Equilibrium Environmental Inc.

SCOPE OF WORK

FRESH WATER AQUATIC LIFE WORKING GROUP

DEVELOPMENT OF A CHLORIDE WATER QUALITY GUIDELINE BASED ON HARDNESS AND ALBERTA *PELECYPODA* SPECIES TOXICOLOGICAL RESPONSE

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1. SCOPE OF RESEARCH

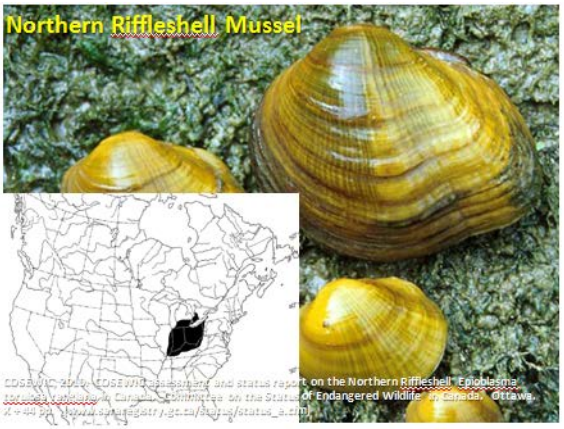
1.1 Background

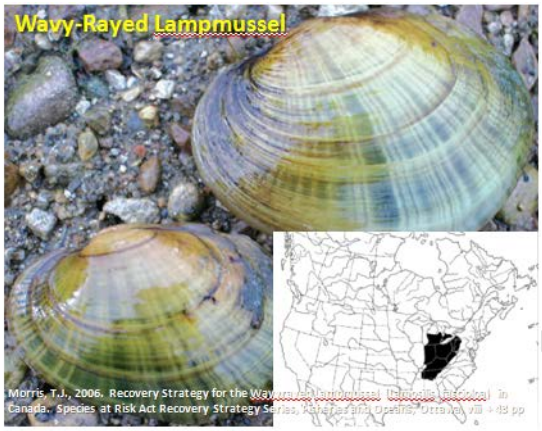
The Canadian Council for Ministers of the Environment (CCME) has recently revised the Water Quality Guideline for chloride (from 230 to 120 mg/L for chronic exposure), applicable to freshwater aquatic life receptors where COSEWIC (Committee on the Status of Endangered Wildlife in Canada) Endangered or Special Concern *Pelecypoda* (clams, mussels) are not present (CCME 2011; *Canadian Water Quality Guidelines – Chloride Ion. Scientific Criteria Document. Canadian Council for Ministers of the Environment, Winnipeg. PN 1460, ISBN 978-1-896997-77-3 PDF*). Toxicity testing data demonstrate that *Pelecypoda* can be sensitive to the adverse effects associated with chloride exposure.

Although *Pelecypoda* are sensitive to chloride toxicity, there is mentionable variability in toxic response, which can vary by more than an order of magnitude between species and by more than four-fold within species and may depend on the water body and associated environmental variables from which the clams or mussels were collected (Gilles 2011[1]). For example, *Lampsilis siliquoidea* collected from Cox Creek produced a chloride Effect Concentration 50% (EC_{50}) of 168 (135 - 198) mg/L for the endpoint of glochidia (larval stage for specific mussel species) survival, whereas the same species collected from the Maitland River produced a chloride EC_{50} of 1,430 (1,350 – 2,953) mg/L for the same endpoint, which is nearly an order of magnitude difference in toxic response for the same species sourced from different water courses. Water hardness was similar as the studies were conducted in reconstituted moderately-hard water (95 – 115 mg/L of $CaCO_3$).

Two of the more sensitive *Pelecypoda* species were classified as COSEWIC Special Concern and Endangered), and had a relatively strong influence on the chloride Species Sensitivity Distribution (SSD) and resulting chloride water quality guideline. The two species are shown below (*Epioblasma torulosa rangiana* or Northern Riffleshell, and *Lampsilis fascioloa* or Wavy-rayed lampmussel). These species are encountered within a localized area of Canada, essentially within the Ontario Great Lakes region (shown in the map inserts below, black marked area of Canada and the United States).

[1] Gilles, P.L. 2011; *Assessing the toxicity of sodium chloride to the glochidia of freshwater mussels: Implications for salinization of surface waters, Environmental Pollution (2011), doi:10.1016/ j.envpol.2011.02.032*





COSEWIC Endangered: Northern Riffleshell (Epioblasma torulosa rangiana).

COSEWIC Special Concern: Wavy-rayed Lampmussel (Lampsilis fascioloa).

The CCME (2011) stated it was aware of potential issues associated the application of mussel toxicity data for an Endangered mussel species and a Special Concern mussel species, which appear to be highly sensitive to salt toxicity. CCME debated whether the two Ontario mussel species should be included in a national dataset, and in the end decided to include the indigenous Great Lakes mussels as they may act as surrogates for other un-tested sensitive species that are found across Canada. They further state that the guideline can be re-run without the Great Lake mussels providing it can be verified that other equally sensitive species do not exist in the water body of concern.

While the COSEWIC Endangered Northern Riffleshell is not located in Alberta or any species within the same genera (Clifford, 2012[2]; Clarke, 1973[3]*)* the *Lampsilis* genera of the Family *Unionidae* is encountered in Alberta. A number of other *Pelecypoda* species are present in Alberta, for which there are no available toxicity information in relation to chloride exposure.

Given the option by CCME (2011) to re-run a guideline without the Great Lake mussels, providing it can be verified that other equally sensitive species do not exist in the water body of concern, and given there are species in Alberta for which no toxicity testing data exists, it is proposed that work by initiated to assess the toxicity of chloride towards Alberta species of *Pelecypoda*. This work could be used to confirm or disaffirm whether there are species in Alberta that are of equivalent sensitivity to chloride as the Special Concern and Endangered Ontario Great Lake mussel species.

[2] *Clifford, H.F. 2012. Aquatic Invertebrates of Alberta. Pelecypoda (Clams). Department of Biological Sciences, University of Alberta. http://sunsite.ualberta.ca/Projects/Aquatic_Invertebrates/?Page=20*

[3] *Clarke, A.H. 1973. The freshwater molluscs of the Canadian Interior Basin. Malacologia* ***13****(1-2): 1-509*

Furthermore, CCME (2011) indicated there was a need for the generation of more toxicity data incorporating aspects of hardness. Given that Alberta surface water bodies in general have relatively harder water compared to many parts of Canada, a second component of proposed work is to provide further research into the relationship between hardness and chloride toxicity towards *Pelecypoda*.

1.2 Unionidae Family of Clams and Mussels in Alberta

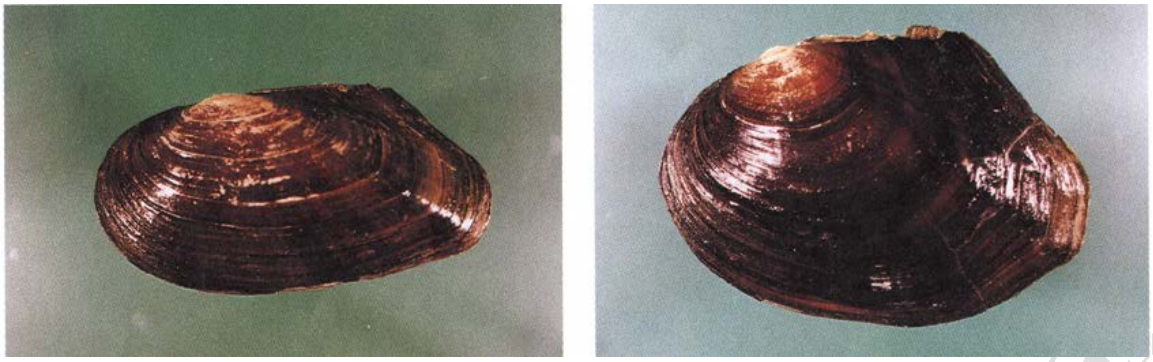
As summarized by Clifford (2012) and Clarke (1973), there are two families of clams and mussels in Alberta:

1) *Unionidae* (large clams or mussels); and,
2) *Sphaeriidae* (fingernail clams).

Within the *Unionidae* Family, there are three genera (*Anodonta, Lampsilis,* and, *Lasmigona*) comprised of five species in Alberta (bolded species are those that had relevant toxicity testing data considered by CCME):

- *Anodonta grandis* (including *A. g. simpsoniana*)
- *Anodonta kennerlyi*
- ***Lampsilis radiata siliquoidea***
- *Lasmigona complanata*
- *Lasmigona compressa*

Lampsilis radiata



left to right: Lampsilis compressa; Lampsilis complanata

These species produce glochidia, which can be a sensitive lifestage of exposure to contaminants (CCME, 2011). Glochidia are a larval stage of development and are released in a cloud formation from an adult when a fish host has been lured into close proximity. The glochidia attached to the host and go through a parasitic phase during which organ development occurs, and after which they detach from their host and settle into their new environment as juveniles (Clarke, 1973).

Toxicity testing data were available for one of the Alberta *Unionidae* species listed above (*Lampsilis siliquoidea* (Fatmucket mussel)), which were included by CCME in the development of a Canada-wide chloride water quality guideline. This species is considered COSEWIC Currently Stable and not of Special Concern or Endangered. An Effect Concentration 10% (EC_{10}) of 1,474 mg/L for the endpoint of glochidia survival was developed by CCME (2011) for this species based on the data of Bringolf *et al.* (2007).

Toxicity data were also available for two other species within the same genera (*Lampsilis fasciola* (Wavy-Rayed lampmussel, the Great Lake Special Concern mussel species) and *Lampsilis cardium* (Plain pocketbook)) – neither of these species has been identified in Alberta. These two species in addition to *Lampsilis siliquiodea* are mantle lure spawners that produce glochidia (CCME, 2011). Toxicity data for Plain pocketbook was not used in the development of a chronic chloride limit, although data for the Wavy-Rayed Lampmussel was utilized. A 24 hour EC_{10} of 24 mg/L for the endpoint of glochidia (larval life stage) survival was developed by the CCME based on the study of Bringolf *et al.* (2007).

Although of a different genera, the Endangered Ontario Great Lake mussel species *Epioblasma torulosa rangiana* (Northern Riffleshell) produces glochidia, and it was a sensitive species in terms of chloride exposure. An EC_{10} of 42 mg/L for the glochidia survival endpoint was derived from the data of Gillis (2010) for the Northern Riffleshell (*Epioblasma torulosa rangiana*) and used by CCME (2011). This species is similarly not encountered in Alberta.

1.3 Sphaeriidae Family of Clams

Within the *Sphaeriidae* Family, there are two genera (*Pisidium* and *Sphaerium*) comprised of approximately 20 species in Alberta (bolded species are those that had relevant toxicity testing data considered by CCME):

- *Pisidium casertanum*
- *Pisidium compressum*
- *Pisidium conventus*
- *Pisidium fallax*
- *Pisidium ferrugineum*
- *Pisidium idahoense*
- *Pisidium lilljeborgi*
- *Pisidium milium*
- *Pisidium nitidum*
- *Pisidium punctatum*
- *Pisidium rotundatum*
- *Pisidium subtruncatum*
- *Pisidium variabile*
- *Pisidium ventricosum*
- *Pisidium walkeri*
- *Sphaerium lacustre*
- *Sphaerium nitidum*
- *Sphaerium rhomboideum*
- ***Sphaerium (Musculium) securis***
- *Sphaerium striatinum*
- *Sphaerium transversum*

Left to Right: Sphaerium (Sphaeriidae); Pisidium (Sphaeriidae)

One the species above (*Sphaerium securis or Musculium secures* (Fingernail clam)) had available toxicity testing data that was incorporated by CCME into the development of a Canada-wide chloride water quality guideline. The species of this Family do not produce glochidia and instead the juveniles develop within the gills of adults. The key difference between genera in the Family is based on the number of different life stages contained at any one time on the adult gills. The toxicological endpoint considered by the CCME for the species *Sphaerium securis* was reduced natality. A 60 to 80 day LOEC of 121 mg/L was derived, although Mackie (1978)[4] produced data that could potentially be used to derive an EC_{10}. This species is not considered Special Concern or Endangered.

1.3 Influence of Water Hardness

Hardness has been identified as an important variable regarding the toxicity of chloride towards aquatic organisms including *Pelecypoda* (CCME, 2011; Gillis, 2011). For example, Gillis (2011) studied the effects of water hardness on *Lampsilis siliquoidea* (Fatmucket mussel), a species found in Alberta. The 24 hour EC_{50} values in soft (47 mg/L $CaCO_3$), moderately hard (99 mg/L $CaCO_3$), hard (172 mg/L $CaCO_3$), and very hard (322 mg/L $CaCO_3$) reconstituted water were 763, 1,430, 1,962, and 1,870 mg/L of chloride. Within this range of hardness, an approximate 2-fold decrease in toxicity was observed with harder water.

The CCME (2011) summarized studies where hardness reduced chloride toxicity (by up to 5-fold) for a variety of aquatic species including the Wavy-rayed lampmussel, water flea, fingernail clam, tubificid worm, snail, and isopod. And Gillis (2011) determined hardness reduced the toxicity of chloride the towards Fatmucket mussel. Similar relationships have been observed for metals such as zinc and cadmium, although the mechanism may be distinct for chloride (CCME 2011). The issue is complicated for chloride by the presence of toxicity studies showing results as equivocal or where the amelioration of chloride toxicity due to increasing water hardness was relatively minor (or negligible), for species such as water flea, fingernail clam, fathead minnow, snail, and damselfly. However, in some studies where minimal hardness effects were observed, the hardness levels were relatively low (*e.g.*, < 100 mg/L $CaCO_3$).

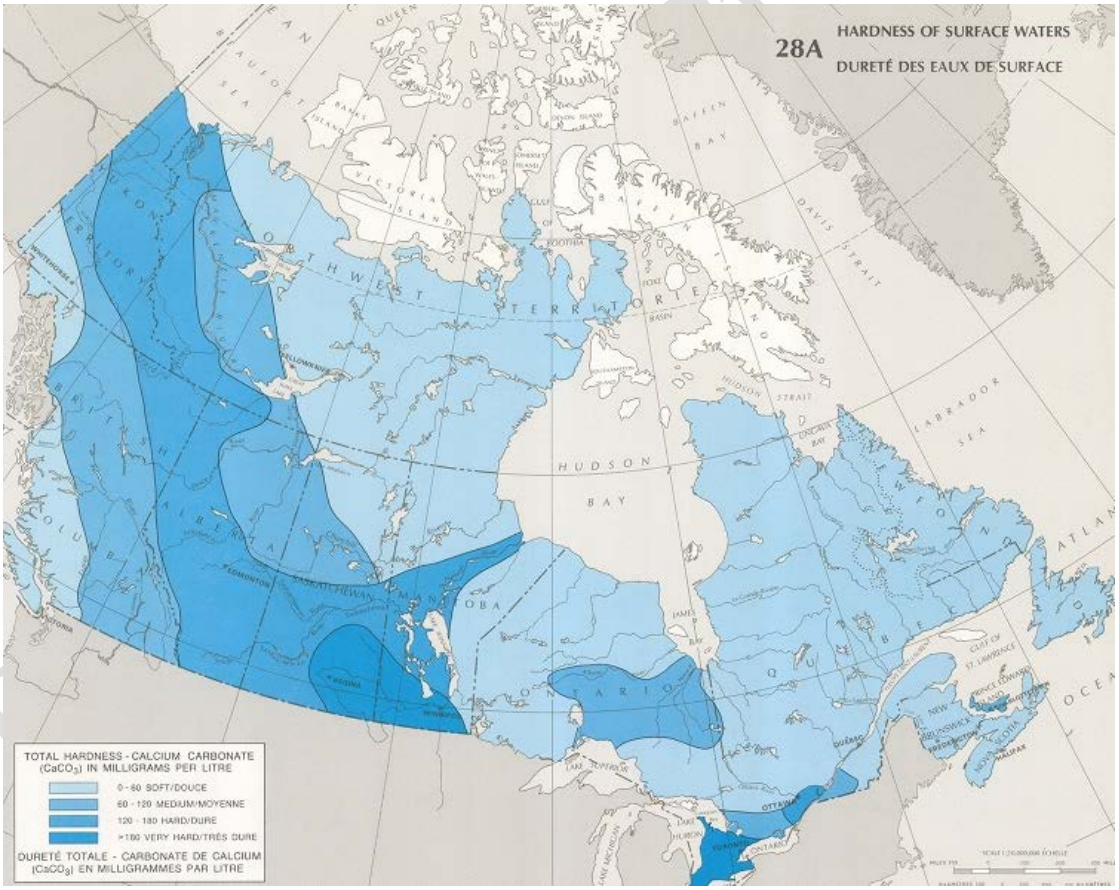
The CCME (2011) stated "*Jurisdictions will have the option of adjusting for site-specific hardness conditions, if they so choose, with the development of site-specific water quality guidelines (or objectives)*". Furthermore, the CCME (2011) stated "*CCME will re-visit the chloride guidelines when sufficient studies are available"*. Studies in this regard refers to chronic toxicity endpoints and a hardness relationship that meets required parameters defined in guidance from the US Environmental Protection Agency (US EPA,

[4] Mackie, G.L. 1978. Effects of pollutants on natality of *Musculium securis* (Bivalvia: Pisidiidae). The Naurulus. **92**: 25-33.

2001[5]), "...*such as the highest hardness is at least 3 times the lowest and the highest hardness is at least 100 mg/L higher than the lowest)*".

Precedent has been set by regulatory agencies for developing a chloride water quality guideline incorporating aspects of hardness. The US EPA in collaboration with the state of Iowa developed an algorithm for the adjustment of a chloride water quality guideline based on hardness. Given this information, an important component of any future research on the toxicity of chloride towards sensitive aquatic organisms is to incorporate varying hardness levels and produce information that can be used to improve the accuracy of guidelines developed on a provincial or national scale.

Variability in water hardness across Canada is graphically presented below, and it should be noted that hardness definitions differ. In Alberta, the following scheme has been used for water hardness (Government of Alberta, 2011[6]): 1) Soft (0 to 50 mg/L); 2) Moderately Soft (50 to 100 mg/L); 3) Moderately Hard (100 to 200 mg/L); 4) Hard (200 to 400 mg/L); 5) Very Hard (400 to 600 mg/L); and, 6) Extremely Hard (> 600 mg/L).



[5] The citation provided by the CCME (2011) for US EPA (2001) is: US EPA. 2001. Update of ambient water quality criteria for cadmium. Washington, D.C., Office of Water.

[6] Government of Alberta. Water Softening. 2011. http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex9354

2. RESEARCH PLAN AND METHODOLOGY

2.1 Alberta Mussel and Clam Species Toxicity Testing Work

The following chloride water concentrations (representing doses) would be considered for the toxicity testing work with mussels and clams: 0, 25, 75, 225, 675, and 2,025 mg/L. This concentration range will provide resolution towards where an EC_{10} may be expected for guideline development and an upper bound of exposure producing more extensive effects for confirmation of toxicity (*e.g.*, EC_{50}). The experiments will be conducted in re-constituted water to allow for comparison with other toxicity testing data and the consistent control of parameters that may relate to toxicity. The mussels and clams to be tested should be sourced from Alberta water bodies that are considered generally pristine and not impacted by sources of contamination such as heavy agricultural runoff, city storm sewer drainage, municipal and county salt storage yards, produced water contaminated sites, livestock operations, *etc.* An appropriate number of animals per dose group will be considered to allow for reproducible results and statistical analysis.

Unionidae Family

An ASTM (2006)[7] method is available for testing the toxicity of substances towards mussels that have a glochidia lifestage, such as for species of the *Unionidae* Family found in Alberta. A suitable third party vendor will be identified for conducting laboratory studies with two species in this Family in order to assess whether they are of equivalent sensitivity to chloride exposure as the Ontario Great Lakes species of Special Concern (Wavy-rayed lampmussel, *Lampsilis fascioloa*) and Endangered (Northern Riffleshell, *Epioblasma torulosa rangiana*) mussels, which all have a glochidia based reproductive cycle.

Of the five Alberta *Unionidae* species, toxicity testing data already exist for the Fatmucket mussel (*Lampsilis radiate siliquoidea*), which has been utilized by CCME (2011) in the development of an aquatic life guideline and for which a relationship has been identified in terms of hardness and chloride toxicity. It is recommended that the two other species for consideration originate from the two other generas of *Unionidae* found in Alberta, specifically one from *Anodonata* (either *Anodonata grandis, Anodonata simpsoniana*, or *Anodonata kennerlyi*), and one from *Lasmigona* (*Lasmigona complanata* or *Lasmigona compressa*). Both *Anodonata* and *Lasmigona* species have sharp hook-like points at the end of the embryonic valves, which differs from the valve structure of *Lampsilis siliquoidea*. Given the concern about the glochidia endpoint from chloride exposure being the ability for valves to close properly leading to attachment

[7] ASTM 2006. ASTM E2455-06 Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels. DOI: 10.1520/E2455-06. http://www.astm.org/Standards/E2455.htm

onto a host fish (CCME 2011), toxicity testing data for these two genera are considered relevant and to be of value for determining the chloride sensitivity of Alberta *Unionidae*.

- *Anodonta grandis* (including *A. g. simpsoniana*)
- *Anodonta kennerlyi*
- ***Lampsilis radiata siliquoidea***
- *Lasmigona complanata*
- *Lasmigona compressa*

Sphaeriidae Family

A methodology is available for assessing the toxicity of chloride towards species of the *Sphaeriidae* Family, which are present in Alberta. The toxicological endpoint of natality as determined by Mackie (1978) was considered appropriate, which is in alignment with CCME (2011). This endpoint is considered a measure of population increase for living young organisms under specific environmental conditions varying with size and composition of population as well as physical environment.

Of the 20 species in Alberta from the *Sphaeriidae* Family grouped into two genera (*Pisidium* and *Sphaerium*), toxicity testing data already exist (Mackie 1978) for the Fingernail clam (*Sphaerium (Musculium) securis*), which has been utilized by CCME (2011) in the development of an aquatic life guideline. The study did evaluate the relative toxicity of sodium chloride versus calcium chloride, but not specifically the toxicity of sodium chloride in the presence of varying water hardness levels. Toxicity testing data for a species of Fingernail clam (*Sphaerium simile*) that has not been identified in Alberta, indicates members of the genera *Sphaerium* and potentially the Family *Sphaeriidae* may demonstrate a substantial effect from water hardness on chloride toxicity based on information summarized by CCME (2011).

It is recommended that toxicity testing work be conducted with *Sphaerium (Musculium) securis* (Fingernail clam) to build upon the work completed by Mackie (1978) and incorporate a more relevant assessment of hardness modifying effects on toxicity. In addition, it is recommended that toxicity testing work be conducted with a species from the second genera of *Sphaeriidae* encountered in Alberta, specifically *Pisidium*. Toxicity testing data for these two genera are considered relevant and to be of value for determining the chloride sensitivity of Alberta *Sphaeriidae*.

- *Pisidium casertanum*
- *Pisidium compressum*
- *Pisidium conventus*
- *Pisidium fallax*
- *Pisidium ferrugineum*
- *Pisidium idahoense*

- *Pisidium lilljeborgi*
- *Pisidium milium*
- *Pisidium nitidum*
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- *Pisidium subtruncatum*
- *Pisidium variabile*
- *Pisidium ventricosum*
- *Pisidium walkeri*
- *Sphaerium lacustre*
- *Sphaerium nitidum*
- *Sphaerium rhomboideum*
- ***Sphaerium (Musculium) securis***
- *Sphaerium striatinum*
- *Sphaerium transversum*

Hardness Modifying Effect of Chloride Toxicity

For each species tested, selected chloride dose levels (n=3) will involve an assessment of chloride toxicity in the presence of varying water hardness levels. The following hardness levels are recommended (expressed as $CaCO_3$): 25 (Soft), 75 (Moderately Soft); 150 (Moderately Hard); 300 (Hard); and, 500 mg/L (Very Hard). Testing under water conditions of Extremely Hard (> 600 mg/L) was considered to be of lesser relevance given it is expected to represent a relatively minor portion of the water hardness environments encountered in Alberta.