Influence of Water Conditions on the Embryonic Survivorship of the Oregon Spotted Frog (*Rana pretiosa*) and Implications for Sustainable Management in BC.

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A thesis submitted in partial fulfillment of
the requirements for the degree of

MASTER OF SCIENCE
in
ENVIRONMENT AND MANAGEMENT

We accept this thesis as conforming
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April 2006

Your file Votre référence
ISBN: 978-0-494-30163-0
Our file Notre référence
ISBN: 978-0-494-30163-0

Abstract

The Oregon spotted frog (*Rana pretiosa*) is an endangered species with only three known populations in Canada located in isolated sites in the extreme south-west corner of British Columbia. One of the populations has shown a steady decline while another has shown an increase during 1997 to 2000. This research examined the question of whether water conditions correlate with the embryonic survivorship at these populations.

At MD Aldergrove, mean embryonic survivorship varied between 9% and 36% at sub site A, 78% and 88% at sub site B and 77% to 84% at Seabird Island. No extreme water quality conditions occur at either of the two study sites and water quality did not significantly correlate with poor embryonic survivorship. Sulphate, pH, chloride and conductivity were the only water chemistry variables that differed significantly (or marginally) among sites. A weak positive correlation was found between chloride and embryonic survivorship and conductivity and embryonic survivorship.

Acknowledgements

Funding for this research was provided by Environment Canada/Canadian Wildlife Service and Pacific Environmental Science Center [PESC].

Firstly I would like to thank my sponsor, Dr Christine Bishop for her help and advice during the field work, her help, advice and reviews of many thesis drafts and for the opportunity to use this project for my thesis. Thank you to my supervisor, Dr Bill Dushenko for his edits, reviews and advice.

I would like to thank the following people who helped me during my research: Sara Ashpole for her help during endless hours of field work, of which many were in the pouring rain, Dennis Knopp for his assistance in finding egg masses, Russ Haycock and Hyla Environmental Services Ltd. for 2002 temperature data, Dan Buffett for his help with the maps, the personnel of MD Aldergrove and the Seabird Island Sto:lo Band for granting me access to their properties to conduct my research. A very special thanks to Dr Christy Morrissey for her help with the statistical analysis and support during my studies.

I would like to thank my husband, Owain for his support during the past two years and his willingness to put our lives on hold so I could pursue my studies. Thank you to my father for his support and a very special thank you to my sister, Elmarie for the many phone calls, e-mails and especially her visit to support me.

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1. Introduction

Worldwide, many amphibian populations have shown a decline in population size and occurrence (Barinaga, 1990; Biek et al., 2002; Blaustein & Wake, 1990; Blaustein et al., 1994a; Davidson et al., 2002; Semlitsch, 2002 and Wake, 1991). Factors contributing to declines include habitat degradation and alteration, disease and pathogens, global warming, chemical contamination, invasive species, physiological effects due to changes in water chemistry, vectors provided by commercial trade, altered hydrology and water quality (Alford & Richards, 1999; Blaustein et al., 1999; Boyer & Grue, 1995; Carey et al., 1999; Duellman & Trueb, 1994; Hayes et al., 1997; Lips, 1998; Marco et al., 1999; Phillips, 1990; Semlitsch, 2002 and Wake, 1991). Since 1988, amphibian declines have also occurred in various protected areas with pristine habitat, which suggests that the decline might also be due to one or more global factors such as increased UV-B radiation or widespread pollution like acid rain (Barinaga, 1990; Blaustein et al., 1999 and Lips, 1998).

Frogs spend the greater part of their development in water and thus they can be directly exposed to chemicals that are in the water (Stebbins & Cohen, 1995). The hydrophilic gel that surrounds amphibian eggs provide very little protection (Pough, 1976) and the developing embryos can be highly sensitive to environmental pollutants (Carey & Bryant, 1995). They are closely related to their environment because they have little physiological control over body temperatures or evaporative water loss and various stages of their biphasic life cycle are sensitive to environmental contaminants (Duellman, 1999 and Duellman & Trueb, 1994). Due to their complex life history in aquatic and terrestrial environments (Stebbins & Cohen, 1995) and their

thin, highly permeable skins (Duellman & Trueb, 1994) deterioration in water quality can therefore have potential lethal or sub-lethal effects on amphibians (Boyer & Grue, 1995).

Some studies suggest that amphibians can be as sensitive as, or even more sensitive, than other vertebrate species when exposed to aquatic contamination (Hall & Henry, 1992 and Holcombe et al., 1987) making amphibians highly sensitive indicators or sentinel species. Factors such as pH (Barr & Babbit, 2002; Beattie et al., 1992; Bishop et al., 2000; Carey, 1993; Pierce, 1985 and Räsänen et al., 2002), temperature (Banks & Beebee, 1988; Broomhall, 2002; Carey, 1993; Howard, 1978; Kaplan, 1992; Licht, 1971 and 1969b and Pahkala et al., 2001), oxygen concentration (Marian et al., 1980; Mills & Barnhart, 1999; Nie et al., 1999 and Warkentin, 2002), aluminium (Clark & Hall, 1985), nitrate and nitrate (Marco et al., 1999 and Rouse et al., 1999) and copper (Herkovits & Helguero, 1998) can have lethal or sub-lethal effects on amphibians. The synergistic effect of parameters such as pH and temperature (Beattie et al., 1992); pH and aluminium (Andrén et al., 1988; Beattie et al., 1992 and Jung & Jagoe, 1995); pesticides, dissolved oxygen and temperature (Mann & Bidwell, 2001); and oxygen and temperature (Nie et al., 1999) can also contribute to increased toxicity to amphibians. Water quality is therefore seen as an important component in sustainable amphibian populations.

In Canada, 18 amphibian species are currently listed as endangered, threatened or of special concern (http://www.cosewic.gc.ca/eng/sct5/index_e.cfm). Water quality is postulated as a contributing factor to several of these declines (http://www.speciesatrisk.gc.ca/default_e.cfm). Among the most threatened species is the Oregon Spotted Frog (*Rana pretiosa*). This species was designated as "Endangered" in an emergency listing in November 1999 by the Committee

on the Status of Endangered Wildlife in Canada (COSEWIC) (Haycock, 2000b). This status was re-examined and confirmed in May 2000 (COSEWIC, 2000), constituting the first emergency listing of a species in Canada (Ministry of Water, Land and Air Protection [WLAP], 2002). The reason for the designation is the fact that the population was reduced to only 348 individuals in 2001 (Haycock, 2001) and occurs at only three isolated sites, each containing very low numbers of individuals (COSEWIC, 2000). This is exacerbated by the harmful effect that habitat loss, exotic species and exotic vegetation have had on the species and these are a continuing threat (COSEWIC, 2000). Water quality is another possible factor influencing *R. pretiosa* survival but has not been examined to date. The three known populations of *R. pretiosa* in Canada occur at Maintenance Detachment (MD) Aldergrove, Mountain Slough in Agassiz and Maria Slough adjacent to Seabird Island in the Lower Fraser Valley, British Columbia. The populations are genetically-isolated by habitat fragmentation and gene movement among sites is unlikely (Haycock, 2000a and 2001).

The MD Aldergrove population, particularly, has shown a steady annual decline from 90 egg masses in 1997 to 33 egg masses in 2001 (Haycock, 2001). The reason for poor fecundity at MD Aldergrove is currently unknown. Adequate reproduction is essential to maintain the adult breeding population, (Semlitsch, 2002) and poor survivorship can alter the recruitment into the next generation, having long-term impacts on population viability (Broomhall, 2002). Unfavourable aquatic conditions can result in multiple years of unsuccessful reproduction leading to population declines or even local extinction (Semlitsch et al., 1996). Conditions that are experienced during the early developmental stages can also have carry over effects on

amphibian performance during later stages including growth, mating success and fecundity (Goater, 1994; Kaplan, 1992; Newman, 1988 and Semlitsch et al., 1988).

In some amphibian species where fecundity is relatively low, egg and embryo mortality can contribute to declines in population size (Blaustein et al., 1994b and Blaustein et al., 1998). Low embryonic survivorship at MD Aldergrove could ultimately result in a decline in the adult breeding population. This suggests that embryonic survivorship may be limiting at the site and that many factors, including water quality, need to be investigated to determine the causes of poor fecundity.

Research Hypothesis and Objectives

This study tested the hypothesis that water conditions correlate with embryonic survivorship of two populations of *R. pretiosa* in British Columbia, Canada. The specific research objectives of this study were to:

- determine the embryonic survivorship of *R. pretiosa* at MD Aldergrove sub site A, MD Aldergrove sub site B and Seabird Island;
- examine differences in embryonic survivorship among sites and sub sites;
- determine which developmental stage has the highest mortality rate;
- assess whether water conditions correlate with embryonic survivorship; and
- determine the water conditions (limnological water chemistry, water temperature, total and fecal coliforms, and trace metals) ideal for successful reproduction in captivity and that correlate with development in the wild.

To test this hypothesis, the embryonic survivorship of *R. pretiosa* at MD Aldergrove and Seabird Island, and the developmental stages with the highest mortality rate were determined during

2002-2005. Water conditions (limnological water chemistry, water temperature, total and fecal coliforms, and trace metals) at these sites were measured and examined for relationships with development stage survivorship.

2. Literature review

2.1 Oregon spotted frog (*Rana pretiosa*)

Research on *R. pretiosa* goes as far back as the early 1900's with Thompson (1913) describing two subspecies of *R. pretiosa* (*R. p. luteiventris* and *R. p. pretiosa),* from Nevada. Svihla (1935) published some general notes on *R. pretiosa* describing their distribution, breeding behaviour and tadpoles. *R. pretiosa* was described as one of the most abundant and widely spread species in Western Washington (Svihla, 1935). This is in sharp contrast with the current situation, where *R. pretiosa* has declined between 80% and 90% from its historic distributions in Washington, California, Oregon and British Columbia (Haycock, 2000a and Hayes, 1994b). In Canada, *R. pretiosa* was designated "Endangered" in 1999 by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) (Haycock, 2000b). In Washington and California the status of *R. pretiosa* is S1 (critically endangered) and in Oregon it is S2 (endangered) (http://www.natureserve.org/explorer). The species also is listed as vulnerable by The World Conservation Union (IUCN) (http://www.natureserve.org/explorer and http://www.redlist.org).

Licht (1986, 1975, 1974, 1971, 1969a and 1969b) studied, among other things, the breeding habits, thermal requirements and embryonic survivorship of *R. pretiosa* in south-western British Columbia and his work is among the most comprehensive collection of studies on *R. pretiosa* and *R. aurora* in south-western British Columbia. Although the *R. pretiosa* populations where he conducted his research are now extirpated, he provided invaluable information regarding the breeding behaviour, vocalization, oviposition sites, water temperature requirements of embryos and tadpoles, hatching success, predation and life history of the south-western British Columbia

populations. He also conducted research on the Red-legged frog (*R. aurora*) (Licht, 1986, 1974, 1971 &1969b) which he found to be a sympatric species at his study sites.

R. pretiosa is a Pacific Northwest species (Corkran & Thoms, 1996 and Green et al., 1997). Historically, its distribution extended from the south-west corner of British Columbia to the north-east of California (Hayes et al., 1997). In British Columbia, *R. pretiosa* historically occurred at three locations, including Nicomen Island in the Fraser River (Carl & Cowan, 1945), the Sumas Prairie (Ministry of Water, Land and Air Protection [WLAP], 2002) and the district of Langley (Haycock, 2000a). The first two populations were recorded prior to the 1950's and the third population was studied in the late 1960's (Haycock, 2001 and WLAP, 2002). Since the 1990's, *R. pretiosa* was not found in any of the previous locations (Haycock, 1999). In British Columbia today, it occurs in three sites in the extreme south-west corner of the province: namely Maintenance Detachment (MD) Aldergrove, Mountain Slough in Agassiz and Maria Slough adjacent to Seabird Island (Haycock, 1998 and 2000a).

R. pretiosa is highly aquatic and occurs in warm-water marshes, shallow ephemeral pools, small floodplain wetlands with permanent water, slow streams, lake edges, and permanent ponds often with abundant vegetation (Corkran & Thoms, 1996; Hayes, 1994a and 1994b; Licht, 1969a and McAllister & Leonard, 1997). Adults are brown or reddish brown with black spots with light centers on the back and head (Corkran & Thoms, 1996 and WLAP, 2002). The throat and abdomen are white with brown, grey or tan spots and the thighs have orange-red pigments also with brown, grey or tan spots (Corkran & Thoms, 1996 and WLAP, 2002). The presence of mottling on at least some of the ventral portions of the abdomen is a distinguishing morphological characteristic that differentiates *R. pretiosa* from all other spotted frogs (Hayes,

1994a). Adults are 44-100 mm (snout to vent length) (Corkran & Thoms, 1996; Licht, 1969b and 1974; Pearl& Hayes, 2002 and WLAP, 2002).

Males arrive first at the breeding ground as early as the winter thaw permits and breeding usually starts in February or March (Licht, 1971). In south-western British Columbia, *R. pretiosa* males gather in small groups and individuals can vocalize in close proximity of each other (Licht, 1969b). This occurs while floating on the surface of shallow water close to the edge of the pond or river (Licht, 1969b). *R. pretiosa* often breeds at the edge of a river, stream or pond (Licht, 1969b).

Females spawn mainly during the daylight (Licht, 1969b). Egg masses are laid in communal oviposition sites on top of each other or side-by-side, and are not attached to any vegetation (Corkran & Thoms, 1996; Licht, 1969b and 1971; McAllister et al., 1993 and Svihla, 1935). This occurs in shallow water with only the bottom half of the mass submerged and the top half exposed above the water line (Corkran & Thoms, 1996 and Licht, 1969b and 1971; McAllister et al., 1993 and Svihla, 1935). Due to their strategy of laying eggs early in the season, the embryos face the dangers of desiccation or freezing. Complete or extensive mortalities can occur during dry and/or cold periods (Hayes, 1994a and Licht, 1974). Adult females breed every year laying a single egg mass (Licht, 1974 and Olson et al., 1997) with an average of 598 to 670 eggs (Haycock, 2000a; Licht, 1974 and McAllister & Leonard, 1997). The egg development period can be between 7 and 18 days (Licht, 1969b and McAllister & White, 2001) and eggs hatch at Gosner stage 19 (Licht, 1971).

In western Oregon, *R. pretiosa* has disappeared from at least 90% of its previous range (Hayes, 1994b). The areas where *R. pretiosa* currently occur are all at high elevation (over 1219 m). These areas have fewer to no hydrological modifications and exotic macro predators, compared to lower elevation sites (Hayes, 1994b). However, only one of these western Oregon populations appears to have both successful recruitment and sufficient adult survivorship (Hayes 1994b). Another study on *R. pretiosa* in Oregon, determined that adult *R. pretiosa* regularly prey on juvenile western toads (*Bufo boreas*) (Pearl & Hayes, 2002). This may suggest that R. *pretiosa* plays a unique ecological role among North American ranids since it is thought that *Bufo* are unfavourable food for ranids (Pearl & Hayes, 2002).

During 1989 to 1991, surveys that were conducted at 60 sites in western Washington where *R. pretiosa* historically occurred revealed only a single specimen (McAllister et al., 1993). Habitat alterations and predators such as exotic bull frogs (*R. catesbeiana)* and centrarchid fishes are probably the main causes for the extirpation of *R. pretiosa* from much of its former range (McAllister et al., 1993). Currently there are four known *R. pretiosa* populations in Washington. Research conducted at one of these populations revealed that optimal *R. pretiosa* spawning habitat consists of water 3.6 cm to 8.2 cm deep and a daytime substrate temperature of 7 °C to 9 °C during mid-February to mid-March (McAllister & White, 2001). *R. pretiosa* also prefer low vegetation and the water's surface must be almost completely exposed to sunlight (McAllister & White, 2001). There is an ongoing research project in the Baker River Watershed to establish the baseline environmental condition of *R. pretiosa* and to determine the effects of continued project operations on the species in the watershed (Kruger & Hamer, 2001).

A number of marking and radio telemetry techniques were used and evaluated on *R. pretiosa* at Dempsey Creek in Washington (McAllister et al., 2004). Passive integrated transponder tags (PITT) were the most effective and reliable marking techniques and radio telemetry were very valuable in determining movements, home ranges and habitat use of individuals; however, the researchers experienced problems with the attachment techniques (McAllister et al., 2004). It was suggested that the aluminum bead chain used for attachment be replaced with a cloth ribbon that should be less abrasive to the skin (McAllister et al., 2004). The researchers also suggest the use of coated antenna wire to give the antenna more stiffness which should prevent kinking, coiling and possible entanglement in vegetation (McAllister et al., 2004).

2.2 Embryonic survivorship

A number of studies have been done on amphibian embryonic survivorship and larvae in laboratories (e.g. Clark & LaZerte, 1985; Davis & Roberts, 2005 and Herkovits & Helguero, 1998) but few studies have been conducted in holding cages in-situ. Previous studies that involved cages (e.g. Clark & Hall, 1985; Cooke, 1981 and Materna et al., 1995) did not provide clear descriptions of the holding cages. Harris and Bogart (1997) did a short-term study on the early development of temperate ranid frogs that involved transferring eggs into holding cages. Substantial detail on cage design was provided, enabling this method to be replicated for this study.

Gosner (1960) described staging tables for the development of anuran embryos and larvae, which is essential for studies involving frog life-history. His article provides clear descriptions and

detailed sketches of each life stage and has been used in many studies that involve the description of amphibian development and life stages.

Previous studies on the embryonic survivorship and larvae survivorship of amphibians provided information on survival rates that will ensure sufficient recruitment into the adult breeding populations to ensure sustainable amphibian populations (Licht, 1974 and 1971 and Turtle, 2000). There has also been some examination of the effects of various environmental stress factors on embryonic survivorship of amphibians including roadside run off (Turtle, 2000), temperature (Licht, 1971), aluminium (Clark & Hall, 1985), copper (Herkovits & Helguero, 1998), nitrate, pH and UV-B (Hatch & Blaustein, 2000) and pH, aluminium and temperature (Beattie et al., 1992).

In a field study in south-western British Columbia, *R. pretiosa* had an embryonic survivorship of 70% and *R. aurora* had an embryonic survivorship of 90% (Licht, 1974). *R. pretiosa* embryos have a temperature tolerance of 6 °C to 28 °C while the sympatric *R. aurora* embryos can tolerate temperatures between 4 °C and 21 °C (Licht, 1971).

The embryonic survival of *Bufo americanus, Rana sylvatica* and *Ambystoma maculatum* were assessed at different sites with different pH and inorganic monomeric aluminium concentrations in the field by Clark & Hall (1985). pH varied from 5.8 to 4.3 and inorganic monomeric aluminium concentrations ranged from 10 µg/l to 46 µg/l with higher aluminium concentrations associated with lower pH (Clark & Hall, 1985). The embryonic survival of *Bufo americanus* decreased from 99% to 11% and *Rana sylvatica* from 95% to 83 % at pH 5.8 and 10 µg/l

aluminium to pH 4.3 and 46 µg/l aluminium. Similarly, the embryonic survivorship of *Ambystoma maculatum* decreased from 57% at pH 5.8 and 10 µg/l aluminium to 34% at pH 4.8 and 37 µg/l aluminium (Clark & Hall, 1985).

The embryonic survivorship of the spotted salamander (*Ambystoma maculatum)* was significantly lower in roadside pools compared to woodland vernal pools in south-eastern New Hampshire. There was also a significant difference in conductivity, sodium and chloride concentrations between roadside and woodland pools (Turtle, 2000). De-icing salts used for highway maintenance contaminate roadside areas and probably contribute to lower embryonic survivorship of *Ambystoma maculatum* in these pools (Turtle, 2000).

A laboratory experiment with *Bufo arenarum* embryos indicated that the 24 hour LC_{50} values for copper were about 0.085 mg/l and LC_{10} values were 0.05 mg/l, while the LC_{90} values were approximately 0.155 mg/l (Herkovits & Helguero, 1998). The LC_{50} and LC_{10} did not change much when the exposure time was extended to 168 hours, but the concentration decreased after 96 hours for LC_{90} (Herkovits & Helguero, 1998). The inclusion of zinc revealed a beneficial effect in which 100% protection was achieved with 30 mg/l zinc for a copper concentration that caused 90% mortality (Herkovits & Helguero, 1998).

In *Rana cascadae*, the survival and activity level of larvae were examined in a laboratory controlled setting when exposed to the separate and combined effects pH, nitrate and UV-B. Treatment variables included pH levels of 5 and 7; nitrate levels of 0, 5, and 20 mg/l; and UV-B absence or presence (Hatch & Blaustein, 2000). There was no significant difference in survival

and activity level of larvae in treatments with individual factors but survival and activity levels were significantly reduced at treatments combining low pH, high nitrate and UV-B (Hatch & Blaustein, 2000).

Another laboratory study on the embryonic survival of *Rana temporaria* testing the individual and combined effects of pH, aluminium and temperature indicated that embryonic survivorship was lower at pH 4.5 than pH 6.0 and was also lower at the highest aluminium concentration (400µg/l) (Beattie et al., 1992). Embryos exposed to varying temperatures also had lower survival than those kept at a constant temperature, especially at pH 4.5 (Beattie et al., 1992).

2.3 Water quality

Water quality plays an important role in amphibian survival and amphibians can be very sensitive to changes in water quality. This is due to their complex life history in aquatic and terrestrial environments (Stebbins & Cohen, 1995) and their thin, highly permeable skins (Duellman & Trueb, 1994). A decline in water quality can therefore have potential lethal or sub-lethal effects on amphibians.

Amphibians are highly sensitive to nitrogen pollution, specifically nitrate (Rouse et al., 1999), pH (Barr & Babbit, 2002; Beattie et al., 1992; Pierce, 1985 and Räsänen et al., 2002), temperature (Banks & Beebee, 1988; Licht, 1971 and 1969b and Pahkala et al., 2001), oxygen concentrations (Marian et al., 1980; Mills & Barnhart, 1999; Nie et al., 1999 and Warkentin, 2002), aluminium (Clark & Hall, 1985) and copper (Herkovits & Helguero, 1998). A combination of factors such as pH and temperature (Beattie et al., 1992), pH and aluminium

(Andrén et al., 1988; Beattie et al., 1992 and Jung & Jagoe, 1995) and oxygen and temperature (Nie et al., 1999) can often result in delayed hatching, abnormalities or mortalities in amphibian eggs and larvae. The combined effects of pH and UV-B are somewhat equivocal. Some studies (e.g. Long et al., 1995) found a reduction in egg survival while other studies found no synergistic effect of pH and UV-B (e.g. Pahkala et al., 2001).

2.4 Global amphibian trends and sustainable amphibian populations

It is important not only to consider the plight of *R. pretiosa* in isolation but to also be aware of global trends in amphibian populations. Worldwide, many amphibian populations have shown a decline in occurrence and population size (Biek et al., 2002 and Semlitsch, 2002). For example, the endemic golden toad in Costa Rica, has not bred there since 1987 (Crump et al., 1992) and huge declines in amphibian distribution and population size have also been reported in Australia (Laurance et al., 1996), Costa Rica (Lips, 1998), Panama (Lips, 1999), Poland (Berger, 1989) and the US (Carey, 1993; Davidson et al., 2002; Drost & Fellers, 1996 and Fisher & Shaffer, 1996). Globally, over 200 species have recently shown declines and 32 species have become extinct (Alford & Richards, 1999; Blaustein & Wake, 1990 and Houlahan et al., 2000). Most of these declines can be linked to anthropogenic causes.

In light of the above, it is clear that amphibian management strategies must be implemented to ensure the sustainability of amphibian populations. Successful management, translocation, recovery of amphibian populations and long term monitoring is required (Semlitsch, 2002). There is a need for basic information including life history requirements, habitat use and population biology of species in order to develop and implement management strategies

(Semlitsch, 2002). Furthermore, it is also important to look at species at both the local and meta-population scale to ensure successful recovery strategies. Only through cooperation among states, provinces and countries can the successful conservation of species be insured (Semlitsch, 2002). The critical elements of a successful recovery plan are defining the spatial and temporal scale of the recovery effort and determining the habitat suitability of translocation sites. This includes both the quality of habitat and historic range of the species (Semlitsch, 2002).

"*The translocation of animals involves several critical factors that can affect the success of recovery efforts: (1) location of source populations, (2) genetic divergence from other populations, (3) whether animals are wild caught or captive-reared, (4) age or life-history stage of founders, and (5) number, frequency, and timing of release.*" (Semlitsch, 2002, pp. 624-625).

It is also important to develop methods for measuring success and long-term management, which will vary among projects depending on available resources, project goals and time (Semlitsch, 2002).

The translocation and reintroduction of an endangered amphibian species, the green and golden bell frog (*Litoria aurea*) in south-eastern Australia, utilized information on habitat variables that could explain the presence of *L. aurea* in one water body and absence from another, and also distinguish between breeding and non breeding sites (Hamer et al., 2002). "*The aim was to produce a habitat model for this species so that existing populations can be managed in-situ and additional habitat created according to those variables with the highest discriminating power.*" (Hamer et al., 2002, p 414). The diversity of vegetation on the banks of water bodies including

Juncus kraussii, Schoenoplectus litoralis and *Sporobolus virginicus* were significant predictors of the presence of *L. aurea.* Other aspects of water body physiognomy and water chemistry, however, could not explain the distribution of *L. aurea* (Hamer et al., 2002). A water body was also more likely to be occupied if there was another water body within 50 m which contained a population of *L. aurea* (Hamer et al., 2002) suggesting the importance of migration and available habitat patches for this species.

In Utah, 31 of 57 ponds that were surveyed indicated the presence of the Columbia spotted frog (*Rana luteiventris*) (Welch & MacMahon, 2005). Conductivity, emergent vegetation, pond hydrology, water temperature and littoral zone depth were monitored at all ponds to determine habitat variables associated with the presence of *R. luteiventris* (Welch & MacMahon, 2005). The results suggested that *R. luteiventris* was more likely to be present in ponds that did not shrink in size, had reasonably constant water temperatures throughout the season, and had tall emergent vegetation (Welch & MacMahon, 2005). There was a marginal association between *R. luteiventris* and littoral depth, and the association with conductivity was mixed but mainly negative (Welch & MacMahon, 2005). It is essential to be aware of these strategies, methodologies and models and to incorporate them into the recovery plan for *R. pretiosa*.

3 Research methodology

3.1 Study sites

The research was conducted at two sites, the Department of National Defence's Maintenance Detachment (MD) Aldergrove (latitude 49° 0' and longitude 122° 29") and Maria Slough (latitude 49º 17' and longitude 121º 42') on the Seabird Island reserve near Agassiz (Fig 3.1, Fig 3.2 & Fig 3.3). MD Aldergrove is approximately 70 km south-east of Vancouver and Seabird Island is approximately 140 km east of Vancouver. Both include warm, shallow marshes with permanent water. MD Aldergrove consists of 2.6 hectares (40%) of shallow, basin marsh and 4 hectares (60%) of shallow basin water (Ward et al., 1992). The larger Maria Slough consists of 17.1 hectares (65%) of stream water and 9.2 hectares (35%) of floodplain marsh (Ward et al., 1992). *R. pretiosa* occurs in the shallow basin marsh at MD Aldergrove and the floodplain at Maria Slough (Haycock, 2000a).

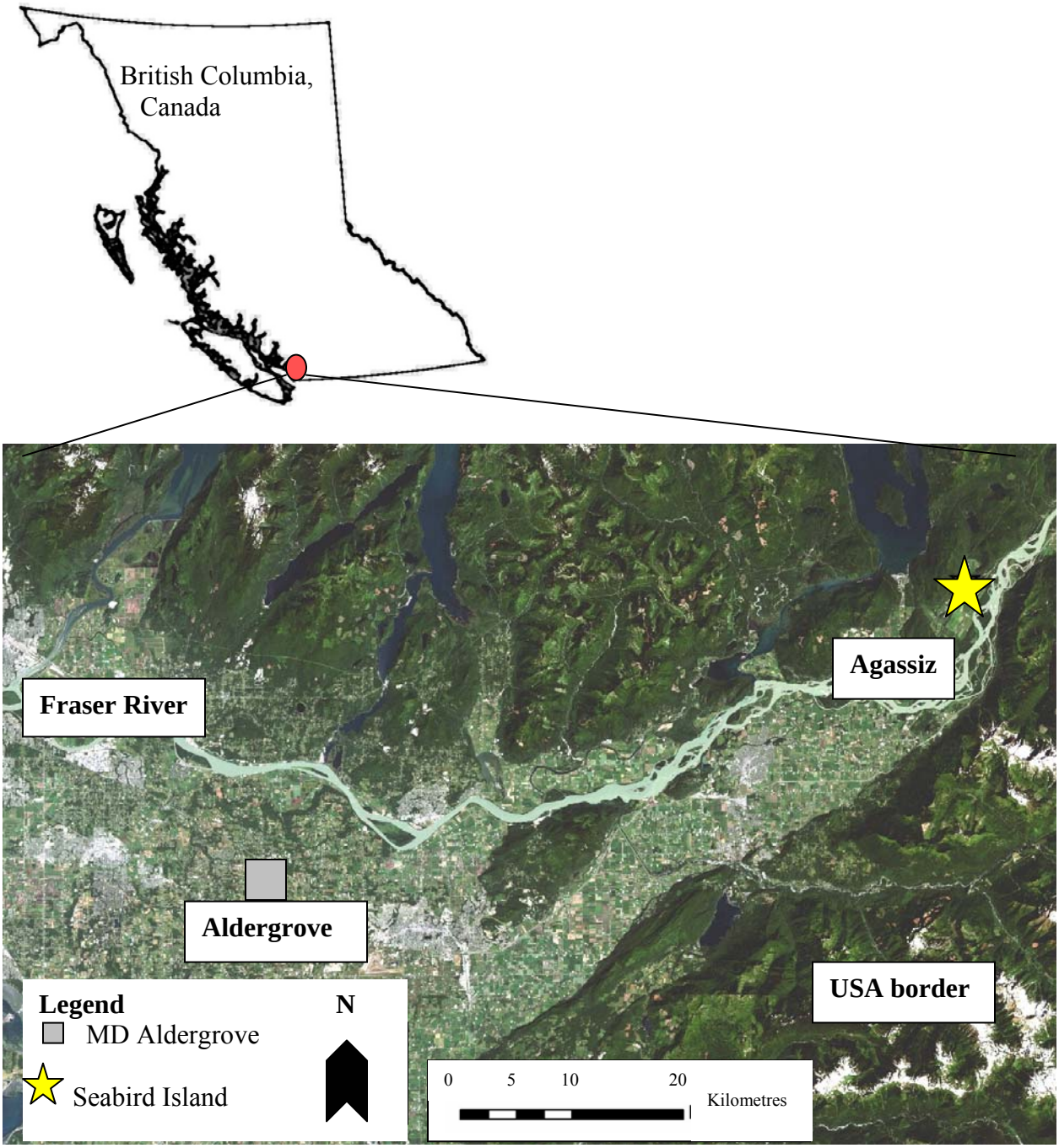

Fig. 3.1 – Fraser Valley, British Columbia, Canada indicating MD Aldergrove and Seabird Island.

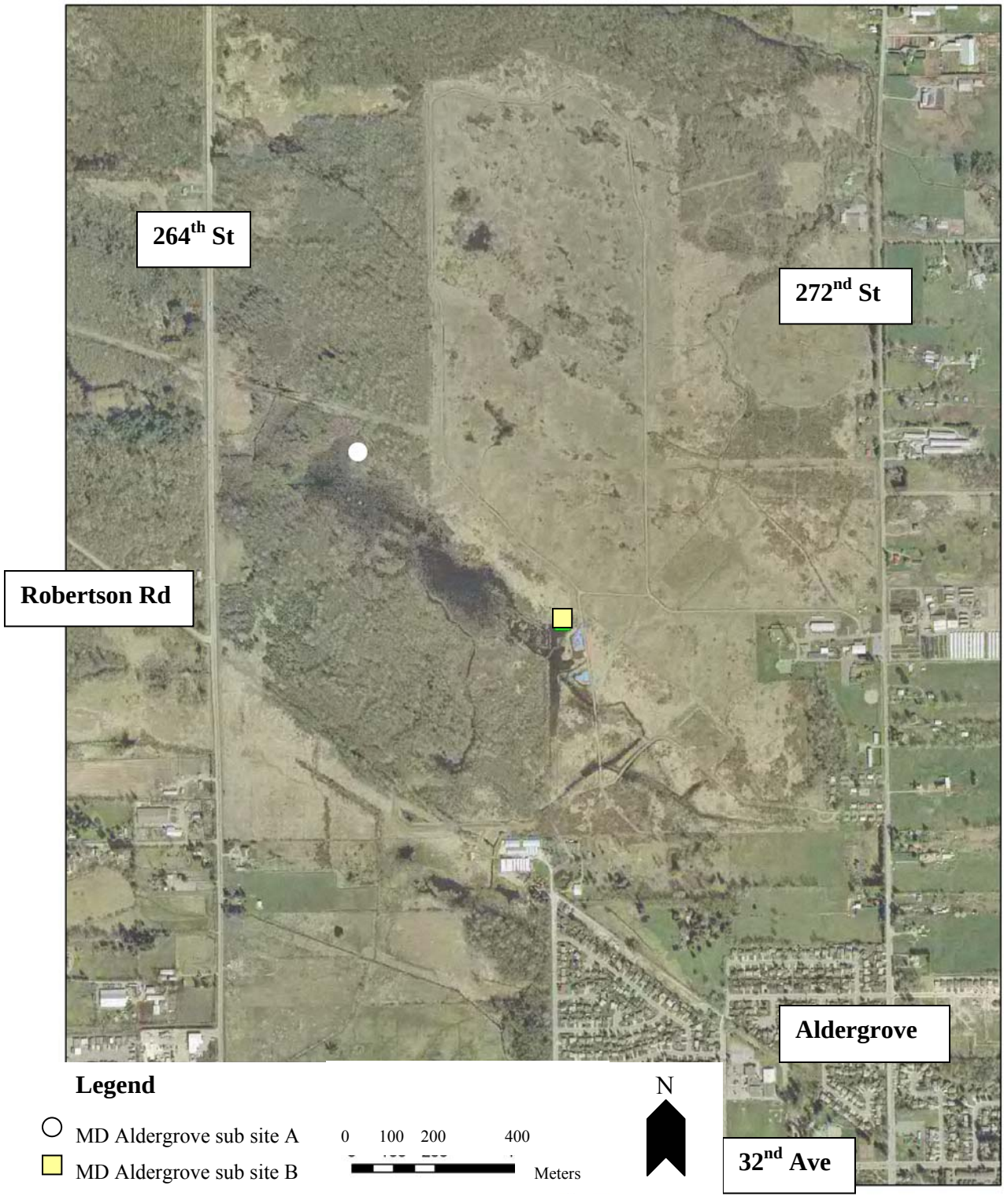

Fig 3.2 – MD Aldergrove indicating sub site A and sub site B, British Columbia, Canada.

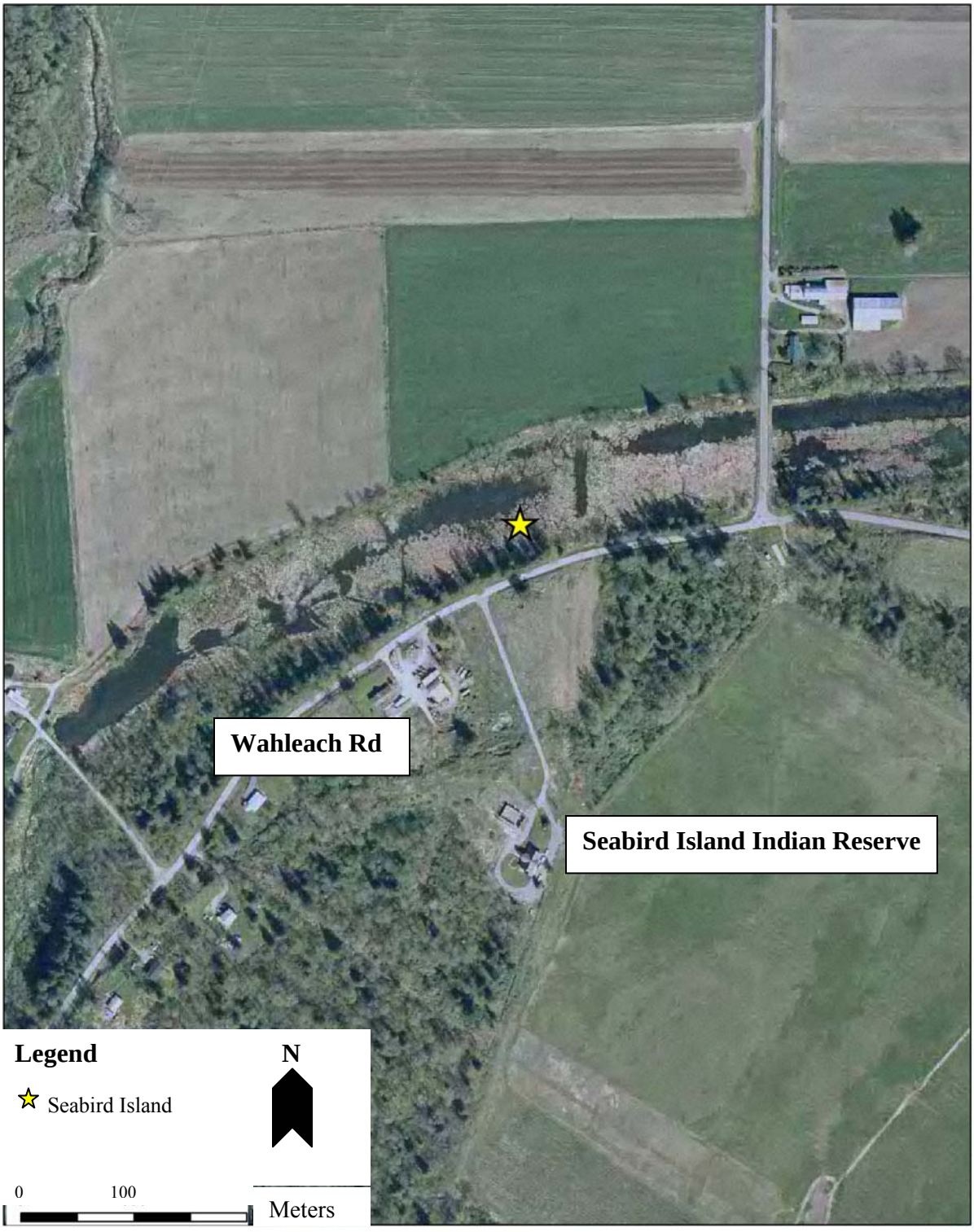

Figure 3.3 - Seabird Island, British Columbia, Canada.

The MD Aldergrove study site consisted of two sub sites, namely sub site A (Fig 3.4) and sub site B (Fig 3.5). Sub site A is a small, open wetland. The dominant shrubs in the wetland are pink spirea (*Spirea douglasii* subsp. d*ouglasii*), blackberry (*Rubus ursinus)*, salmonberry (*Rubus spectabilis*), beaked hazelnut (*Corylus cornuta*), willow (*Salix* spp.), wild rose (*Rosa* sp.), black twinberry (*Lonicera involucrata)* and black gooseberry (*Ribes lacustre)*. A variety of grass and forbs species including skunk cabbage (*Lysichiton americanus*), white water buttercup (*Ranunculus aquatilis*), hard stemmed bulrush (*Scirpus lacustris*), common rush (*Juncus effusus),* reed canary grass (*Phalaris arundinacea*), Bracken fern (*Pteridium aquilinum*), Pacific bleeding heart (*Dicentra formosa),* false lily of the valley (*Maianthemum dilatatum*) and horsetail (*Equisetum* sp.) occur in the wetland. On the edge of the wetland is mountain alder (*Alnus incana* subsp. *tenuifolia*), paper birch (*Betula papyrifera)* and vine maple (*Acer circinatum).*

Fig 3.4 – *Rana pretiosa* breeding site at MD Aldergrove sub site A, British Columbia, Canada.

Fig 3.5 – *Rana pretiosa* breeding site at MD Aldergrove sub site B, British Columbia, Canada.

Sub site B was altered in 2002 and now consists of a large body of open water with pink spirea (*Spirea douglasii* subsp. d*ouglasii*), willow (*Salix* spp.) and mountain alder (*Alnus incana* subsp. *tenuifolia*) on the edge and hard stemmed bulrush (*Scirpus lacustris*) and grass leaved pondweed (*Potamogeton gramineus)* inside the littoral zone of the wetland. MD Aldergrove wetland consists of 30% submerged aquatic vegetation, 20% floating aquatic vegetation, 20% grass, 15% tall brush, and 5% of each of hardwood trees, tall shrub and forbs (Ward et al., 1992). The oviposition sites at sub site A and sub site B are in full sun and no more than 10% of the entire wetland is shaded. The exotic bull frog (*Rana catesbeiana)* occurs at MD Aldergrove (Haycock, 2001).

The Seabird Island site (Fig 3.6) consists of a wetland about 1m deep bordered by a deeper channel on the north side and a paved road on the south side. The wetland is dominated by reed canary grass (*Phalaris arundinacea*) interspersed with cattail (*Thypha latifolia)* with red alder (*Alnus rubra*), black cottonwood (*Populus balsamifera* ssp. *trichocarpa)* and big-leaf maple (*Acer macrophyllum*) on the edge. The wetland and upland consist of 25% tall shrub, 25% grass, 20% submerged aquatic vegetation and 30% non vegetative (Ward et al., 1992). The oviposition sites are in full sun and no more than 15% of the entire wetland is shaded. The exotic green frog (*Rana clamitans)* occurs at Seabird Island (Haycock, 2001). Both study sites have limited, low intensity anthropogenic development in the immediate vicinity of the wetlands. The MD Aldergrove site is surrounded by mowed fields, natural areas and roads while the Seabird Island site is surrounded by roads, residential housing, small scale agriculture and natural areas.

Fig 3.6 – *Rana pretiosa* breeding site at Seabird Island, British Columbia, Canada.

3.2 Embryonic survivorship of *Rana pretiosa*

To measure embryonic survivorship of *R. pretiosa*, the study sites were visited twice a week from the beginning of March to the end of April in 2002 to 2005. The data that was collected during 2002 and 2003 by the researcher occurred before the thesis project began. In 2003, no research was conducted at MD Aldergrove because access was prohibited for any research on *R. pretiosa*. This was due to the concerns for a possible outbreak of an amphibian disease at MD Aldergrove. Concerns were raised due to the low number of egg masses found that year but no disease vector was identified (Bishop, pers comm.). During 2005, access to the Seabird Island site was prohibited and embryonic survivorship was therefore only monitored at MD Aldergrove by the researcher.

To quantify the embryonic survivorship of *R. pretiosa* from early development through to hatching, sub-samples of egg masses were placed in holding cages set up in-situ near the wild egg mass. It is almost impossible to quantify the actual embryonic survivorship of a complete frog egg mass without extensive handling and separation of the eggs. Individual *R. pretiosa* eggs are also held tightly together by the viscous jelly of the neighbouring egg which makes it almost impossible to separate an individual egg from its neighbour (Svihla, 1935). To provide a representative measure of the egg mass survivorship, clumps of 15-30 eggs were sub-sampled from randomly selected wild egg masses and percent survivorship, time to hatching and rate of hatching was determined. On a few occasions when it would have taken too much handling of an egg mass to separate 15-30 eggs, more than 30 eggs were put into a holding cage. Where possible, egg clumps were removed from different locations within masses to ensure sub-samples were taken from both the outside and inside of the egg mass.

Using gloved hands (disposable latex), a small clump of eggs (15-30 eggs) was separated from the main egg mass and placed in plastic sieves within the Nytex cages. These enclosures exclude smaller invertebrate predators, except for bacteria and viruses (Harris & Bogart, 1997), and effectively exclude larger vertebrate predators (Fig 3.7 & 3.8).

Fig 3.7 – Nytex holding cages

Fig 3.8 – *Rana pretiosa* eggs inside plastic sieve.

The cages were made of inert Nytex which does not leach any toxic chemicals into water surrounding the eggs, but allows for adequate water circulation and light penetration. The cages were attached loosely to small wooden dowels on each side by means of plastic cable ties. This was done in such a manner that the cages moved vertically with changes in the water depth to prevent dehydration. Egg sub-samples were transferred into holding cages and egg development was monitored. Because this species is endangered, it was decided, prior to handling, that no more than 30 egg masses would be sampled at Seabird Island and no more than 10 egg masses would be sampled at MD Aldergrove. At MD Aldergrove, the number of egg masses oviposited was low, ranging from five to twelve (pers. obs.) and most egg masses were sampled.

Amphibian eggs and larvae require specific levels of dissolved oxygen, temperature and

light intensity (Duellman & Trueb, 1994); this was considered when using the cages. Samples inside the Nytex holding cages were placed within 1 to 3 m of the wild egg mass to ensure control for local micro-environment conditions during egg development monitoring. The holding cages were placed in such a way that the wild egg masses were not disturbed when the cages were monitored.

The cages were visited two times per week during the three to four week period of development to hatching. Hatchling development was described using Gosner (1960) staging. Stages one through 25 contain the embryonic series. Stage 1 represents fertilization and is indicated by rotation of the embryo. The second polar body is expelled during stage 2 and a lightening appears on part of the pigmented hemisphere. This is followed by seven cleavage stages (Stage 3-9). Stage 10 is the beginning of gastrulation and stages 11-12 represent blastopore formation. During stage 13, the neural plate develops and at stage 14 the embryo becomes elongated and the neural fold is visible. The neural groove narrows and ciliary rotation of the embryo takes place during stage 15. The neural folds are closed at stage 16 and a recognizable head appears. The tail bud appears during stage 17. During stages 18-20, muscular responses are visible and the external gills and tail develop (Gosner, 1960) followed by hatching of *R. pretiosa* in late stage 19 (Licht, 1971). During stages 21-25 the hatchlings develop into a feeding and free swimming tadpole (Gosner, 1960). Free swimming hatchlings, Gosner stages 21-25 (Gosner, 1960) were released at the wild egg masses.

To ensure no cross contamination took place between study sites, all equipment were stored separately, disposable gloves were worn at all times, new gloves were used at each site and

different pairs of waders were worn at each site. Water and sediment movement around the egg masses was limited as much as possible to ensure minimum disturbance to egg masses and vegetation, and to avoid sediments being suspended in the water and settling on the eggs. All handling of the eggs were conducted with disposable gloves.

3.3 Water quality

Water samples were collected for water chemistry, trace metal and bacterial coliform analyses. Water samples were collected at each site where egg masses were oviposited. These were collected at one period each year about half way through the three to four week egg development period. *R. pretiosa* eggs are laid on the water surface and water samples were therefore also taken at surface level. Water analysis was done by CANTEST and Pacific Environmental Science Center [PESC]. Samples were couriered on ice to CANTEST and Pacific Environmental Science Center [PESC] for analysis on the same day that the samples were collected. During 2005, Dissolved Oxygen measurements (DO) were taken at Aldergrove during each visit throughout the season. Measurements were taken with a HANNA HI9142 portable waterproof dissolved oxygen meter.

Trace metal analyses were performed by PESC and analysed for aluminium (Al), antimony (Sb), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi), boron (B), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), silver (Ag), strontium (Sr), thallium (Tl), tin (Sn), uranium (U), vanadium (V) and zinc (Zn). An inductively coupled plasma mass spectrometry (ICP-MS) test was used to analyze the water samples for trace metals ([PESC,

1999). Calcium (Ca), iron (Fe), potassium (K), silicon (Si), sodium (Na), sulphur (S) and titanium (Ti) were analyzed using an inductively coupled plasma (ICP) test (PESC, 1999).

Samples for water chemistry were analyzed using standard methods (PESC, 1999) for pH, conductivity, ammonia (NH3), total nitrogen (TN), phosphorous o-PO4 dissolved, total dissolved phosphorus (TDP), total phosphorous (TP), turbidity, chloride (Cl), fluoride (F), sulphate (SO_4), bromide (Br), nitrate (NO_3), nitrite (NO_2), phosphate (PO_4) and biochemical oxygen demand (BOD).

Water chemistry method detection limit (MDL) for pH was 0.01 pH units, conductivity was 2 µS/cm and turbidity was 0.05 nephelometric turbidity units (NTU). MDL for the remaining water chemistry variables ranged from 0.002 mg/l to 5 mg/l (Table 4.1). Trace metal MDL for calcium, iron, potassium, sodium, silicon, sulphur and titanium ranged from 0.002mg/l to 0.1mg/l. MDL for the remaining trace metals ranged from 0.002 µg/l to 0.1 µg/l (Table 4.2).

The MDL for total coliform ranged from 1 col/100ml to 10 col/100ml, while fecal coliform ranged from 1 col/100ml to 2 col/100ml (Table 4.3). Total and fecal coliform analysis were performed by CANTEST using the Membrane Filtration Method (Ministry of Water, Land and Air Protection, 1994 and Clesceri et al., 1998).

Water temperature was measured at all sites where egg masses were oviposited and cages were deployed. During 2002, water temperature was measured using Stowaway® Temperature loggers (Hoskin Scientific Ltd). Temperature was recorded every 5 minutes during egg

development and the temperature logger's accuracy was two decimal places (± 0.01 ºC). During 2005, water temperature was measured using a Vee Gee Brand minimum/maximum temperature logger. Minimum, maximum and current water temperatures were recorded at each site twice per week. The temperature logger's accuracy was ± 1 ºC. The two different instruments were not compared between years but a Kruskal-Wallis Test (Zar, 1996) of median values indicated that there were no significant differences in temperature between years within sites ($p \leq 0.05$).

3.4 Statistical analysis

3.4.1 Embryonic survivorship

The embryonic survivorship proportions were arcsine transformed only to determine whether the data was normally distributed. The Shapiro-Wilk W Test indicated that the data was not normally distributed and non-parametric statistics were therefore used for data analyses (Zar, 1996). Embryonic survivorship data among years and among MD Aldergrove sub site A, MD Aldergrove sub site B and Seabird Island were compared using the Kruskal-Wallis Test to determine differences among years, sites and sub sites (Zar, 1996). The data for MD Aldergrove sub site A and sub site B were also combined and compared with Seabird Island using a Wilcoxon rank sum test to determine differences among years, sites and sub sites (Zar, 1996).

3.4.2 Water quality

Due to the small sample size, non-parametric statistics were used to analyse data. Water chemistry and coliform data at MD Aldergrove sub site A and sub site B and Seabird Island were compared using the Kruskal-Wallis Test to determine differences among sites and sub sites. Due to the small sample size, no statistical analysis was performed on trace metal, temperature and

dissolved oxygen data. At MD Aldergrove, the sample size for trace metals and water temperature were two and at Seabird Island the sample size for both was one. Dissolved oxygen was only measured during 2005 and only at MD Aldergrove. No statistical analysis was performed on nitrite, bromide and phosphate since all measurements for all study years at all sites and sub sites were below the MDL. Where necessary, for values that were below MDL, the values were changed to half the detection limit for statistical analysis. The Spearman Rank correlation was performed on embryonic survivorship and water chemistry variables that showed a significant or marginally significant difference among sites and sub sites (Zar, 1996).

Statistical analysis was performed using the software JMP IN version 4 (SAS Institute Inc.).

4. Results

4.1 Embryonic survivorship of *Rana pretiosa*

During 2002 and 2004-2005, the mean embryonic survivorship of *R. pretiosa* at MD Aldergrove sub site A ranged from 9 % to 35%, and at sub site B it ranged from 78% to 88% (Fig 4.1, Table A1, Appendix A). At Seabird Island, the mean embryonic survivorship for the study years 2002 to 2004 varied between 77% and 84% (Fig 4.1, Table A2, Appendix A).

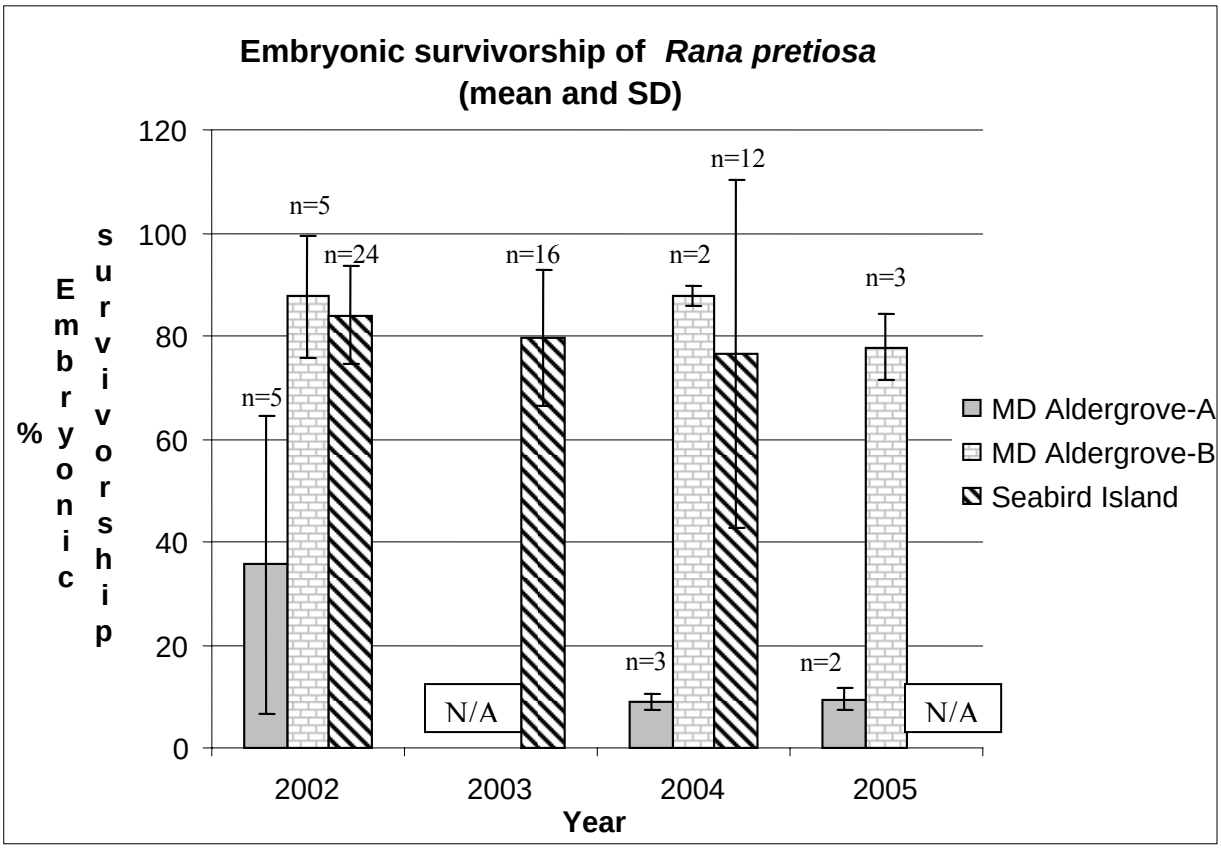

Fig. 4.1 - Mean [± standard deviation (SD)] embryonic survivorship of *Rana pretiosa* at MD Aldergrove sub site A, sub site B and Seabird Island, British Columbia, Canada during 2002 to 2005. N/A = years when no data was collected for a site/sub site. n = number of cages.

At MD Aldergrove sub site A, all eggs developed until Gosner stage 12-13 (late gastrulation and neural plate development). The highest percentage of eggs (about 50%) died between Gosner stages 14-17 (neural fold to tail bud development) (Fig 4.2).

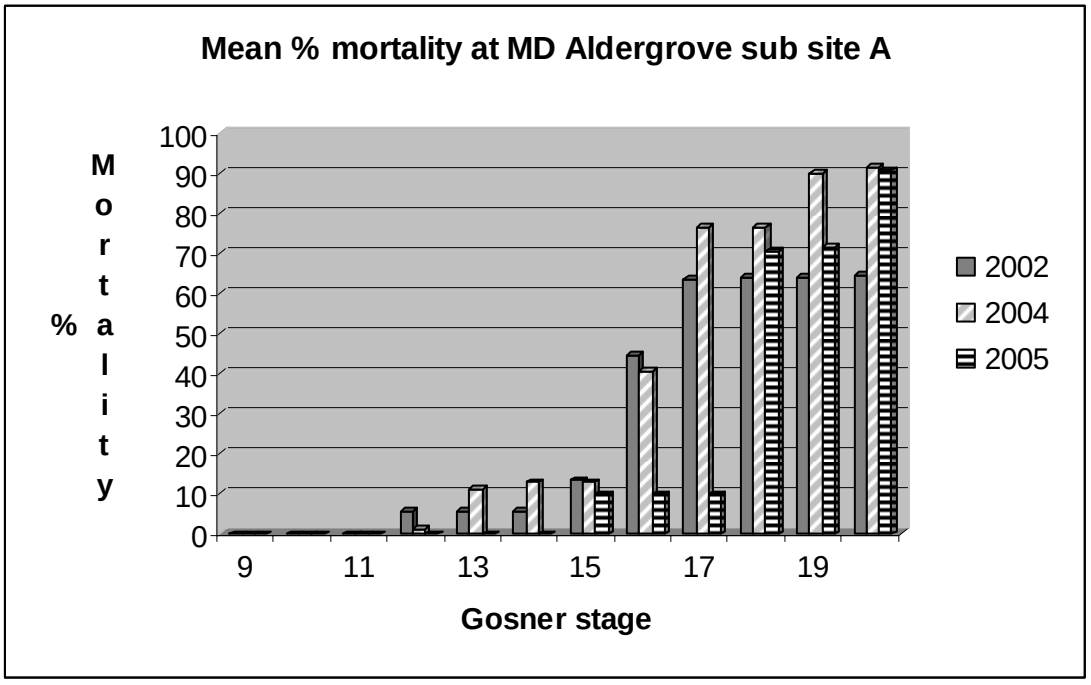

Fig 4.2 - Mean % mortality at each Gosner stage (stage 9 to hatching) for *Rana pretiosa* at MD Aldergrove sub site A, British Columbia, Canada during 2002, 2004 to 2005.

At MD Aldergrove sub site B, most eggs developed normally until stage 14 (when the neural fold becomes visible) and the highest percentage (5-10%) died between Gosner stages 15 (ciliary rotation of the embryo) and hatching (Fig 4.3).

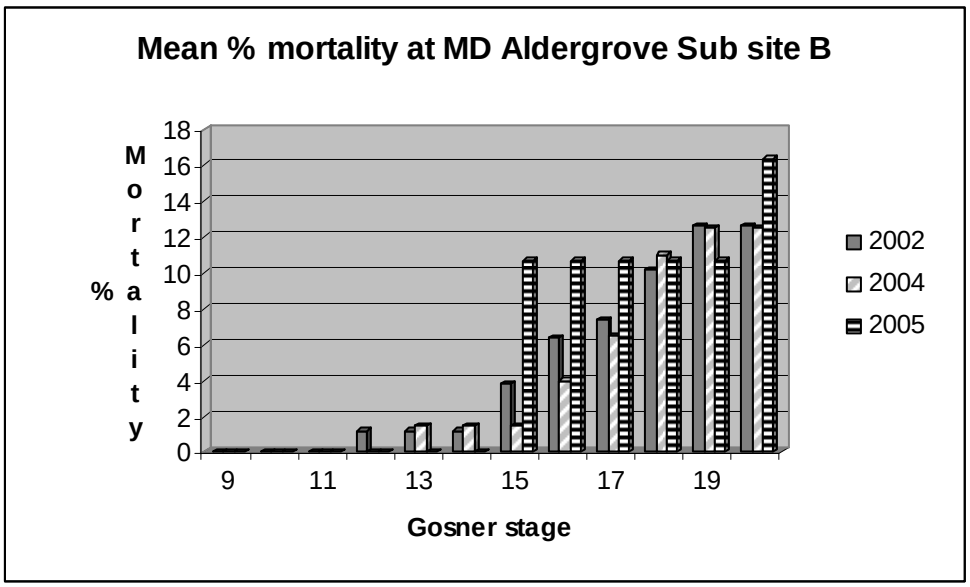

Fig 4.3 - Mean % mortality at each Gosner stage (stage 9 to hatching) for *Rana pretiosa* at MD Aldergrove sub site B, British Columbia, Canada during 2002, 2004 to 2005.

At Seabird Island, by comparison, eggs died throughout development with a slight emphasis on mortality at Gosner stage 17 (tail bud development) to hatching (Fig 4.4).

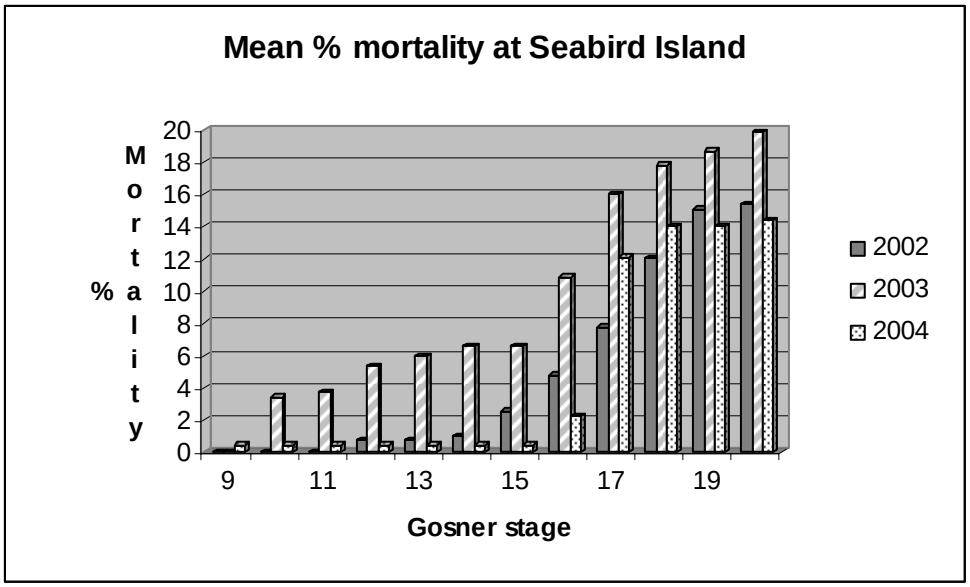

Fig 4.4. Mean % mortality at each Gosner stage (stage 9 to hatching) for *Rana pretiosa* at Seabird Island, British Columbia, Canada during 2002 to 2004.

To determine if there was a year effect on embryonic survivorship, data for Seabird Island and MD Aldergrove were pooled and results were compared among years (2002-2005). Median embryonic survivorship for pooled data for MD Aldergrove and Seabird Island for all years was 82.63% with a range of 100%. Median embryonic survivorship for pooled data for MD Aldergrove and Seabird Island for 2002 was 82.63% with a range of 91.84%, and 86.36% with a range of 100% for 2004. No significant difference in embryonic survivorship, however, was found among years (Kruskal-Wallis Test, $\chi^2 = 3.93$; df = 3; p = 0.27).

In order to examine the representative population at MD Aldergrove, sub site A and sub site B were examined as a single mega-population initially and then individually thereafter. The median embryonic survivorship for pooled data for MD Aldergrove sub site A and sub site B for 2002 was 75.42% with a range of 91.84%, for 2004, the median was 10% with a range of 92.37%, and in 2005 the median was 71.43% and range of 76.32%. Embryonic survivorship at MD Aldergrove (combined data for sub sites A and B), however, was not significantly different among these study years (Kruskal-Wallis Test, $\chi^2 = 1.49$; df = 2; p = 0.47). Median embryonic survivorship for Seabird Island for 2002 was 86.34% with a range of 32%, for 2003 the median was 82.85% with a range of 47.44%, and in 2004 the median was 92.37% with a range of 100%. Embryonic survivorship at Seabird Island, however, was also not found to be significantly different among years (2002-2004) (Kruskal-Wallis Test $\chi^2 = 2.03$ df = 2; p = 0.36).

A comparison of survivorship was also conducted among years for MD Aldergrove sub site A and sub site B separately during 2002, 2004 and 2005. The median for MD Aldergrove sub site A for 2002 was 33.33% with a range of 73.32%, for 2004 the median was 9.52% and range of

2.86% and for 2005 the median was 9.5% and a range of 3.22%. The median for MD Aldergrove sub site B for 2002 was 91.3% with a range of 28.57%, for 2004 the median was 87.78% and range was 2.83%, and in 2005 the median was 77.78% with a range of 12.78%. There was, again, no significant difference in embryonic survivorship among years within either of the two sub sites (Kruskal-Wallis Test, MD Aldergrove sub site A: $\chi^2 = 3.67$; df = 2; p = 0.16; MD Aldergrove sub site B: $\chi^2 = 2.56$; df = 2; p = 0.28), which might be due to the small sample sizes involved.

Median survivorship at MD Aldergrove sub site A and sub site B combined for all years (2002, 2004 and 2005) was also compared with Seabird Island for the same time period. The median embryonic survivorship for MD Aldergrove combined for all years was 71.43% with a range of 92.86% and for Seabird Island the median was 85% and a range of 100%. Embryonic survivorship at MD Aldergrove was significantly lower than Seabird Island over this same time period (Wilcoxon rank sum test, $\chi^2 = 7.62$; df = 1; $p < 0.01$). However, when comparing MD Aldergrove with Seabird Island by year, there was no significant difference in embryonic survivorship data obtained during 2002 or 2004 (Wilcoxon rank sum test, for 2002: $\chi^2 = 2.02$; df = 1; p = 0.16; for 2004: $\chi^2 = 3.2309$; df = 1; p = 0.07). This might be due to the small sample sizes involved for these time periods.

Upon combining data for all years within each sub site (2002-2005) embryonic survivorship was found to be significantly different among MD Aldergrove sub site A, sub site B and Seabird Island (Kruskal-Wallis Test, $\chi^2 = 19.96$, df = 2, $p < 0.0001$). MD Aldergrove sub site A (median 10.56% with a range of 74.37%) exhibited significantly lower survivorship than MD Aldergrove

sub site B (85.29% and range of 28.57%) and Seabird Island (85% and range of 100%); no significant differences were found between sub site B and Seabird Island.

In 2002, embryonic survivorship was significantly different among MD Aldergrove sub site A, sub site B and Seabird Island (Kruskal-Wallis Test, $\chi^2 = 9.58$, df = 2, p = 0.008). The embryonic survivorship at MD Aldergrove sub site A (median 33.3% and a range of 73.3%) was significantly lower than the embryonic survivorship at MD Aldergrove sub site B (91.3% and range 28.5%) and Seabird Island (median 86.34% and range 32%); again, no significant difference was found between the latter two sites. No significant difference in embryonic survivorship among MD Aldergrove sub site A (median of 9.52% and range of 2.86%), sub site B (median of 87.78% and range of 2.83%) and Seabird Island (median: 92.37% and range of 100%) was found in 2004 (Kruskal-Wallis Test, $\chi^2 = 5.23$, df = 2, p = 0.07). Embryonic survivorship at MD Aldergrove sub site A (median: 9.5% and range of 3.22%) was also not significantly lower than embryonic survivorship at MD Aldergrove sub site B (77.78% and range of 12.78%) during 2005 (Wilcoxon rank sum test, $\chi^2 = 3.0$, df = 1, p = 0.0833). The fact that there was no statistical significant difference detected in embryonic survivorship among sites and sub sites during 2004 and 2005 may again be due to the smaller sample sizes involved. Because few clutches were oviposited, the number of egg masses sampled at sub site A and sub site B ranged from two to five per year.

4.2 Water quality

4.2.1 Water chemistry

There were few differences in water chemistry among MD Aldergrove sub site A, sub site B and Seabird Island during 2002 to 2005, based on samples taken half way through the two to three

week breeding season each year. At MD Aldergrove sub site A and sub site B; pH, BOD, chloride, fluoride, sulphate, nitrate, phosphorous (dissolved o-PO4) and conductivity did not show a substantial change in concentration of more than an order of magnitude or at least 100% among study years (2002, 2004 and 2005). Given there is little or no literature on the impacts of these chemicals on *R. pretiosa*, it is assumed here that an order of magnitude change would have the potential to start affecting the species in some capacity. Within sub site A, ammonia, total nitrogen, total dissolved phosphorous, total phosphorous and turbidity were the only parameters to vary at least by 100% or up to an order of magnitude between years. At sub site B, ammonia, total dissolved phosphorous, total phosphorous and turbidity, again, where the only parameters to vary at least by 100% or up to an order of magnitude between years (Table B1, Appendix B). No perceptible trends could be identified with water parameters among study years.

Parameters including BOD, fluoride, sulphate, total nitrogen, conductivity and turbidity were the only parameters to vary at least by 100% or up to an order of magnitude in concentration between years at Seabird Island. Others including pH, chloride, nitrate, phosphorous (o-PO4 dissolved), total dissolved phosphorous and total phosphorous showed no substantial change among study years (2002-2004) (Table B2, Appendix B). No noticeable trends, again, could be identified among water parameters among study years.

For parameters in which Canadian Water Quality Guidelines (CWQG) for the protection of freshwater aquatic life (Canadian Council of Ministers of the Environment [CCME], 2003) were available, only pH was below the CWQG range at MD Aldergrove sub site A and sub site B. At

both sub sites, pH was below CWQG by 1 pH unit in 2002. At sub site A, pH was also below CWQG by 0.04 pH units in 2003 and by 0.24 pH units in 2005. All other variables for MD Aldergrove sub site A and sub site B and Seabird Island were within or below the CWQG for all study years (Table 4.1).

Table 4.1. Mean [± standard deviation (SD)] water chemistry at MD Aldergrove sub site A, sub site B and Seabird Island, British Columbia, Canada during 2002-2005 with method detection limit (MDL) and Canadian Water Quality Guidelines (CWQG). Water samples were collected once during the breeding season during 2002, 2004 to 2005 at MD Aldergrove and at Seabird Island 2002 to 2004.

Compound	**Units**	**MDL**	**CWQG**	**MD Aldergrove sub site A**	**MD Aldergrove sub site B**	**Seabird Island**
pH	pH Units	0.01	6.5-9.0	6.08 ± 0.49	6.5 ± 0.83	7.02 ± 0.08
BOD	mg/L	5	-	6.33 ± 0.58	8 ± 2	11.33 ± 4.04
Cl	mg/L	0.1	-	1.03 ± 0.32	0.63 ± 0.49	1.83 ± 0.32
F	mg/L	0.01	0.12	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
SO_4	mg/L	0.5	-	< 0.5	< 0.5	3.30 ±3.17
Br	mg/L	0.05	-	<0.05	<0.05	<0.05
NO_3	mg/L	0.002	13	0.004 ± 0.002	0.002 ± 0.002	0.0043 ± 0.0031
NO_2	mg/L	0.005	0.06	<0.005	<0.005	<0.005
PO_4	mg/L	0.05	-	<0.05	<0.05	<0.05
NH_3	mg/L	0.005	1.37-2.2	0.007 ± 0.005	0.01 ± 0.011	0.15 ± 0.25
Nitrogen Total	mg/L	0.04	-	1.48 ± 0.89	0.5 ± 0.09	1.71 ± 1.91
Phos. o-PO4 diss.	mg/L	0.001	-	0.001 ± 0.0009	0.001 ± 0.0009	<0.001
Total Dissolved Phosphorus	mg/L	0.002	-	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.002
Total Phosphorus	mg/L	0.002	-	0.21 ± 0.15	0.13 ± 0.15	0.08 ± 0.1
Conductivity	µS/cm	2	-	29.33 ± 8.14	37.33 ± 5.51	125.33 ± 47.18
Turbidity	NTU*	0.05	-	11.22 ± 11.79	3.22 ± 1.85	2.3 ± 1.03

*NTU, Nephelometric turbidity units. "-"= no CWQG exists.

The water chemistry data for 2002, 2004 and 2005 at MD Aldergrove sub site A and sub site B

and for 2002-2004 at Seabird Island were combined within years and compared among sites and sub sites. Parameters including BOD, fluoride, nitrate, ammonia, total nitrogen, phosphorous o-PO4 dissolved, total dissolved phosphorous, total phosphorous and turbidity were not significantly different among sites for all years. Only sulphate was significantly different among MD Aldergrove sub site A and sub site B (both < 0.5 mg/l) and Seabird Island (0.9 mg/l to 6.9 mg/l) for all years (Kruskal-Wallis Test, $\chi^2 = 7.62$, df = 2, p=0.022). Chloride ranged from 0.8 to 1.4 mg/l at MD Aldergrove sub site A, from 0.3 to 1.2 mg/l at sub site B and from 1.6 to 2.2 mg/l at Seabird Island. At MD Aldergrove sub site A, pH ranged from 5.53 to 6.46, at sub site B from 5.54 to 7.04 and at Seabird Island from 6.93 to 7.07. Conductivity varied between 20 and 35 µS/cm at MD Aldergrove sub site A, 32 and 43 µS/cm at sub site B, and between 71 and 156µS/cm at Seabird Island. Chloride (Kruskal-Wallis Test, $\chi^2 = 5.96$, df = 2, p = 0.051), pH (Kruskal-Wallis Test, $\chi^2 = 5.11$, df = 2, p=0.078) and conductivity (Kruskal-Wallis Test, $\chi^2 = 5.96$, df = 2, p=0.051) were marginally significantly different among sites.

Of these four water chemistry variables that were significantly different (or marginally so) among sites and sub sites, significant correlations with embryonic survivorship were not found for pH (Spearman Rank correlation, r = 0.319, p = 0.07) and sulphate (Spearman Rank correlation, r = 0.169, p = 0.12) A weak but significant positive correlation, however, was found between chloride and embryonic survivorship (Spearman Rank correlation, r = 0.278, p = 0.005) (Fig 4.5.) as well as between conductivity and embryonic survivorship (Spearman Rank correlation, r = 0.336, p = 0.031) (Fig 4.6.).

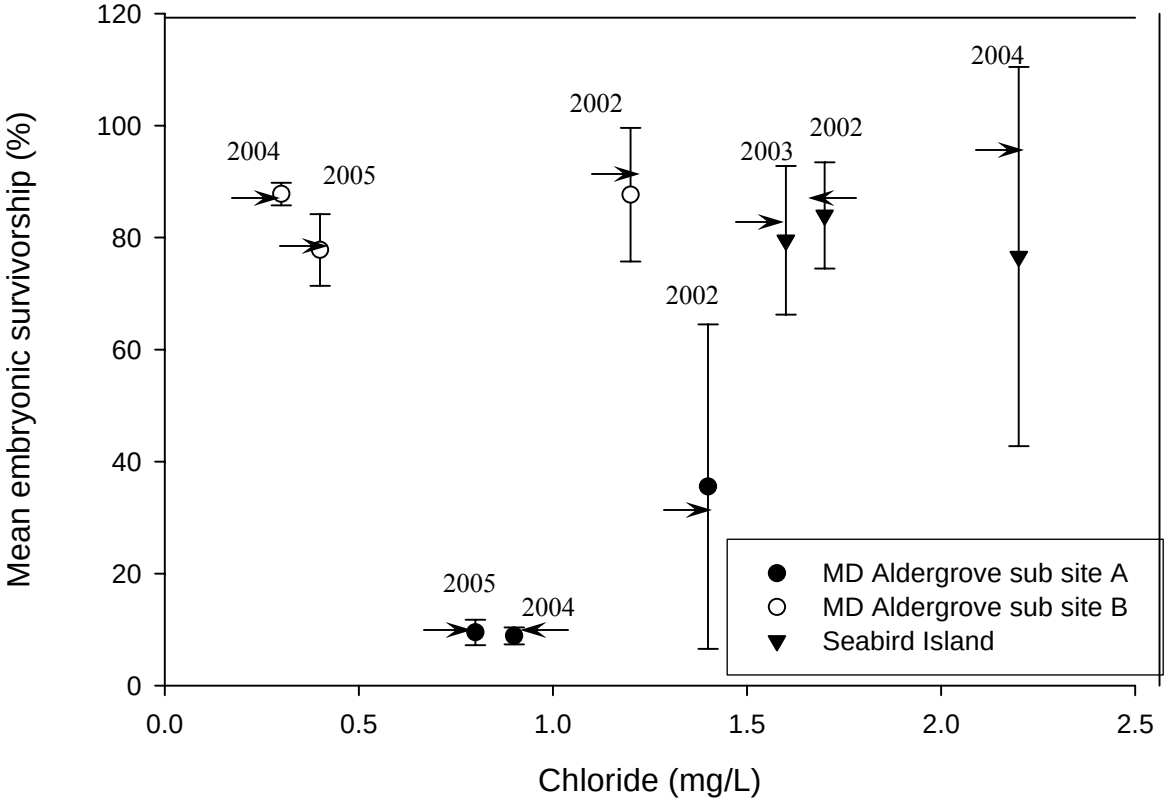

Fig 4.5 - Spearman Rank correlation between chloride and mean embryonic survivorship (%) [± standard deviation (SD)] of *Rana pretiosa*. Median values are indicated with an arrow (→).

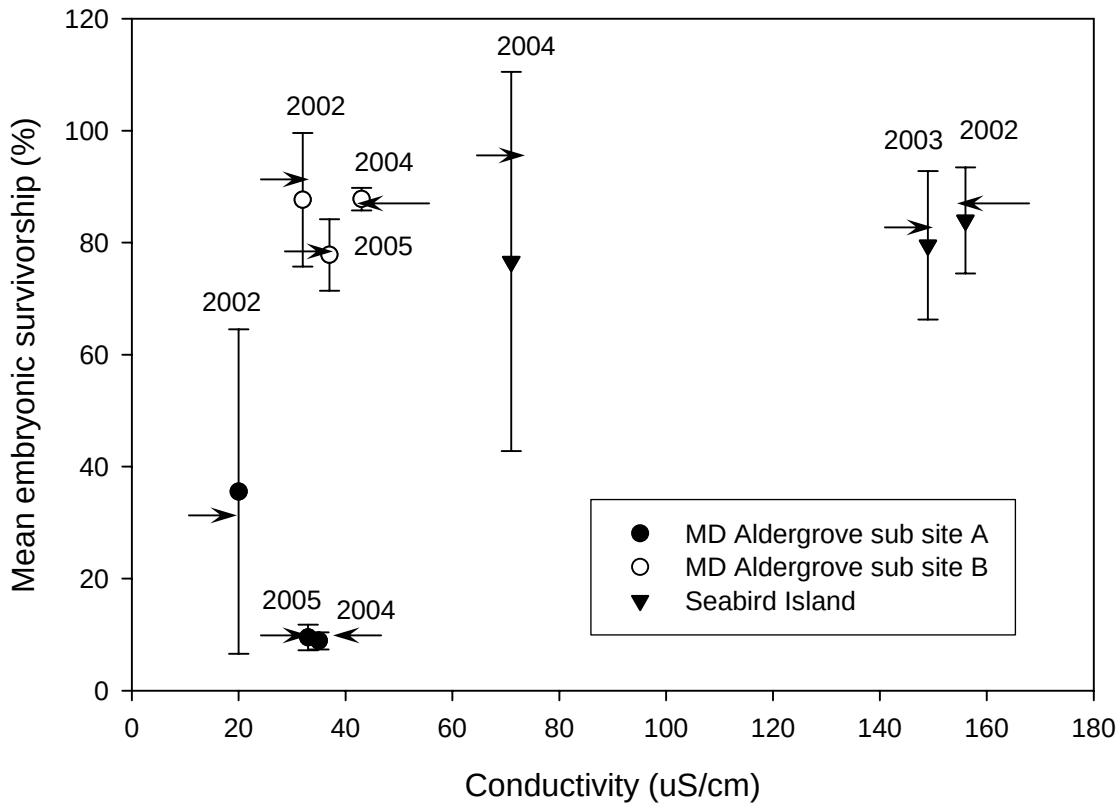

Fig 4.6 - Spearman Rank correlation between conductivity and embryonic survivorship (%) [± standard deviation (SD)] of *Rana pretiosa*. Median values are indicated with an arrow (→).

There was also a weak but significant positive correlation between chloride and conductivity (Spearman Rank Correlation, $r = 0.420$, $p < 0.0001$).

4.2.2 Trace metals

Most trace metals did not show a substantial change in concentration of more than an order of magnitude or at least 100% among study years (2004 and 2005). At sub site A, iron, silicon, cadmium, manganese and selenium were the only parameters to vary by at least 100% or up to an order of magnitude in concentration between years. At sub site B, aluminium, cadmium,

manganese, nickel and selenium were the only parameters to vary by at least 100% or up to an order of magnitude between years (Table B3, Appendix B). No trends could be identified among trace metals between study years.

At MD Aldergrove sub site A, iron exceeded the CWQG by a small degree in 2004 and 2005, and aluminium exceeded the CWQG by a larger degree in 2004 and in 2005. At sub site B, iron exceeded the CWQG by a small degree in 2004 and 2005 and aluminium exceeded the CWQG by a larger degree in 2004 and 2005. At sub site A, cadmium exceeded the CWQG in 2004 and, in 2005, chromium and copper exceeded the CWQG by a small degree (Table 4.2.).

Table 4.2. Mean [± standard deviation (SD)] trace metals at MD Aldergrove sub site A, sub site B and Seabird Island, British Columbia during 2004 and 2005 with method detection limit (MDL) and Canadian Water Quality Guidelines (CWQG). Water samples were collected once during the breeding season during 2004 and 2005 at MD Aldergrove and during 2004 at Seabird Island. "-"= No CWQG available.

Compound	**Units**	**MDL**	**CWQG**	**Sub site A**	**Sub site B**	**Seabird Island**
Calcium (Ca)	mg/l	0.1	-	3.60 ± 0.28	3.45 ± 0.21	7.8
Iron (Fe)	mg/l	0.006	0.3	1.01 ± 0.66	0.37 ± 0.0035	0.516
Potassium (K)	mg/l	0.1	-	0.80 ± 0.57	0.45 ± 0.07	1.4
Silicon (Si)	mg/l	0.06	-	1.98 ± 1.05	1.44 ± 0.14	0.96
Sodium (Na)	mg/l	0.1	-	1.8 ± 0.28	1.55 ± 0.07	0.8
Sulphur (S)	mg/l	0.06	-	0.46 ± 0.35	0.15 ± 0.01	0.31
Titanium (Ti)	mg/l	0.002	-	0.02 ± 0.01	0.01 ± 0.01	<0.002
Aluminium (Al)	µg/l	0.2	5-100	442.5 ± 135.06	249.0 ± 128.69	23.3
Antimony (Sb)	µg/l	0.005	-	0.07 ± 0.02	0.05 ± 0.02	0.059
Arsenic (As)	µg/l	0.1	5	0.50 ± 0.14	0.45 ± 0.07	0.9
Barium (Ba)	µg/l	0.02	-	10.69 ± 3.56	5.57 ±0.42	11.5
Beryllium (Be)	µg/l	0.002	-	0.05 ± 0.03	0.05 ± 0.04	0.052
Bismuth (Bi)	µg/l	0.02	-	0.02 ± 0.01	0.02 ± 0.01	<0.02
Boron (B)	µg/l	2	-	5.0 ± 1.41	6.0 ± 2.83	7
Cadmium (Cd)	µg/l	0.01	0.017	0.01 ± 0.01	0.01 ± 0.0035	0.01
Chromium (Cr)	µg/l	0.2	1	1.2 ± 0.57	0.6 ± 0.14	<0.02
Cobalt (Co)	µg/l	0.005	-	0.46 ± 0.36	0.17 ± 0.06	0.125
Copper (Cu)	µg/l	0.05	2-4	3.69 ± 0.62	3.05 ± 0.91	2.54
Lead (Pb)	µg/l	0.01	1-7	0.54 ± 0.09	0.29 ± 0.04	0.33
Lithium (Li)	µg/l	0.05	-	0.27 ± 0.14	0.14 ± 0.02	0.24
Magnesium (Mg)	µg/l	0.05	-	1460.0 ± 14.14	1575.0 ± 134.35	1610
Manganese (Mn)	µg/l	0.005	-	53.15± 47.45	36.05±27.93	36.8
Molybdenum (Mo)	µg/l	0.05	73	0.11 ± 0.08	0.14 ± 0.08	0.59
Nickel (Ni)	µg/l	0.05	25-150	2.14 ± 1.03	4.58 ± 5.25	5.61
Selenium (Se)	µg/l	0.2	1	0.26 ± 0.35	0.21 ± 0.28	<0.2
Silver (Ag)	µg/l	0.02	0.1	0.06 ± 0.01	0.05 ± 0.01	0.03
Strontium (Sr)	µg/l	0.005	-	25.55 ± 3.18	23.1 ± 1.41	27.5
Thallium (Tl)	µg/l	0.002	0.8	0.02 ± 0.01	0.01 ± 0.01	0.004
Tin (Sn)	µg/l	0.01	-	0.11 ± 0.06	0.15 ± 0.06	0.11
Uranium (U)	µg/l	0.002	-	0.02 ± 0.0007	0.01 ± 0.0035	0.016
Vanadium (V)	µg/l	0.05	-	1.21 ± 0.31	0.69 ± 0.21	0.31
Zinc (Zn)	µg/l	0.1	30	11.95 ± 2.62	3.15 ± 0.78	3.4

At Seabird Island, trace metals were only measured in 2004 during which titanium, bismuth, chromium, and selenium were below the MDL (MDL for titanium was 0.002 mg/l, bismuth 0.02µg/l, chromium and selenium 0.2 µg/l). Iron exceeded the CWQG by 0.216 mg/l in 2004 (Table 4.2, see alsoTable B4, Appendix B).

4.2.3 Coliforms

Total coliforms at MD Aldergrove during 2002 and 2004 to 2005 ranged from less than the MDL (< 1 col/100ml) to 370 col/100 ml and from < 1 to 350 col/100ml at Seabird Island during 2002 to 2004. Fecal coliforms ranged from less than the MDL (< 1 col/100ml) to 16 col/100ml within the MD Aldergrove sub sites and, at Seabird Island, it was consistently less than the MDL (Table 4.3). No CWQG guidelines currently exist for coliforms. The summary for total and fecal coliforms for MD Aldergrove sub site A, sub site B and for Seabird Island during 2002 to 2005 is provided in Tables B5 and B6 (Appendix B).

Table 4.3. Mean [± standard deviation (SD)] total and fecal coliforms at MD Aldergrove sub site A, sub site B and Seabird Island, British Columbia, Canada during 2002-2005 with method detection limit. No CWQG available. At MD Aldergrove water samples were collected once during the breeding season during 2002, 2004 to 2005 and at Seabird Island 2002 to 2004. "-"= No CWQG available.

Compound	**Units**	**CWQG**	**Sub site A**	**Sub site B**	**Seabird Island**
Total coliforms	col/100ml	-	133.5 ± 205.35	42.83 ± 66.93	116.7 ± 202.04
Fecal coliforms	col/100ml	>200*	6.83 ± 8.13	2.17 ± 1.76	<MDL all years

Year	**MDL Total coliform**	**MDL Fecal coliform**
2002	1 col/100ml	1 col/100ml
2003	1 col/100ml	1 col/100ml
2004	10 col/100ml	2 col/100ml
2005	2 col/100ml	2 col/100ml

* From Water quality evaluation of agricultural runoff in the Lower Fraser Valley (1994).

At MD Aldergrove sub site A, total coliforms ranged from less than the MDL in 2002, peaking at 370 col/100ml in 2004 and decreasing again to 30 col/100ml in 2005. Total coliforms at sub site B showed a similar trend, increasing from less than the MDL in 2002, peaking to 120 col/100ml in 2004 and then a decreasing again to 8 col/100ml in 2005. Fecal coliform concentrations changed slightly among years at sub site A, increasing from less than the MDL in 2002 to 16 col/100ml in 2005. At sub site B, fecal coliforms also increased slightly from less than the MDL in 2002 to 4 col/100ml in 2005. At Seabird Island, only total coliforms showed a substantial change by increasing from less than the MDL, in 2002 and 2003, to 350 col/100ml in 2004.

Total coliforms for 2002, 2004 and 2005 combined did not differ significantly among MD Aldergrove sub site A, sub site B and Seabird Island (Kruskal-Wallis Test, $\chi^2 = 0.51$, df = 2, p=0.7753); neither did fecal coliform (Kruskal-Wallis Test, $\chi^2 = 2.06$, df = 2, p = 0.3579).

4.2.4 Water temperature and dissolved oxygen

MD Aldergrove sub site A and sub site B minimum mean water temperature was slightly cooler (1 to 1.5 °C) than Seabird Island (Table 4.4). The maximum mean water temperature at Seabird Island was between 4 and 6 °C warmer than at MD Aldergrove sub site A and sub site B, while the mean water temperature was between 1.5 and 2.5 °C warmer.

Table 4.4. Mean [± standard deviation (SD)] water temperature at MD Aldergrove sub site A, sub site B and Seabird Island, British Columbia, Canada during 2002 and 2005. Water temperature was measured at MD Aldergrove during 2002 and 2005 and at Seabird Island during 2002. During 2002, water temperature was recorded every 5 minutes and during 2005, twice a week. n = # of year temperature data collected.

Temperature	Sub site A	Sub site B	Seabird Island
Mean ºC	10.01 ± 1.67	9.12 ± 1.27	11.58 ± 1.90
Minimum ºC	6.36 ± 2.28	6.74 ± 1.85	7.71 ± 1.92
Maximum ºC	11.58 ± 1.90	7.71 ± 1.92	18.8 ± 4.57
n (sample size/years)	2	2	1

At MD Aldergrove sub site A, the minimum water temperature in 2002 varied between 4.17 and 10.84 ºC, the maximum water temperature varied between 8.21 and 21.47 ºC, and the mean water temperature varied between 6.72 and 12.17 ºC. During 2005, the trend at this sub site was similar with minimum water temperature ranging from 3 to 9 ºC, the maximum water temperature ranging from 14 to 18 ºC and the average water temperature ranging from 8 to14 ºC.

At MD Aldergrove sub site B, the minimum water temperature varied between 3.79 and 10.63 ºC, the maximum water temperature varied between 8.02 and 15.95 ºC and mean water temperature varied between 7.09 and 11.26 ºC during 2002. Again, during 2005, the trends were similar to 2002 with minimum water temperature ranging from 3 to 9 ºC, the maximum water temperature ranging from 11 to 18 ºC and the average water temperature ranging from 7.33 to 14 ºC within MD Aldergrove sub site B.

At Seabird Island the minimum water temperature ranged from 4.79 to 10.86 ºC, the maximum water temperature ranged from 13.93 to 29.14 ºC and the average water temperature ranged from 8.41 to 14.83 ºC during 2002.

Dissolved oxygen (DO) during 2005 at MD Aldergrove sub site A ranged from 5 to 11.7 mg/l. At sub site B, the DO ranged from 10.8 to 15.8 mg/l. The low value of 5mg/l at sub site A was below the CWQG and the high value of 11.7 mg/l exceeded the CWQG. All values at sub site B exceeded the CWQG by 1.3 mg/l to 6.3 mg/l (Table 4.5).

Table 4.5. Mean [± standard deviation (SD)] dissolved oxygen (DO) at MD Aldergrove sub site A and sub site B, British Columbia, Canada for 2005.
DO readings were taken twice a week during the 3 to 4 week of egg development

Compound	**Units**	**CWQG**	**Sub site A**	**Sub site B**
DO	mg/l	5.5-9.5	7.18 ±2.33	12.73 ± 2.12

4.3 Summary

In summary, embryonic survivorship at MD Aldergrove sub site A was consistently and significantly lower than at MD Aldergrove sub site B and at Seabird Island during 2002 to 2005. Embryonic survival at MD Aldergrove sub site B and Seabird Island was consistently similar. Field observations indicated that all eggs were fertile and developed normally until about Gosner stage 12, after which mortalities occurred. There was no significant difference in most of the water chemistry parameters among sites and sub sites. Water temperature was also within the tolerable limits of 6 – 28 °C for *Rana pretiosa* (Licht, 1971). Sulphate, pH, conductivity and chloride were the only water chemistry parameters which differed significantly (or marginally significantly) among sites and sub sites. MD Aldergrove sub site A and sub site B had lower chloride and conductivity levels than Seabird Island. There was a weak, but significantly positive correlation between chloride and embryonic survivorship and between conductivity and

embryonic survivorship and a weak put significantly positive correlation between chloride and conductivity.

5. Discussion

5.1 Embryonic survivorship

At MD Aldergrove sub site B and Seabird Island, mean embryonic survivorship was 77% to 88%. In a study on embryonic survivorship of *R. pretiosa* and *R. aurora* in British Columbia, Licht (1974), determined embryonic survivorship of *R. pretiosa* in the wild, and not in captivity, to be between 68% and 74% (Licht, 1974). McAlister & White (2001) estimated that embryonic survivorship of *R. pretiosa* at most sites at Beaver Creek in Washington to be higher, at 99% to 100%. The embryonic survivorship at MD Aldergrove sub site B and Seabird Island would appear to be within the range reported by many studies of Ranids and salamanders in the wild, including *R. sylvatica* with 92%-97% success (Seigel, 1983), *R. aurora* with 91%-92% success (Licht, 1974), *R. macrocnemis* with 62% to 88% success (Tarkhnishvili & Gokhelashvili, 1999) and spotted salamander (*Ambystoma maculatum)* with 80% success (Portnoy, 1990).

R. pretiosa lay eggs in communal oviposition sites in shallow water, usually with the top row of eggs exposed (Corkran & Thoms, 1996; Licht, 1969b; McAlister et al., 1993 and Svihla, 1935). One of the major threats to embryos survival is desiccation or freezing which can result in mass mortality of *R. pretiosa* embryos (Haycock, 2000; Licht, 1971 and 1974). However, this was not the case during the study years 2002, 2004 and 2005 at MD Aldergrove sub site A and the low survival rate of 9% to 35% can not be explained by desiccation or freezing. Temperatures were never low enough to cause freezing and no ice was detected during any of the visits.

R. pretiosa usually breed in February and March, soon after snow melt (Licht, 1971). During this time, there is usually a wide temperature variation with low and often freezing temperatures

during the night and high temperatures during the day; therefore, the embryos must withstand low and fluctuating temperatures (Licht, 1971). The lethal thermal limits for young *R. pretiosa* embryos are about 6 – 28 °C (Licht, 1971). As the embryos become older, the tolerance limit broadens and older *R. pretiosa* embryos can survive short term exposure to normally lethal, cold temperatures (Licht, 1971). During this study, temperatures at all sites and sub sites were within the temperature tolerance limits for *R. pretiosa*. Mean minimum temperature at MD Aldergrove sub site A and sub site B were both 6 °C and 7 °C at Seabird Island, while the mean maximum temperature varied between 12 °C and 18 °C among the sites and sub sites. On a few occasions (2-4 times during a study year), minimum temperatures dropped to 4 °C for four to six hours but this would not have a negative effect on embryonic survivorship because *R. pretiosa* embryos can withstand temperatures of 1 °C for up to eight hours (Licht, 1971).

Water levels ranged between 6 and 21 cm and were therefore high enough during the breeding season to prevent desiccation (pers. obs.). When water levels dropped for a short period of time at Seabird Island, during 2002, vegetation underneath the egg masses was carefully removed to prevent desiccation (Haycock, 2003).

Field observations indicated that egg mortality at MD Aldergrove sub site A occurred mainly during Gosner stages 14 to 17. At MD Aldergrove sub site B, the highest mortality was between Gosner stages 16 to hatching and at Seabird Island mortality occurred throughout development with a slightly higher percentage during Gosner stage 17 to hatching. During stages 14 to 15, the neural folds become ridges lateral to the neural groove, the body becomes elongated and the embryo begins to rotate (Duellman & Trueb, 1994). During stage 16, the neural folds close to

form the neural tube which is the origin of the central nervous system (brain and spinal cord) (Duellman & Trueb, 1994 and Villee, 1977). The tail bud, adhesive organs and gills develop during stages 17 to hatching. The sensitive stages in this study are slightly in contrast with other studies that found mortality occurred at early stages such as Gosner stages 8 to 10 (mid cleaveage to early gastrulation) (Beattie & Tyler-Jones, 1992) in *Rana temporaria* and Gosner stage 10 to12 (gastrulation) in *Pseudophryne* (Woodruff, 1976). In *Rana temporaria,* at pH 4.5 and pH 6.0, the most sensitive stages were Gosner stage 10 to 12 (gastrulation) and hatching (Beattie et al., 1992). In another study, most eggs of *R. temporaria* at a low pH of 4.5 died at earlier stages (Gosner 7 to12), while at a moderate pH of 5 the highest mortality occurred at later stages (Gosner 18 to 25) (Leuven et al., 1986). In *Heleioporus albopunctatus*, no relationship was found between egg mortality and developmental stage (Davis & Roberts, 2005). More studies appear warranted to determine the most sensitive stage for *R. pretiosa.*

5.2 Water quality

Sulphate, pH, conductivity and chloride were the only water chemistry parameters that differed significantly (or marginally significantly) among sites and sub sites. Amphibians are most sensitive to low pH during the fertilization and embryonic development stage (Beattie & Tyler-Jones, 1992; Beattie et al., 1992; Clark & Hall, 1985 and Pierce et al., 1984). Low pH can cause developmental abnormalities (Leuven et al., 1986; Pierce, 1985; Pierce et al., 1984; Portnoy, 1990 and Pough & Wilson, 1977) causing embryos to become curled within the shrunken perivitelline space (the space between the egg yolk and membrane) and failing to hatch (Dunson & Connell, 1982; Freda & Dunson, 1985; Leuven et al., 1986 and Pierce et al., 1984). Low pH levels can also cause a delay in hatching (Clark & LaZerte, 1985), delay in embryonic

development (Andrén et al., 1988; Pahkala et al., 2001 and 2002; Portnoy, 1990 and Räsänen et al., 2002) and increased fungal growth on eggs (Clark & LaZerte, 1985; Leuven, et al., 1986 and Strijbosch, 1979). Acidity can even cause the embryos to stop developing altogether, leading to high mortalities, and low hatching success (Andrén et al., 1988; Beattie et al., 1992; Dunson & Connell, 1982; Freda & Dunson, 1985; Leuven et al., 1986; Pahkala et al., 2001 and Rowe & Dunson, 1993). This can affect the overall survivorship of amphibian populations (Saber & Dunson, 1978). These lethal and sub lethal effects of pH were usually observed at pH < 4.5 (Beattie et al., 1992; Dunson & Connell, 1982; Freda & Dunson, 1985; Leuven et al., 1986; Pierce et al., 1984; Potnoy, 1990 and Pough & Wilson, 1977;). In this study, deformities were negligible and the lowest pH was relatively high at pH 5.53, indicating pH was not a factor alone in hatching success.

pH, in combination with aluminium, can also cause embryonic mortalities (Beattie & Tyler-Jones, 1992; Beattie et al., 1992 and Clark & LaZerte, 1985). These lethal effects were usually observed at a pH less than 4.5 (Beattie & Tyler-Jones, 1992; Beattie et al., 1992; Clark & LaZerte, 1985; Jung & Jagoe, 1995; and Leuven et al., 1986). Inorganic monomeric aluminium that was toxic to *Rana sylvatica* and *Bufo americanus* eggs at a pH less than 4.5 had no effect at a pH more than 4.5 (Clark & LaZerte, 1985) and aluminium that might have been toxic at pH 4.3 was not toxic at pH 4.8 (Clark & Hall,1985). In another experiment, using a pH of 4.5 and aluminium concentration of 400 µg/l, mortality of newly hatched tadpoles of the green tree frog (*Hyla cinerea*) was more than 80%, while mortality at the same aluminium concentration decreased to less than 10% at pH 5.5 (Jung & Jagoe, 1995).

Aluminium undergoes speciation with changing pH (Beattie et al., 1992). Inorganic monomeric aluminium, which predominantly occurs at low pH, has the greatest aquatic toxicity (Beattie et al., 1992; Clark & LaZerte, 1985 and Driscoll et al., 1980). Due to the solubility of aluminium, it is also expected that there will be a higher concentration of dissolved aluminium in water with a low pH (Rowe & Dunson, 1993). The lowest pH measured in this study was pH 5.53 and aluminium concentrations were between 23.3 µg/l at Seabird Island and 538 µg/l at MD Aldergrove sub site A. A study on *R. temporaria* embryos indicated that even aluminium levels as high as 1600 µg/l at pH 4 did not affect survival (Olsson et al, 1987). Embryonic survival of Swedish brown frogs (*R. arvalis, R. temporaria* and *R. dalmatina*) was also not affected by aluminium concentration as high as 800 µg/l at pH 4, 5 and 6; but there was an increase in deformities (Andrén et al., 1988). Wood frog (*R. sylvatica*) embryo survival was more than 80% at 300 µg/l and pH 4.6, and it is suggested that aluminium is not a major threat to most amphibian populations (Corn et al., 1989). Given that pH in this study was never below pH 5.5, it is unlikely that the combination of pH and resulting (monomeric) aluminium concentrations would have had any significant effect on embryonic survivorship observed in this study.

Conductivity is an indication of water's ability to conduct an electric current and is directly linked to the amount of total dissolved salt (TDS) (Wetzel, 1975 and http://waterontheweb.org). The majority of TDS comes from four positive ions; namely, calcium, magnesium, sodium and potassium; and from four negative ions namely, bicarbonate, carbonate, chloride and sulphate (Wetzel, 1975). Since chloride is one of the major negative ions contributing to conductivity (Wetzel, 1975 and http://waterontheweb.org), it probably explains the positive correlation between chloride and conductivity that was found in this study.

Once amphibian eggs are laid, the capsule swells because of the uptake of water. This is important as a reservoir for the developing embryos (Duellman & Trueb, 1994). The ionic concentration of the water affects the rate and extent of the swelling of the capsule surrounding the egg (Duellman & Trueb, 1994). Beattie (1980) found a negative correlation between capsule size and ionic concentration. Changes in ion concentration represented by conductivity could also affect embryos because differences in ion concentration between the inner egg membrane and the outside water influence the flow of water across the egg membrane (Zug, 1993). This flow is important because it can provide dissolved oxygen to the embryos as well as flushing toxic ammonia waste from the inner capsule (Duellman & Trueb, 1994). Embryonic enzymatic system functioning might also be affected by high ion concentrations (Freda & Dunson, 1985). Ions are also essential for cell development, cell functions and maintaining the osmotic relationship between the cell and its environment (Hovingh, 1993 and Villee, 1977).

Conductivity levels that are too high or too low can have a negative influence on the survival, growth or reproduction of aquatic organisms (Michaud, 1991; Welch, 2005 and http://waterontheweb.org). Spawning site selection by *R. temporaria* depended upon conductivity, salinity values and chloride concentration (Viertel, 1999). Studies on spawning site selection indicated that water with a conductivity of 664 µS/cm (Viertel, 1999) and 606 µS/cm (Beebee, 1983) were selected by amphibians as spawning sites. Embryonic mortality of *R. temporaria* was low (< 5%) in water with conductivity levels as high as 3100 µS/cm (Viertel, 1999). *Ambystoma maculatum* bred successfully (80% success) in ponds with a mean conductivity of 57 µS/cm (Portnoy, 1990). *Ambystoma maculatum*, conversely, had a significantly higher embryonic survivorship in ponds with conductivity ranging from 20 µS/cm

to 38 µS/cm than in ponds with conductivity ranging from 470 µS/cm to 1654 µS/cm (Turtle, 2000). Turtle (2000) also found a pattern of high specific conductance and sodium and chloride concentrations, and low embryonic survivorship. In Utah, the associations between the presence of Columbia spotted frogs (*Rana luteiventris*) and conductivity were mixed (Welch, 2005). Four out of the six study sites showed a negative association while two showed a positive association (Welch, 2005). Conductivity at MD Aldergrove sub site A and sub site B ranged from 20 µS/cm to 43 µS/cm, while at Seabird Island conductivity was higher varying from 71µS/cm to 159 µS/cm. These levels seem to be within the levels associated with successful amphibian reproduction. The site with the lowest conductivity had the lowest embryonic survivorship, which is in contrast with other studies (Turtle 2000). There seems to be inconsistency among studies regarding the effect of conductivity and amphibians because different species have different tolerance levels to salt and conductivity (Viertel, 1999). The effects of conductivity might also be confounded by other chemical, biological and/or physical factors (Wetzel, 1975 and http://waterontheweb.org).

Based on Canadian Water Quality Guidelines (CWQG), there were no exceptionally high levels of trace metals at any of the sites and most of the trace metals were within the CWQG for aquatic life. CWQG are based on the most sensitive plant and animal species that occur in Canadian waters and the guidelines are expected to protect 100% of species in Canadian water, 100% of the time (Canadian Council of Ministers of the Environment [CCME], 2003). The only exceptions were aluminium, iron, cadmium, chromium and copper. Although these trace metals exceeded the CWQG, when compared with a different set of water quality guidelines, namely the U.S. National Ambient Water Quality Criteria for the Protection of Aquatic Life (NAWQC),

they were all still within these guidelines (Suter, 1996 and http://www.epa.gov). Although these guidelines are less stringent than the CWQG, these guidelines should still protect most aquatic species for the majority of time with sound confidence (Suter, 1996). In most cases, toxicity tests for these guidelines were performed on fish, daphids, aquatic plants and, in a few cases, on nondaphid invertebrates. Cadmium concentrations in this study were also still far below the limits of cadmium of 0.786 µg/l that cause mortality in bull trout (*Salvelinus confluentus*) embryos reported elsewhere (Hansen et al, 2002). Copper concentrations, similarly, were below levels of 0.085 mg/l that caused mortality in *Bufo arenarum* embryos (Herkovits & Helguero, 1998). It is therefore expected that these trace metals were still within acceptable levels for anurans.

Oxygen is also critical to the development of amphibian eggs (Duellman & Trueb, 1994) and oxygen can be a trigger for hatching (DiMichele & Taylor, 1980; Petranka et al., 1982 and Warkentin, 2002). Low environmental oxygen can cause accelerated hatching in amphibians and fish (DiMichele & Powers, 1984; Garside, 1959 and Petranka et al., 1982). In this study, DO levels were within or above the CWQG by a small margin and it is therefore not expected that low oxygen levels were a factor in embryonic survivorship of *R. pretiosa.* DO levels were also above the levels of 5.8mg/l reported critical for *R. cyanophylictis* (Marian et al., 1980) and 4.3mg/l for *R. pipiens* (Wassersug & Seibert, 1975).

Not all water quality parameters were measured during this study. For example, pesticides were not measured because there is no known large scale or point source close to any of the study sites. The MD Aldergrove site is surrounded by mowed fields, natural areas and roads while the Seabird Island site is surrounded by roads, residential housing, small scale agriculture and

natural areas. However, due to long-distance atmospheric transport of anthropogenic contaminants, e.g., from intensive agricultural land use in the Lower Fraser Valley, pollutants can still enter the watershed and bioaccumulate in amphibians. This has been documented in numerous studies in the Arctic (Bard, 1999; Barrie, 1986 and Rahn, 1981), amphibians in the Sierra Nevada mountain range (Angermann et al., 2002) and in agricultural areas in the Lower Fraser valley in British Columbia (de Solla, 2002). Although it is probably not a major factor contributing to water quality in this study, it cannot be wholly disregarded.

5.3 Project implications

The Oregon spotted frog Recovery Team (OSFRT) was established in 1999 to coordinate projects to protect *R. pretiosa* and it consists of members from a variety of organizations including federal and provincial governments, first nations and universities (WLAP, 2002). One of the team's first priorities is to monitor breeding sites (WLAP, 2002) and this study is therefore part of and contributes to the ongoing monitoring of the breeding of *R. pretiosa*. The OSFRT was also responsible for the Oregon spotted frog recovery plan. The long term goal of the OSFRT is to down-list the species from "Endangered" to "Threatened" (Haycock, 2001). Endangered species face imminent extinction or extirpation (http://www.cosewic.gc.ca/eng/sct5/index_e.cfm) and by down listing *R. pretiosa* to "Threatened" removes the species one step further away from extinction or extirpation. The OSFRT also determined a number of short term goals including the monitoring and protection of the existing populations, habitat protection and monitoring, on going surveys for new sites and conducting research that will contribute to the recovery of the species (Haycock, 2001). The team is also involved in captive breeding and is also considering reintroduction programs (WLAP, 2002).

This project contributes towards achieving some of the short term goals of the OSFRT. There are still huge data gaps in the knowledge of the existing populations of *R. pretiosa* (Haycock, 2001). The embryonic survivorship data in this study provides data for evaluating the current reproductive and health status of the populations. This is essential since survivorship can affect adult recruitment into the population. The research also provides survivorship data for population viability analyses (PVA)[1]. Survival and fecundity data is important for population viability analyses (Akçakaya, 2002).

Through this project, it was determined that MD Aldergrove sub site B and Seabird Island have normal embryonic survivorship while MD Aldergrove sub site A has a relatively low embryonic survivorship. MD Aldergrove sub site A should therefore be a high priority for future projects to determine other factors contributing to low embryonic survivorship and mitigate these. Embryonic survivorship for *R. pretiosa* in the wild in south-western British Columbia has not been determined since the late 1960's and early 1970's. This study has now provided more recent embryonic survivorship data.

It is important to have knowledge of the physical habitat and environmental parameters that might affect population demographics in order to develop successful conservation strategies and promote sustainability of the species (Scribner et al., 2001 and Welch, 2005). There is also a need for assessing the qualitative and quantitative habitat requirements of *R. pretiosa* as part of the recovery criteria of *R. pretiosa* (Haycock, 2001). Oviposition habitat was identified as critical

[1] PVA is the process of identifying the threats faced by a species, usually threatened or endangered, evaluating the likelihood of that species existing for a specific time into the future, and assessing the chances of recovery for the species (Akçakaya, & Sjögren-Gulve, 2000 and http://www.ramas.com/pva.htm).

habitat (Haycock, 2000) and it is therefore essential to determine the characteristics of the oviposition habitat. The monitoring of water quality provided data on some of the microclimatic characteristics of the three breeding sites and sub sites. This water quality data can be used as bench marks to evaluate future reintroduction sites. This data can also be used in future captive breeding programs to determine the optimal water quality conditions for successful reproduction. One of the possible environmental factors that might have contributed to poor survivorship at MD Aldergrove sub site A has now been explored and resources can now be focused on other possible factors, e.g. genetics and disease. However, the possible role of pesticides should still be investigated.

6. Conclusions and Recommendations

This research has indicated that water conditions do not likely significantly influence the embryonic survivorship of *R. pretiosa* at two study sites in British Columbia, Canada and therefore do not primarily contribute to the low embryonic survivorship, population decline and potential extirpation at MD Aldergrove sub site A. However, due to the small number of egg masses oviposited at MD Aldergrove, the sample size is small which might have limited the study's analytical power. The fact that the species is endangered also limits the research that can be conducted on the species, which also makes it more challenging to provide recommendations. Optimum water quality conditions for successful reproduction of *R. pretiosa* in these study sites include pH between 5.5 and 7, water temperature between 6 and 18 º C, conductivity between 20 and 156 µS/cm, chloride levels between 0.3 and 2.2 mg/l and sulphate 0.5 to 6.9 mg/l. (A complete list of water quality parameters is presented in Appendix B.)

The MD Aldergrove sub site A has very low embryonic survivorship and future research projects and resources should therefore focus on MD Aldergrove sub site A to determine factors contributing to low embryonic survivorship and to improve hatching success. Only through successful embryonic survivorship can the sustainability of *R. pretiosa* be ensured. The population has declined from an estimated 180 individuals in 1997 to 66 in 2001. A number of other possible factors may also be contributing to poor fecundity and population decline at MD Aldergrove sub site A.

It is recommended that future research focus on other possible factors, such as genetics, that might influence embryonic survivorship, fecundity and population decline. Many amphibian

species show very strong site fidelity (Duellman & Trueb, 1994 and Sinsch, 1990) and various studies have indicated that individuals that were displaced several meters from their home range returned to their original sites (Holomuzki, 1982; Jameson, 1957 and Kleeberger & Werner, 1982). This suggests that it might be the same individuals that return year after year to breed at each of the sites and sub sites, resulting in a potential genetic- bottleneck effect. A decline in genetic diversity can occur in species that have declined in number and spatial distribution (Scribner, 2001). If low fecundity at MD Aldergrove sub site A is due to a genetic deficiency, low embryonic survivorship might, therefore, occur year after year. It is therefore recommended that research projects, be initiated to investigate genetic aspects of *R. pretiosa* at MD Aldergrove sub site A, sub site B and Seabird Island. Studies, for example, should be done to determine and compare genetic structure and diversity and determine mutation rates through mitochondrial DNA and microsatellites of the different populations (Grewe & Hampton, 1998 and Howes & Lougheed, 2005).

Bullfrogs occur at MD Aldergrove and may be another factor contributing to the population decline at MD Aldergrove. Bullfrogs, which are exotic, not only prey on *R. pretiosa* (Licht, 1974 and Pearl et al., 2004) but can also act as vectors of disease such as chytrid fungus (Govindarajulu et al., 2005). Projects should be initiated to eradicate the bull frog population at MD Aldergrove and determine their role of vectors in the spread of disease. This can be done by screening tissue samples for fungus from native frogs, and bull frogs, and comparing levels of the prevalence of fungus in bull frog-infested and bull frog-free areas (Govindarajulu et al., 2005).

The current water quality conditions allow for the successful reproduction of *R. pretiosa.* It is therefore important that continuous monitoring of the water quality be conducted to ensure optimal water quality conditions remain and ensure the long term health of the populations. Other water quality parameters e.g. pesticides should be measured to determine whether they contribute to low embryonic survival at MD Aldergrove sub site A.

R. pretiosa, plays an important ecological, economic and social role. *R. pretiosa,* like all other amphibians is an important component of many diverse ecosystems (Wake, 1991) and amphibians are among the most abundant of terrestrial vertebrates (Duellman & Trueb, 1994). Amphibians serve as a predator to many insects and small mammals and are also prey to many animals like birds, reptiles and mammals (Stebbins & Cohen, 1995). The diet of adult *R. pretiosa* includes leaf, rove and ground beetles, spiders, syrphid and long-legged flies, ants and water strides (Licht, 1986) while tadpoles feed on algae, decaying vegetation and detritus (Licht, 1974). Amphibians in different life stages fall prey to carnivorous insects, birds, fish, snakes, mammals, like raccoons and river otters (Cockran & Thoms, 1996) and even to carnivorous plants (Venus flytrap) (Duellman and Trueb, 1994).

Amphibians are important links in a variety of aquatic and terrestrial food webs and they occupy different levels in the food web. Many amphibians that undergo metamorphosis are primary consumers (herbivores) in their larvae stage and become secondary consumers (predators) after metamorphosis (Corkran & Thoms, 1996). They therefore are a crucial part of many food chains and more complex food webs in the ecosystem. Due to their important role in the ecosystem, a decline in amphibian populations can have serious negative effects on other animal populations

and the many different ecosystems that they inhabit (Stebbins & Cohen, 1995). Such a decline in a sentinel species can indicate other general environmental problems (Wake, 1991).

R. pretiosa has no direct known economical or social importance, is not hunted or commercially exploited and there is little public interest in the species in British Columbia (Haycock, 2000). However, indirectly, *R. pretiosa* has important economic and social importance as an indicator of environmental health. Amphibians are sensitive to environmental conditions (Blaustein et al., 1994a) and therefore useful as indicators of ecotoxicology, water quality (Boyer and Grue, 1995) and a variety of environmental contaminants (Blaustein et al., 1994a). Many amphibians can thus be used as bioindicators to provide information regarding the state of the health of the environment (Wake, 1991). A decline in frogs can therefore possibly be used as an indicator of degrading water quality, and poor water quality can cause far reaching negative effects to other animals and humans (Stebbins & Cohen, 1995).

Clean water is vital for human survival and has an impact on the social and economic health of communities. Contaminated water can cause serious illnesses or even death (http://www.who.int/en/). Polluted water not only has a negative effect on the environment but can also have important social and economic impacts on society. For example, nitrate is the most common agricultural pollutant and can contaminate water sources (Freedman, 2004.) It can cause illnesses and even death in humans (Freedman, 2004) and the economic cost involved in the remediation of polluted water can be significant. Annually thousands of people contract diseases caused by polluted water and the medical cost associated with treating these patients can be in the millions (http://www.who.int/en/).

Salmon is dependent on clean water and is a very important component of many societies. It provides jobs and has an important cultural value to First Nations Peoples (http://www.cnie.org/NAE/northwest.html). Salmon stocks declining due to contaminated water can cause large negative effects on the social and economic well being of societies.

Lakes, rivers and streams are used for recreational purposes by thousands of people in the form of sports angling, swimming, river-rafting and boating. Should water bodies become contaminated, it can reduce the recreational values of these areas.

To achieve a sustainable *R. pretiosa* population it is essential that the current OSFRT continues its role to bring together stakeholders including First Nations to discuss the importance and management of the species and to coordinate research and recovery programs. It is also important to reach out to communities to create an interest in and educate the public regarding the importance of *R. pretiosa* and its role in a healthy ecosystem. South-western British Columbia is the only known location where *R. pretiosa* occurs in Canada and this is a golden opportunity for local communities to participate in research programs and prevent extirpation of the species in Canada. With co-operation among all different government levels, private sector, non-governmental organizations, local communities and First Nations; sustainable populations for future generations can be achieved.

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Appendix A
Summary of embryonic survivorship of *Rana pretiosa.*

Table A1. Mean [± standard deviation (SD)] embryonic survivorship of *Rana pretiosa* at MD Aldergrove sub site A and sub site B, British Columbia, Canada during 2002, 2004 to 2005. Sample size of eggs per cage at sub site A was 20-54 eggs per cage and sub site B 21-38 eggs per cage.
N/A = years when no data were collected for a site/sub site.

Year	Sub site	Mean embryonic survivorship (%)	SD	N (Cages)
2002	A	35.54	28.97	5
	B	87.66	11.93	5
2002	**Pooled (A and B)**	**61.60**	**34.51**	**10**
2003	A	N/A		
	B	N/A		
2004	A	8.89	1.53	3
	B	87.78	2.00	2
2004	**Pooled (A and B)**	**40.44**	**43.23**	**5**
2005	A	9.50	2.27	2
	B	77.80	6.39	3
2005	**Pooled (A and B)**	**50.48**	**37.70**	**5**

Table A2. Mean [± standard deviation (SD)] embryonic survivorship of *Rana pretiosa* at Seabird Island, British Columbia, Canada during 2002 to 2004. Sample size of eggs per cage was 16-34 eggs per cage.
N/A = years when no data were collected for a site/sub site.

Year	Sub site	Mean embryonic survivorship (%)	SD	N (cages)
2002	Seabird Island	83.97	9.47	24
2003	Seabird Island	79.53	13.26	16
2004	Seabird Island	76.62	33.85	12
2005	Seabird Island	N/A		

Appendix B
Summary of water quality data.

Table B1. Summary of raw data for water chemistry at MD Aldergrove sub site A and sub site B, British Columbia, Canada during 2002, 2004 to 2005 with method detection limits (MDL) and Canadian Water Quality Guidelines (CWQG).

Compound	Units	MDL	CWQG	Sub site A					Sub site B				
				2002	2004	2005	Mean	SD	2002	2004	2005	Mean	SD
pH	pH Units	0.01	6.5-9.0	5.53	6.46	6.26	**6.08**	**0.49**	5.54	6.91	7.04	**6.50**	**0.83**
BOD	mg/L	5	/	6	7	6	**6.33**	**0.58**	10	6	8	**8**	**2**
Cl	mg/L	0.1	/	1.4	0.9	0.8	**1.03**	**0.32**	1.2	0.3	0.4	**0.63**	**0.49**
F	mg/L	0.01	0.12	0.01	0.02	0.03	**0.02**	**0.01**	0.01	0.02	0.02	**0.02**	**0.01**
SO_4	mg/L	0.5	/	< 0.5	< 0.5	< 0.5	**< 0.5**	**n/a**	< 0.5	< 0.5	< 0.5	**< 0.5**	**n/a**
Br	mg/L	0.05	/	< 0.05	< 0.05	< 0.05	**<0.05**	**n/a**	< 0.05	< 0.05	< 0.05	**<0.05**	**n/a**
NO_3	mg/L	0.002	13	< 0.002	0.005	0.005	**0.004**	**0.002**	< 0.002	< 0.002	0.005	**0.002**	**0.002**
NO_2	mg/L	0.005	0.06	< 0.005	< 0.005	< 0.005	**<0.005**	**n/a**	< 0.005	< 0.005	< 0.005	**<0.005**	**n/a**
PO_4	mg/L	0.05	/	< 0.05	< 0.05	< 0.05	**<0.05**	**n/a**	< 0.05	< 0.05	< 0.05	**<0.05**	**n/a**
NH_3	mg/L	0.005	1.37-2.2	< 0.005	0.005	0.013	**0.007**	**0.005**	0.022	< 0.005	0.013	**0.01**	**0.011**
Nitrogen Total	mg/L	0.04	/	2.5	0.83	1.12	**1.48**	**0.89**	0.6	0.44	1.12	**0.5**	**0.09**
Phos. o-PO4 diss.	mg/L	0.001	/	0.002	< 0.001	< 0.001	**0.001**	**0.0009**	0.002	< 0.001	< 0.001	**0.001**	**0.0009**
Total DissPhosphorus	mg/L	0.002	/	0.027	0.007	0.013	**0.02**	**0.01**	0.032	0.007	0.013	**0.02**	**0.01**
Total Phosphorus	mg/L	0.002	/	0.24	0.034	0.045	**0.21**	**0.15**	0.067	0.031	0.045	**0.13**	**0.15**
Conductivity	µS/cm	2	/	20	35	33.0	**29.33**	**8.14**	32	43	33.0	**37.33**	**5.51**
Turbidity	NTU*	0.05	/	24.80	3.70	5.15	**11.22**	**11.79**	5.34	2.39	5.15	**3.22**	**1.85**

*NTU, Nephelometric turbidity units

Table B2. Summary of raw data for water chemistry at Seabird Island, British Columbia, Canada during 2002 to 2004 with method detection limits (MDL) and Canadian Water Quality Guidelines (CWQG).

Compound	**Units**	**MDL**	**CWQG**	**Seabird Island**				
				2002	**2003**	**2004**	**Mean**	**SD**
pH	pH Units	0.01	6.5-9.0	7.07	7.07	6.93	**7.02**	**0.08**
BOD	mg/L	5	/	15	7	12.0	**11.33**	**4.04**
Cl	mg/L	0.1	/	1.7	1.6	2.2	**1.83**	**0.32**
F	mg/L	0.01	0.12	<0.01	0.02	0.02	**0.02**	**0.01**
SO_4	mg/L	0.5	/	2.1	6.9	0.9	**3.30**	**3.17**
Br	mg/L	0.05	/	< 0.05	<0.05	< 0.05	**<0.05**	**n/a**
NO_3	mg/L	0.002	13	< 0.002	0.005	0.007	**0.0043**	**0.0031**
NO_2	mg/L	0.005	0.06	< 0.005	<0.005	< 0.005	**<0.005**	**n/a**
PO_4	mg/L	0.05	/	< 0.05	<0.05	< 0.05	**<0.05**	**n/a**
NH_3	mg/L	0.005	1.37-2.2	0.438	<0.005	< 0.005	**0.015**	**0.25**
Nitrogen Total	mg/L	0.04	/	3.9	0.42	0.80	**1.71**	**1.91**
Phos. o-PO4 diss.	mg/L	0.001	/	< 0.001	<0.001	< 0.001	**<0.001**	**n/a**
Total DissPhosphorus	mg/L	0.002	/	0.003	0.005	0.007	**0.005**	**0.002**
Total Phosphorus	mg/L	0.002	/	0.2	0.016	0.032	**0.08**	**0.10**
Conductivity	µS/cm	2	/	156	149	71	**125.33**	**47.18**
Turbidity	NTU*	0.05	/	3.04	1.13	2.74	**2.30**	**1.03**

*NTU, Nephelometric turbidity units

Table B3. Summary of raw data for trace metals at MD Aldergrove sub site A and sub site B, British Columbia, Canada during 2004 and 2005 with method detection limits (MDL) and Canadian Water Quality Guidelines (CWQG).

Compound	Units	MDL	CWQG	Sub site A				Sub site B			
				2004	2005	Mean	SD	2004	2005	Mean	SD
Calcium (Ca)	mg/l	0.1	/	3.4	3.8	**3.6**	**0.28**	3.3	3.6	**3.45**	**0.21**
Iron (Fe)	mg/l	0.006	0.3	0.548	1.48	**1.01**	**0.66**	0.371	0.376	**0.374**	**0.004**
Potassium (K)	mg/l	0.1	/	0.4	1.2	**0.8**	**0.57**	0.4	0.5	**0.45**	**0.07**
Silicon (Si)	mg/l	0.06	/	1.24	2.72	**1.98**	**1.05**	1.34	1.54	**1.44**	**0.14**
Sodium (Na)	mg/l	0.1	/	1.6	2.0	**1.8**	**0.28**	1.5	1.6	**1.55**	**0.07**
Sulphur (S)	mg/l	0.06	/	0.21	0.71	**0.46**	**0.35**	0.14	0.15	**0.145**	**0.007**
Titanium (Ti)	mg/l	0.002	/	0.013	0.026	**0.02**	**0.009**	0.02	0.007	**0.014**	**0.009**
Aluminium (Al)	µg/l	0.2	5-100	347	538	**442.5**	**135.06**	340	158	**249**	**128.69**
Antimony (Sb)	µg/l	0.005	/	0.05	0.085	**0.07**	**0.025**	0.042	0.065	**0.054**	**0.016**
Arsenic (As)	µg/l	0.1	5	0.4	0.6	**0.5**	**0.141**	0.5	0.4	**0.45**	**0.071**
Barium (Ba)	µg/l	0.02	/	8.17	13.2	**10.69**	**3.56**	5.86	5.27	**5.565**	**0.417**
Beryllium (Be)	µg/l	0.002	/	0.025	0.066	**0.05**	**0.03**	0.074	0.016	**0.045**	**0.041**
Bismuth (Bi)	µg/l	0.02	/	< 0.02	0.02	**0.02**	**0.01**	< 0.02	0.02	**0.02**	**0.01**
Boron (B)	µg/l	2	/	4	6	**5**	**1.41**	4	8	**6**	**2.83**
Cadmium (Cd)	µg/l	0.01	0.017	0.02	< 0.01	**0.01**	**0.01**	0.01	< 0.01	**0.008**	**0.004**
Chromium (Cr)	µg/l	0.2	1	0.8	1.6	**1.2**	**0.57**	0.5	0.7	**0.6**	**0.14**
Cobalt (Co)	µg/l	0.005	/	0.198	0.712	**0.46**	**0.36**	0.213	0.128	**0.17**	**0.06**
Copper (Cu)	µg/l	0.05	2-4	3.25	4.13	**3.69**	**0.62**	2.41	3.69	**3.05**	**0.91**
Lead (Pb)	µg/l	0.01	1-7	0.47	0.60	**0.54**	**0.09**	0.31	0.26	**0.29**	**0.04**
Lithium (Li)	µg/l	0.05	/	0.17	0.37	**0.27**	**0.14**	0.12	0.15	**0.14**	**0.02**
Magnesium (Mg)	µg/l	0.05	/	1470	1450	**1460**	**14.14**	1480	1670	**1575**	**134.35**
Manganese (Mn)	µg/l	0.005	/	19.6	86.7	**53.15**	**47.45**	55.8	16.3	**36.05**	**27.93**
Molybdenum (Mo)	µg/l	0.05	73	0.05	0.16	**0.11**	**0.08**	0.08	0.19	**0.14**	**0.08**
Nickel (Ni)	µg/l	0.05	25-150	2.86	1.41	**2.14**	**1.03**	8.29	0.86	**4.58**	**5.25**
Selenium (Se)	µg/l	0.2	1	< 0.2	0.5	**0.26**	**0.35**	< 0.2	0.4	**0.21**	**0.28**
Silver (Ag)	µg/l	0.02	0.1	0.05	0.07	**0.06**	**0.01**	0.04	0.05	**0.045**	**0.007**
Strontium (Sr)	µg/l	0.005	/	23.3	27.8	**25.55**	**3.18**	22.1	24.1	**23.1**	**1.14**
Thallium (Tl)	µg/l	0.002	0.8	0.013	0.028	**0.02**	**0.01**	0.016	0.008	**0.01**	**0.01**
Tin (Sn)	µg/l	0.01	/	0.07	0.15	**0.11**	**0.06**	0.11	0.19	**0.15**	**0.06**
Uranium (U)	µg/l	0.002	/	0.018	0.017	**0.018**	**0.001**	0.017	0.012	**0.015**	**0.004**
Vanadium (V)	µg/l	0.05	/	0.99	1.43	**1.21**	**0.31**	0.84	0.54	**0.69**	**0.21**
Zinc (Zn)	µg/l	0.1	30	10.1	13.8	**11.95**	**2.62**	2.6	3.7	**3.15**	**0.78**

Table B4. Summary of raw data for trace metals at Seabird Island, British Columbia, Canada for 2004 with method detection limits (MDL) and Canadian Water Quality Guidelines (CWQG).

Compound	**Units**	**MDL**	**CWQG**	**Seabird Island**
				2004
Calcium (Ca)	mg/l	0.1	/	7.8
Iron (Fe)	mg/l	0.006	0.3	0.516
Potassium (K)	mg/l	0.1	/	1.4
Silicon (Si)	mg/l	0.06	/	0.96
Sodium (Na)	mg/l	0.1	/	0.8
Sulphur (S)	mg/l	0.06	/	0.31
Titanium (Ti)	mg/l	0.002	/	< 0.002
Aluminium (Al)	µg/l	0.2	5-100	23.3
Antimony (Sb)	µg/l	0.005	/	0.059
Arsenic (As)	µg/l	0.1	5	0.9
Barium (Ba)	µg/l	0.02	/	11.5
Beryllium (Be)	µg/l	0.002	/	0.052
Bismuth (Bi)	µg/l	0.02	/	< 0.02
Boron (B)	µg/l	2	/	7
Cadmium (Cd)	µg/l	0.01	0.017	0.01
Chromium (Cr)	µg/l	0.2	1	< 0.2
Cobalt (Co)	µg/l	0.005	/	0.125
Copper (Cu)	µg/l	0.05	2-4	2.54
Lead (Pb)	µg/l	0.01	1-7	0.33
Lithium (Li)	µg/l	0.05	/	0.24
Magnesium (Mg)	µg/l	0.05	/	1610
Manganese (Mn)	µg/l	0.005	/	36.8
Molybdenum (Mo)	µg/l	0.05	73	0.59
Nickel (Ni)	µg/l	0.05	25-150	5.61
Selenium (Se)	µg/l	0.2	1	< 0.2
Silver (Ag)	µg/l	0.02	0.1	0.03
Strontium (Sr)	µg/l	0.005	/	27.5
Thallium (Tl)	µg/l	0.002	0.8	0.004
Tin (Sn)	µg/l	0.01	/	0.11
Uranium (U)	µg/l	0.002	/	0.016
Vanadium (V)	µg/l	0.05	/	0.31
Zinc (Zn)	µg/l	0.1	30	3.4

Table B5. Summary of raw data for total and fecal coliforms at MD Aldergrove sub site A and sub site B, British Columbia, Canada during 2002, 2004 to 2005 with method detection limits (MDL).

Compound	**Units**	**Sub site A**					**Sub site B**				
		2002	**2004**	**2005**	**Mean**	**SD**	**2002**	**2004**	**2005**	**Mean**	**SD**
Total coliforms	col/100ml	<1	370	30	**133.5**	**205.4**	<1	120	8	**42.83**	**66.93**
Fecal coliforms	col/100ml	<1	4	16	**6.83**	**8.13**	<1	2	4	**2.17**	**1.76**

Year	**MDL Total coliform**	**MDL Fecal coliform**
2002	1 col/100ml	1 col/100ml
2003	1 col/100ml	1 col/100ml
2004	10 col/100ml	2 col/100ml
2005	2 col/100ml	2 col/100ml

Table B6. Summary of raw data for total and fecal coliforms at Seabird Island, British Columbia, Canada during 2002 to 2004 with method detection limits (MDL).

Compound	**Units**	**Seabird Island**				
		2002	**2003**	**2004**	**Mean**	**SD**
Total coliforms	col/100ml	<1	<1	350	**116.7**	**202.04**
Fecal coliforms	col/100ml	<1	<1	<2	**<MDL**	**n/a**

Year	**MDL Total coliform**	**MDL Fecal coliform**
2002	1 col/100ml	1 col/100ml
2003	1 col/100ml	1 col/100ml
2004	10 col/100ml	2 col/100ml
2005	2 col/100ml	2 col/100ml