacts) Table 3.8
200-1100
JEB/mid
9/7/22, 12/4/23

Table 3.8
Acute Toxicity of Sodium Chloride (NaCl) to the Water Flea *Ceriodaphnia dubia* Exposed for 48 Hours at Different Concentrations of Sulfate (SO_4^{2-})

Average Sulfate Concentration (mg/l)	Range of Sulfate Concentrations (mg/l)	Average LC50 (mg/l Chloride)
26	23-28	1,356
55	50-60	1,489
112	107-117	1,317
234	229-239	1,357
472	461-482	1,154
712	694-729	1,192

Source: D.J. Soucek, T.K. Linton, C.D. Tarr, A Dickinson, N. Wickramanayake, C.G. Delos, and, L.A. Cruz, "Influence of Water Hardness and Sulfate on the Acute Toxicity of Chloride to Sensitive Freshwater Invertebrates," Environmental Toxicology and Chemistry, *30:930-938, 2011*

Table 3.9
Acute Toxicity of Three Chloride Salts to the Asian Clam *Corbicula fluminea* Exposed for 192 Hours

Chloride Salt	LC50 (mg Cl^-/l)
Sodium chloride ($NaCl$)	10,069
Calcium chloride ($CaCl_2$)	2,235
Magnesium chloride ($MgCl_2$)	1,769

*Source: K.D. Coldsnow and R. Relyea, "Toxicity of Various Road-Deicing Salts to Asian Clams (*Corbicula fluminea*), *Environmental Toxicology*, doi.org/10.1002.etc.4126, 2018*

#262874 – TR-62 (Chloride Study Impacts) Table 3.10
200-1100
JEB/mid
5/11/22, 12/4/23

Table 3.10
Acute Toxicity of Four Chloride Salts to the Water Flea *Daphnia magna* Exposed for 100 Hours

Chloride Salt	LC50 (mg Cl^-/l)
Magnesium chloride ($MgCl_2$)	2,595
Sodium chloride (NaCl)	1,889
Calcium chloride ($CaCl_2$)	415
Potassium chloride (KCl)	323

Source: B.F. Dowden and H.J. Bennett, "Toxicity of Selected Chemicals to Certain Animals," Journal of the Water Pollution Control Federation, *37:1,308-1,326, 1965*

Table 3.11
Acute Toxicity of Three Chloride Salts to the Diatom *Nitzschia linearis* Exposed for 120 Hours

Chloride Salt	LC50 (mg Cl^-/l)
Calcium chloride ($CaCl_2$)	2,000
Sodium chloride (NaCl)	1,474
Potassium chloride (KCl)	701

Source: R. Patrick, J.J. Cairns, and A. Scheier, "The Relative Sensitivity of Diatoms, Snails, and Fish to Twenty Common Constituents of Industrial Wastes," The Progressive Fish-Culturist, *30:137-140, 1968*

#265056 – TR-62 (Chloride Study Impacts) Table 3.12
200-1100
JEB/LKH/mid
9/29/22, 8/7/23, 12/4/23

Table 3.12
Wisconsin's Water Quality Criteria for Free Cyanide (HCN and CN^-)

Criteria	Coldwater Systems	All Others
Acute Toxicity[a] (µg/l)	22.40	45.80
Chronic Toxicity[b] (µg/l)	5.22	11.47

[a] *The maximum daily concentrations of free cyanide is not to exceed the acute toxicity criterion more than once every three years.*

[b] *The four-day maximum concentration of free cyanide is not to exceed the chronic toxicity criterion more than once every three years.*

Source: Wisconsin Department of Natural Resources

Table 3.13
Acute Toxicity of Sodium Chloride (NaCl) to Wood Frog (*Lithobates sylvatica)* Tadpoles Exposed for 72 Hours

Gosner Stage[a]	LC50 (mg Cl^-/l)
Stage 19	112
Stage 22	116
Stage 26	472
Stage 29	1,558
Stage 33	1,812

[a] *Gosner stages are stages in tadpole development. Higher numbered stages occur later in development.*

Source: A.L. Copan, Acute Toxicity of Deicing Compounds and Personal Care Products to Early Amphibian Life Stages *Master's Thesis, Saint Mary's University, Halifax, Nova Scotia, Canada, 2016.*

#262877 – TR-62 (Chloride Study Impacts) Table 3.14
200-1100
JEB/mid
5/11/22, 12/4/23

Table 3.14
Acute Toxicity of Calcium Chloride ($CaCl_2$) to Rohu Carp (*Labeo rohita)* Life Stages Exposed for 96 Hours

Life Stage	LC50 (mg Cl^-/l)
Eggs	19
Spawn	804
Fry	4,072
Fingerlings	6,300

*Source: A. Mallick, B.C. Mohapatra, and N. Sarangi, "Acute Toxicity of Calcium Chloride on Different Stages (Egg, Spawn, Fry and Fingerling) of Rohu (*Labeo rohita, *Hamilton)*," Research Journal of Animal, Veterinary and Fishery Science, *2:11-16, 2014*

Table 3.15
Acute Toxicity of Sodium Chloride (NaCl) to Different Clones of the Water Flea *Daphnia longispina* Exposed for 48 Hours

Clone	LC50 (mg Cl^-/l)
N116	1,729
E89	1,711
N91	1,698
E99	1,517
N31	1,504

Source: J. Leitao, R. Ribeiro, A.M.V.M. Soares, and I. Lopes, "Tolerance to Copper and Salinity in Daphnia Longispina*: Implications within a Climate Change Scenario,"* PLoS One, *8:e68702, 2013*

#265761 – TR-62 (Chloride Study Impacts) Table 3.16
200-1100
JEB/mid
11/14/22, 12/4/23

Table 3.16
Percentage of Atlantic Salmon Alevins Showing Deformities at Different Sodium Chloride Concentrations

Sodium Chloride Concentration (mg/l)	Percent of Eggs Showing Deformities
0	0
100	10
500	15
1,000	27

*Source: U. Mahrosh, M. Kleiven, S. Meland, B.O. Rosseland, B. Salbu, and H.-C. Teien, "Single and Multiple Stressor Effect of Road Deicing Salt (NaCl) and Copper (Cu) to Fertilization and Early Development Stages of Atlantic Salmon (*Salmo salar*) Alevins from Hatching to Swim-up,"* Journal of Environmental Sciences*, 66:368-378, 2018*

Table 3.17
Some Chloride Concentration Thresholds for Changes in Biological Communities

Chloride Concentration (mg/l)	Reported Impact	References
5-40	Decreased reproduction and increased mortality in six *Daphnia* Species	Arnott et al., 2020, *Environmental Science and Technology*, 54:9,398-9,407.
16	Reduced bacteria density in biofilms	Cochero et al., 2017, *Science of the Total Environment*, 579:1,496-1,503.
33-108	Reductions in fish diversity	Morgan et al., 2012, *North American Journal of Fisheries Management*, 32:941-952.
35	Substantial changes in composition of periphytic diatom assemblages	Porter-Goff et al., 2013, *Ecological Indicators*, 32:97-106
54	Reductions in wetland plant species richness	Richburg et al., 2001, *Wetlands*, 21:247-255.
100	Decrease in photosynthetic production in common waterweed	Zimmerman-Timm, 2007, In: Lozar, et al., *Water Uses and Human Impacts on the Water Budget*
185	Substantial shift in phytoplankton community composition and reduction in ciliates	Astorg et al., 2023, *Limnology and Oceanography Letters*, 8:38-47.
250	Reductions in zooplankton abundance and diversity	Sinclair and Arnott, 2018, *Freshwater Biology* 63:1,273-1,286.
250-260	Wood frogs and spring peepers stop using ponds for breeding	Sadowski, 2002, *Prairie Perspectives*, 5:144-162; Gallagher et al., 2014, *Wetlands Ecology and Management*, 22:551-564
2,000	Inhibition of denitrification in forested wetlands	Lancaster et al., 2016, *Environmental Pollution*

Source: SEWRPC

#267561 – TR-62 (Chloride Study Impacts) Table 3.18
200-1100
JEB/mid
3/20/23, 12/4/23

Table 3.18
Reductions in Zooplankton Abundance Relative to the USEPA Recommended Criterion Continuous Maximum Concentration

Zooplankton Group	Percent of Sites Showing 50 Percent Reductions at Chloride Concentrations Below 230 mg/l	Range of Reductions Observed at a Chloride Concentration of 230 mg/l (percent)
Cladocera	86	22-83
Calanoid copepods	90	15-96
Cyclopoid copepods	60	13-96
Rotifers	82	10-100

Source: W.D. Hintz et al., "Current Water Quality Guidelines Across North America Do Not Protect Lakes from Salinization," Proceedings of the National Academy of Sciences,*" 119:e2115033119, 2022*

SEWRPC Technical Report No. 62

IMPACTS OF CHLORIDE ON THE NATURAL AND BUILT ENVIRONMENT

Chapter 3

IMPACTS OF CHLORIDE ON BIOLOGICAL SYSTEMS

FIGURES

Figure 3.1
Aquatic Organisms Used in Acute Toxicity Testing

Water Flea (*Ceriodaphnia dubia*)

Water Flea (*Daphnia magna*)

Sludge Worm (*Tubifex tubifex*)

Scud (*Hyalella azteca*)

Fathead Minnow (*Pimephales promelas*)

Wood Frog Tadpole (*Lithobates sylvatica*)

Source: Wikimedia Commons

Figure 3.2
Examples of Algae Found in Freshwater

Several Species of Diatoms

Credit: Flickr User Klaus Kemp

Cyanobacteria (*Anabaena* sp.)

Credit: Flickr User Hakan Kvarnstrom

Green algae (*Scenedesmus* sp.)

Credit: Flickr User Don Loarie

Green algae (*Nitella* sp.)

Credit: Wikimedia Commons User Andre Hosp

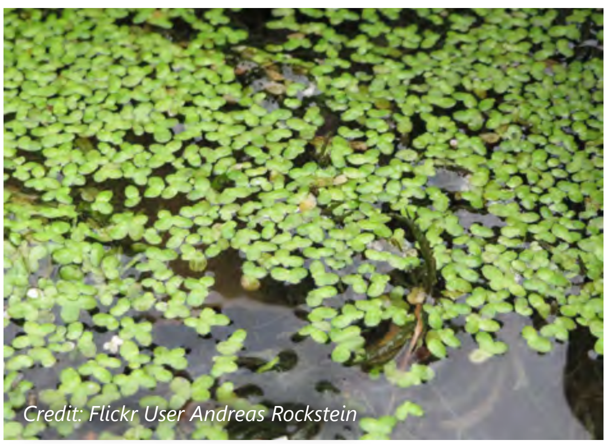
Source: Flicker and Wikimedia Commons

Figure 3.3
Examples of Aquatic Macrophytes

Common Waterweed (*Elodea canadensis*)

Coontail (*Ceratophyllum demersum*)

Duckweed (*Lemna minor*)

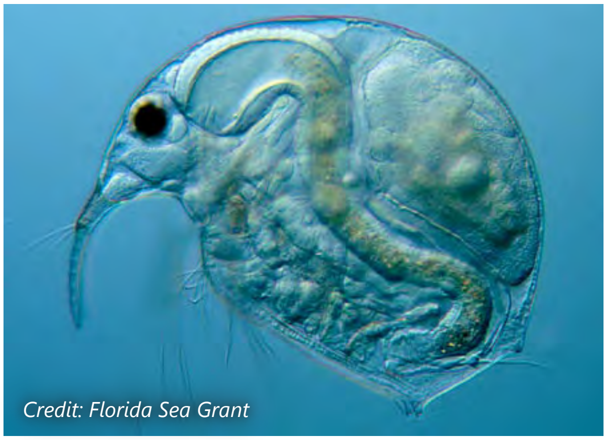


Long-Leaf Pondweed (*Potamogeton nodosus*)

Source: Flicker and Wikimedia Commons

Figure 3.4
Examples of Freshwater Zooplankton

Cladoceran (*Bosmina* sp.)



Credit: Florida Sea Grant

Copepod (*Cyclopse* sp.)

Credit: Wikimedia Commons User Marek Mir

Ostracod (unknown species)

Credit: Flickr User Peter Maguire

Rotifer (*Brachionus calyciflorus*)

Credit: Flickr User Jurgen Dendorfer

Source: Florida Sea Grant, Flickr, and Wikimedia Commons

Figure 3.5
Examples of Aquatic Macroinvertebrates

Fingernail Clam (*Musculium transversum*)

Credit: Flickr User Roger Boyd

Mayfly Larva (*Isonychia* sp.)

Credit: Flickr User Bob Henricks

Isopod (*Lirceus* sp.)

Credit: Flickr User Andrew Hoffman

Mosquito Larva (*Aedes aegypti*)

Credit: Flickr User Cesar Favacho

Backswimmer (*Notonecta glauca*)

Credit: Flickr User Andrew Hoffman

Eastern Elliptio Mussel (*Elliptio complanata*)

Credit: U.S. Fish and Wildlife Service

Source: Flickr and U.S. Fish and Wildlife Service

Figure 3.6
Examples of Fish Reported to be Impacted by Chloride Salts

Common Carp (*Cyprinus carpio*)

Credit: Flickr User Fred Salamin

Least Darter (*Ethostoma microperca*)

Credit: John Lyons, WDNR

Northern Redbelly Dace (*Chrosomus eos*)

Credit: Flickr User ftfriley

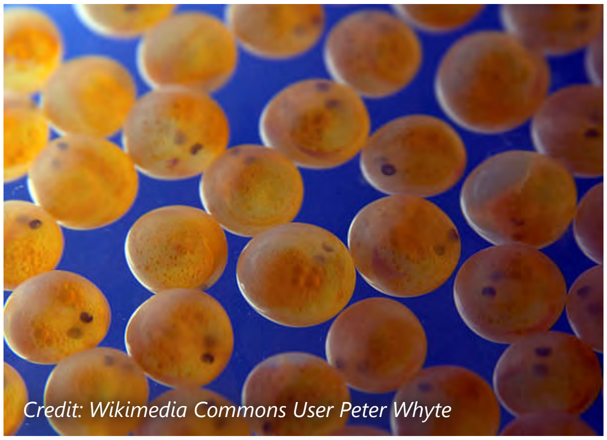
Rainbow Trout (*Oncorhynchus mykiss*)

Credit: Flickr User Keith Bielat

Source: Flickr and WDNR

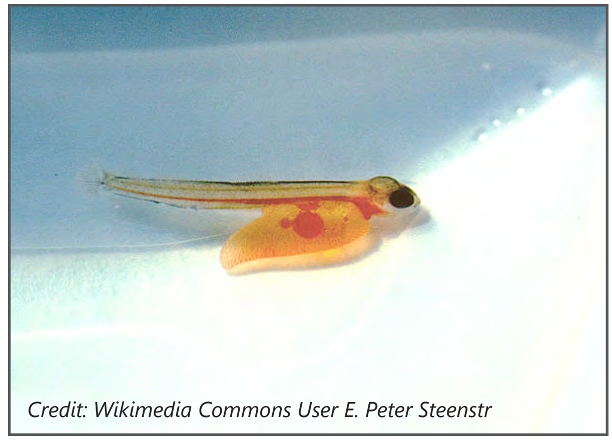
Figure 3.7
Some Early Life Stages of Fish

Salmon Eggs



Credit: Wikimedia Commons User Peter Whyte

Salmon Alevins (Sac Fry)

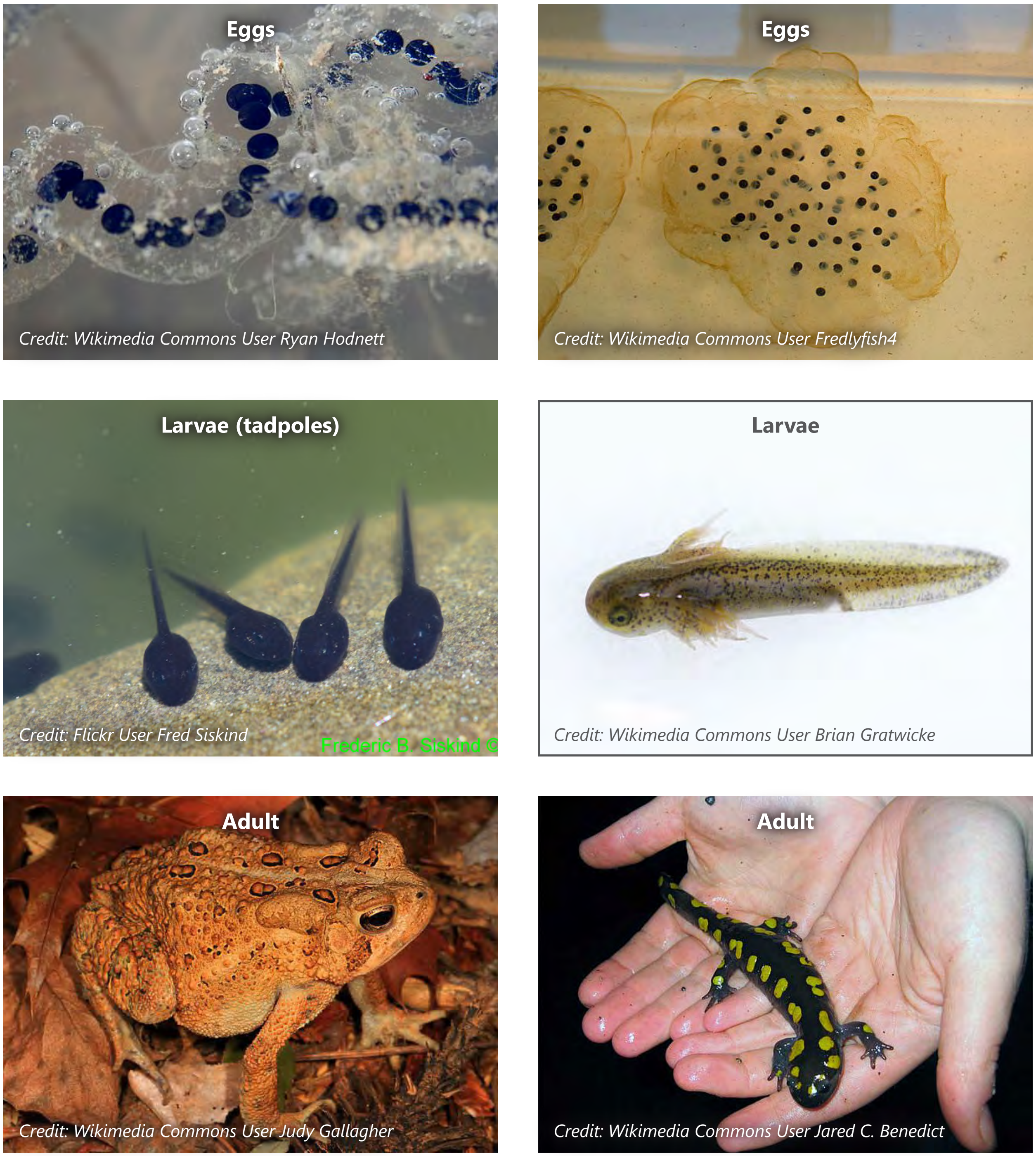


Credit: Wikimedia Commons User E. Peter Steenstr

Source: Wikimedia Commons

Figure 3.8
Life Stages of Two Amphibian Species

American Toad (*Bufo americanus*)	Spotted Salamander (*Ambystoma maculatum*)



Source: Flickr and Wikimedia Commons

Figure 3.9
Some Wisconsin Amphibians

Wood Frog (*Lithobates sylvatica*)

Credit: Wikimedia Commons User Warren Bielenberg

Blue-Spotted Salamander (*Ambystoma laterale*)

Credit: Wikimedia Commons User Greg Schechter

Spring Peeper (*Lithobates crucifer*)

Credit: Wikimedia Commons User Peter Paplanus

Source: Wikimedia Commons

Figure 3.10
Some Wisconsin Turtles

Snapping Turtle (*Chelydra serpentina*)

Credit: Joseph Boxhorn, SEWRPC

Common Musk Turtle (*Sternotherus odoratus*)

Credit: Wikimedia Commons User Ontley

Blanding's Turtle (*Emydoidea blandingii*)

Credit: Gregor Schuurman, WDNR

Painted Turtle (*Chrysemys picta*)

Credit: Joseph Boxhorn, SEWRPC

Source: Wikimedia Commons, WDNR, and SEWRPC

Figure 3.11
Examples of Plant Damage from Chloride Salts in Soil

Chlorosis on Raspberry Leaf	Elm Leaves Showing Necrosis

Source: Wikimedia Commons

Figure: 3.12
Bird Nest Fern (*Asplenium nidus*)
Showing Salt Damage

Source: Wikimedia User Toyoba2

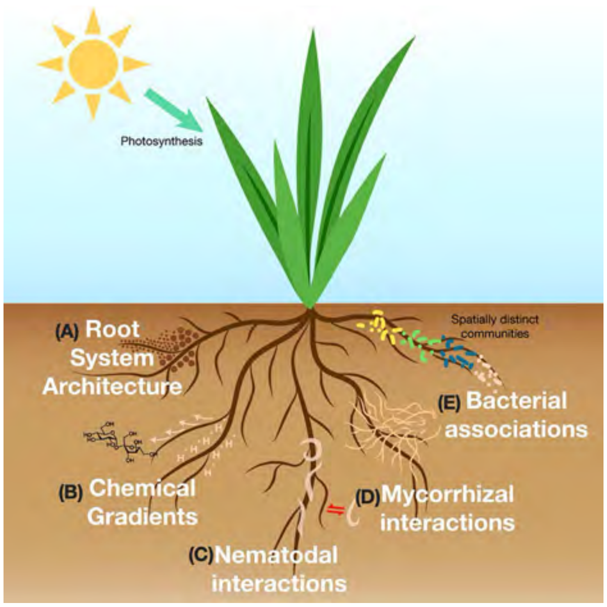
Figure: 3.13
Arbor Vitae Trees Damaged by Salt in Snow Piles

Source: Laura Herrick, SEWRPC

Figure: 3.14
Pine Needles Showing Salt Damage

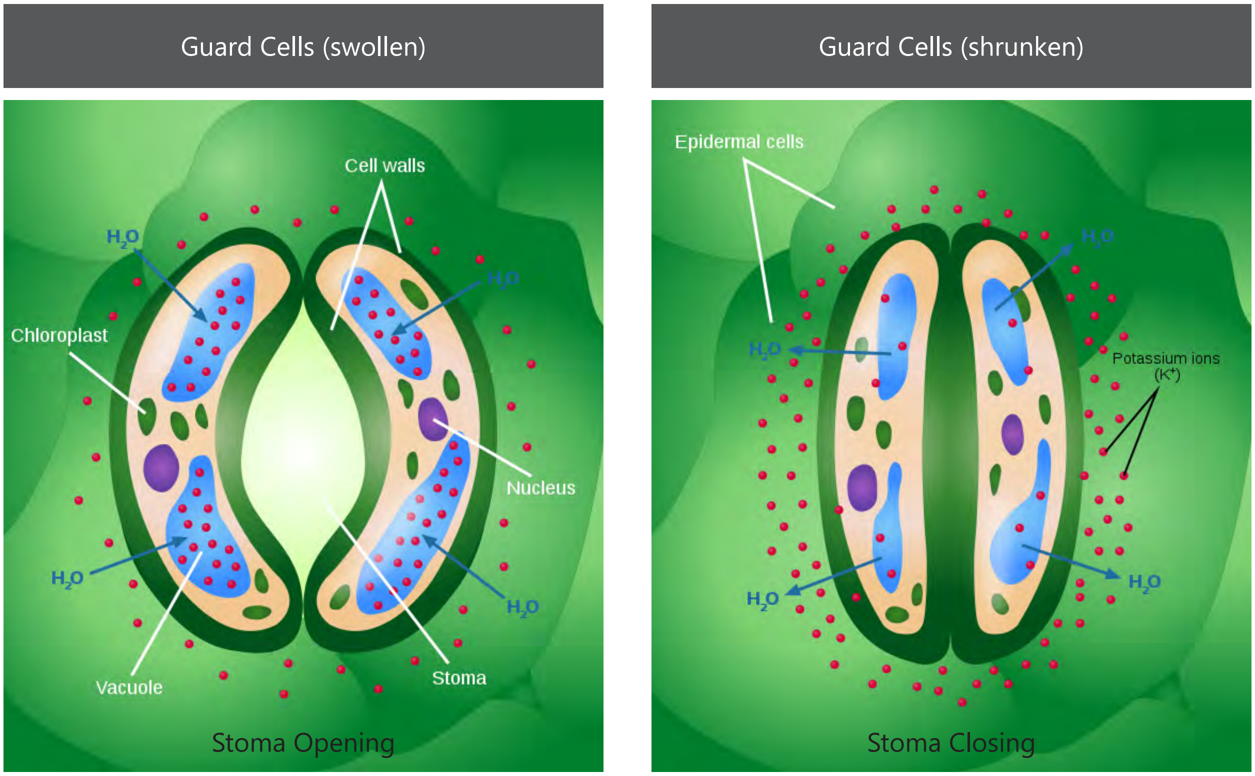
Source: Flickr User Mary Lou Fairweather

Figure: 3.15
Plant Rhizosphere



Source: Wikimedia Commons User M. O. Yee

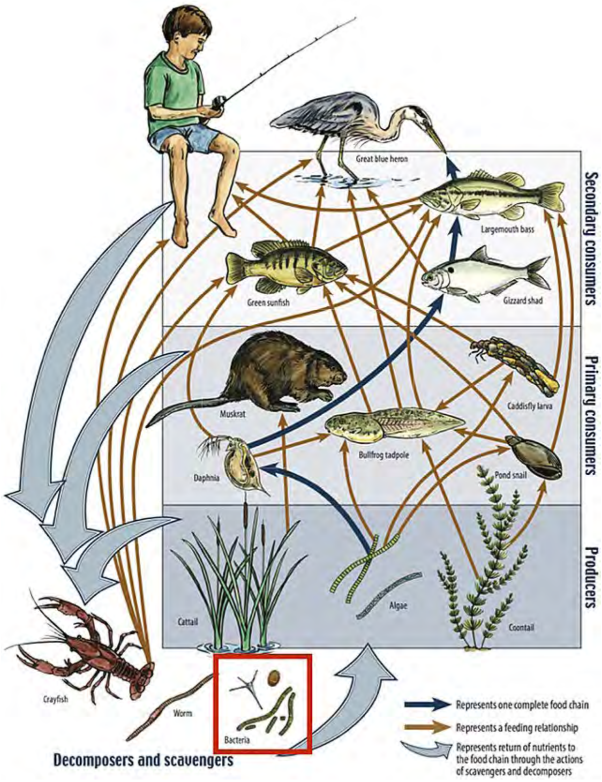
Figure: 3.16
Stomates Opening and Closing on Plant Leaves



Water availability in leaves open and close stomates. When water availability is high, water flows into adjacent cells, opening the stoma pore. When water is less availble, water flows out of the adjacent cells, closing the pore.

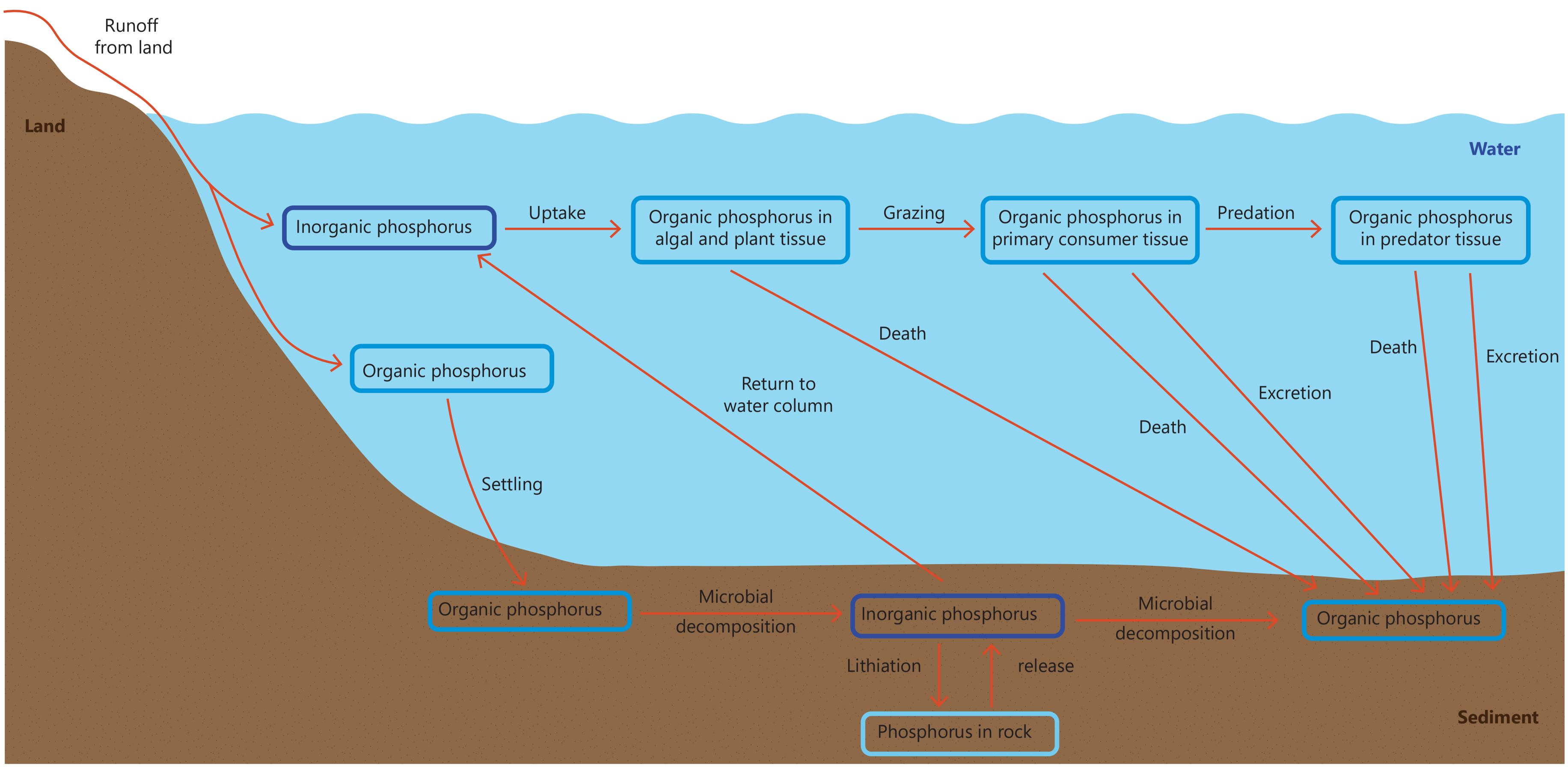
Source: Wikimedia User Ali Zifan

Figure: 3.17
A Simplified Aquatic Food Web



Source: Missouri Department of Conservation

Figure 3.18
Ecosystem Movement of Phosphorus Through a Pond Ecosystem



Source: SEWRPC

SEWRPC Technical Report No. 62

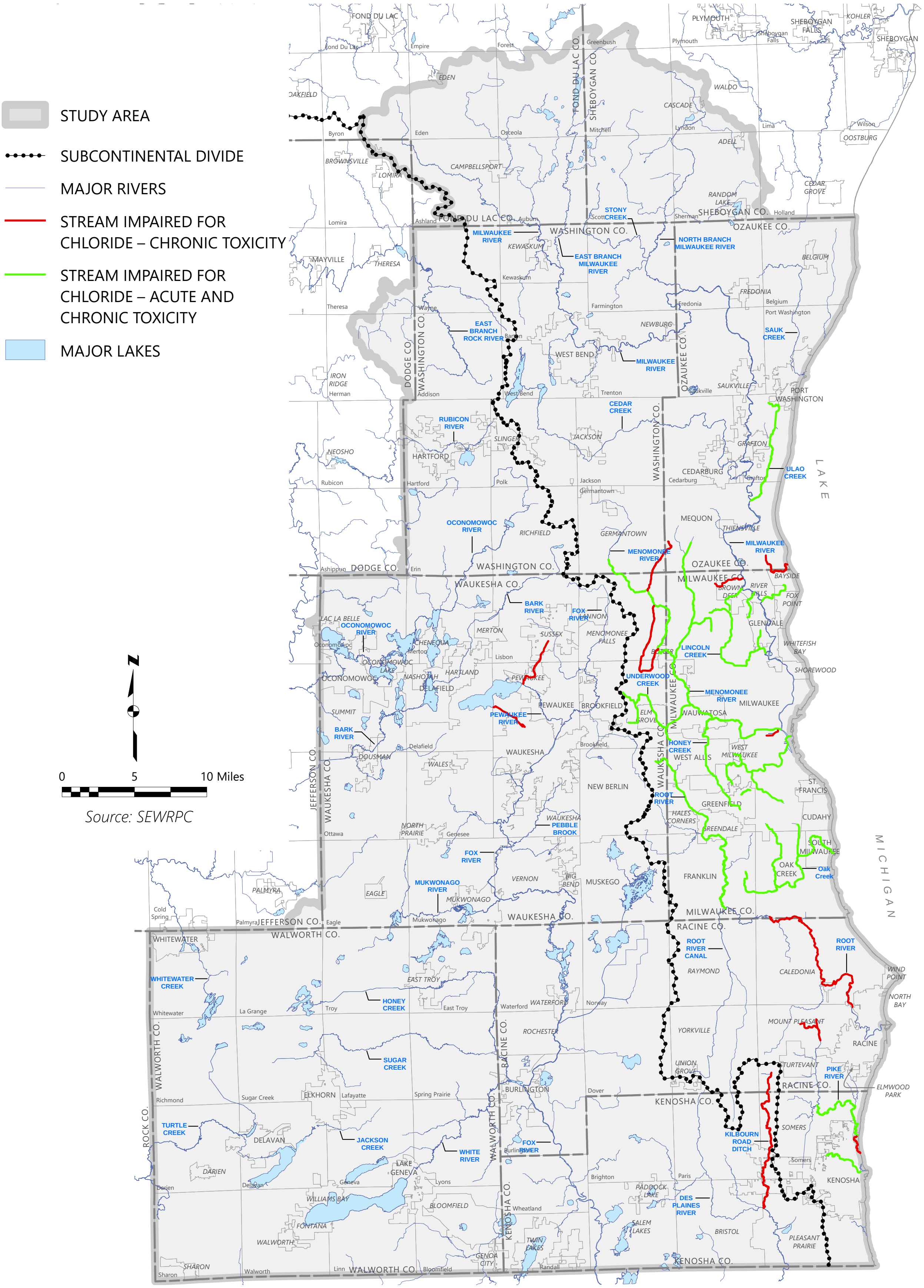
IMPACTS OF CHLORIDE ON THE NATURAL AND BUILT ENVIRONMENT

Chapter 3

IMPACTS OF CHLORIDE ON BIOLOGICAL SYSTEMS

MAPS

Map 3.1
Waterbodies Impaired for Chloride: 2022



Source: SEWRPC