Integrating Human, Animal and Environmental Health

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EXAMINING THE POTENTIAL IMPACTS OF AMERICAN BULLFROGS (*RANA CATESBEIANA*) ON DRINKING WATER QUALITY IN THE GREATER VICTORIA WATER SUPPLY AREA

Prepared for the Capital Regional District
Water Services
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EXECUTIVE SUMMARY

Background

The American bullfrog, *Rana catesbeiana*, was introduced into western North America in the early 1900's as a business enterprise to supply a culinary demand for frog's legs. Unfortunately, bullfrogs escaped or were released from these farms and by the 1970s, wild bullfrog populations were endemic in many reservoirs, lakes, ponds and wetlands (henceforth collectively referred to as 'water bodies'). Bullfrogs have been present in the Greater Victoria area since the 1960s. As with other invasive species, founding bullfrog colonies typically enter suitable habitats through migration (natural or human-assisted) and undergo very rapid population growth, aided by an absence of natural predators and new sources of un-acclimatized prey. American bullfrogs can reach exceedingly high densities in certain water bodies. Negative impacts on native amphibian populations via competition for resources and predation have been documented. Although American bullfrogs have yet to invade the Capital Regional District's (CRD) Water Supply Area, active bullfrog surveillance and control activities have found them in water bodies within 3 km of Japan Gulch Reservoir.

CRD Water Services uses a multiple barrier approach to protect drinking water quality including extensive watershed protection programs, limited public access to the CRD Water Supply Area, complete bans on industrial and agricultural activities within the catchment area, and extensive water quality monitoring. The CRD does not filter the water for bacteria, viruses or parasites, but instead disinfects the water prior to distribution with ultraviolet (UV) light, and then free chlorine and ammonia (in a process called chloramination). Source water protection is a cornerstone of the CRD approach to protecting drinking water; therefore, it is important to maintain the health and integrity of the ecosystem from which the water is extracted.

In this report, we examine three ways by which bullfrogs could potentially negatively affect the quality of CRD drinking water:

- Directly by changing water quality parameters
- Indirectly by damaging water supply infrastructure
- Indirectly by altering aquatic ecosystems, with potential downstream impacts on human health

We identified a number of key water quality parameters (e.g. bacteria, parasites, virus, odour, taste, dissolved solids, nitrogen, phosphate and primary production) and ecosystem health determinants (e.g. biodiversity, habitat restructuring, introduction of novel pathogens, and alteration of nutrient inputs or timing) on which to assess the potential impacts of bullfrogs. A full list is presented in Appendix 1.

Probability of Bullfrog Impacts

Based on elevation and terrain obstacles to bullfrog establishment on Vancouver Island, we concluded that Sooke Reservoir and Japan Gulch Reservoir are at the greatest risk for bullfrog colonization. However, our analysis of the ecological characteristics and limited productivity of Sooke Reservoir suggests that only small bullfrog densities are likely to be supported, which greatly reduces the probability of bullfrog impacts. Although Japan Gulch Reservoir may be able to support greater bullfrog densities, water from this reservoir is utilized for short periods of time in January and February (which correspond to peak hibernation). Therefore, the probability of bullfrog impacts on drinking water quality in Japan Gulch is expected to be negligible.

Nature and Magnitude of Bullfrog Impacts

Water Quality

The bacteria species that have been described from amphibians are known to exist in the environment or gastrointestinal tract of numerous wildlife species. Bullfrogs may be carriers and shedders of bacteria capable of causing disease in humans, but there are no published reports describing amphibians as effective amplifiers of the bacteria. Amphibians are known to carry a number of viruses, parasites and fungi that are largely host-adapted to amphibians, fish and reptiles and pose very little risk to humans. We found a very small number of case reports of human infections by pathogens isolated or described from amphibians, but these are frequently incidental findings with no apparent link between human infections and amphibians.

The pathogen loads of bullfrog populations in general and on Vancouver Island specifically have not been systematically studied, resulting in gaps in our knowledge of the full list of potential pathogens that bullfrogs may carry into the CRD Water Supply Area. Given that bullfrog pathogens have not yet been linked to community outbreaks in humans in any other area where they have been introduced or are endemic, and human disease associated with amphibians in general are few, it is unlikely that this deficit in information has an important impact on risk assessment conclusions.

We did not find any peer-reviewed or grey literature to support the hypothesis that bullfrogs will have adverse impacts on the physical characteristics, on non-metallic inorganic chemical (phosphorus and nitrogen) characteristics, or on algal populations of aquatic ecosystems as a result of the successful colonization of bullfrogs into a new aquatic ecosystem.

Water Supply Infrastructure

Amphibians, including American bullfrogs, have not been reported in the literature or our consultations as causing damage to water intakes, pipes and drains. Consequently we do not believe that an invasion of American bullfrogs into the GVWSA will pose any threat to the water supply infrastructure.

Ecosystem Health

We did not find any peer-reviewed or grey literature to support the hypothesis that bullfrogs will restructure habitats, alter nutrient inputs or timing, or facilitate changes to trophic structures (whether it be aquatic, riparian or terrestrial) in ways that could affect the health of the ecosystem. American bullfrogs have few predators at any life stage, and are unlikely to attract new mammalian and bird species to the lake margin that would in turn be a source of increased public health risk.

Bullfrogs have been documented in the peer-reviewed literature to negatively affect species biodiversity and to introduce new pathogens (e.g., chytrid fungus and ranavirus) that may impact native amphibians. However, there is conflicting evidence as to the actual impact that invasive bullfrogs have on native frog species numbers and composition.

Conclusions and Recommendations

Overall, there was a noticeable lack of published research on bullfrog impacts on water quality, water supply infrastructure and ecosystem health, with the exception of bullfrog impacts on amphibian biodiversity and threatened species.

Based on our review, the risk to public health from the invasion of bullfrogs into the CRD water supply is negligible. The predicted small densities of bullfrogs likely to be established in nutrient poor water bodies, and lack of documented public health effects of bullfrogs in temperate areas, are the basis for this conclusion.

Given this risk conclusion, current bullfrog surveillance and eradication activities in the bullfrog control corridor to the south east of the CRD Water Supply Area are not necessary for the protection of drinking water quality in the GVWSA. This conclusion **does not take into account** the possible ecological impacts on native and endangered amphibian species. It also does not consider policy or opinions regarding management of invasive species in general.

Because of public concerns and prevailing uncertainties, we recommend the following to the CRD Water Services:

1. Consider the possible ecological impacts of bullfrogs on native and endangered amphibian species, and best practices for the management of invasive species when making final policy decisions on bullfrog control.

2. Periodically review current and newly published bullfrog-relevant literature. This report recognizes that bullfrog research is ongoing, and that changes in climate patterns over time may alter bullfrog ecology and behaviours and affect water quality parameters.
3. Support (financial or in-kind) of local research on bullfrog ecology, habitat preference and migratory patterns relevant to the Greater Victoria Water Supply Area

Should the CRD Water Services decide to continue with the current policy of preventing bullfrog colonization of the GVWSA, prediction models for habitat suitability and migration would be invaluable towards concentrating surveillance and eradication to maximize effectiveness and decrease costs.

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1 INTRODUCTION

1.1 Project Background

The American bullfrog (*Rana catesbeiana*) was first recorded in the Greater Victoria Area at Elk and Beaver lakes in the District of Saanich sometime in the 1960s (Govindarajulu, 2004). In the early 1990s, bullfrogs became established in Prospect Lake – from here they gained access to the irrigation ponds, small lakes and wetlands of the Thetis Lake system (Govindarajulu, 2004), and have since spread north up the Saanich Peninsula and west to the City of Langford.

Populations of these large non-native frogs can reach very high densities in suitable water bodies, aided by an absence of natural predators and new sources of un-acclimatized prey. American bullfrogs are known to disperse over great distances to colonize new water bodies and wetlands. Govindarajulu (2004) recorded average range expansions in the Greater Victoria area of 2 km/year (with a range of between 1 and 5 km/year). Bullfrog dispersals into previously non-colonized water bodies are also aided by human activities.

In 2004, a local amphibian biologist suggested American bullfrogs posed a threat to the Greater Victoria Water Supply Area because they carried parasites known to affect humans and could create water quality problems. In response, Capital Regional District (CRD) Water Services convened a meeting in 2004 between the water department staff and other shareholders to discuss the potential for bullfrogs to affect drinking water quality. The primary conclusion from this meeting was that American bullfrogs in the Water Supply Area would be very unlikely to affect human health and drinking water quality.

CRD Water Services have also discussed the potential effects of American bullfrogs on the ecology of drinking water supply reservoirs with a number of aquatic ecologists and amphibian biologists. This group suggested that while bullfrogs would have impacts on species in shoreline habitats, it was very unlikely that the ecology of drinking water supply reservoirs would be affected.

Nevertheless, precautionary measures were recommended due to the nature of the water protection system and the necessity for careful management of the source water supply and supporting ecosystem. The CRD Water Services has therefore endeavoured to prevent bullfrogs from establishing in the Water Supply Area.

At the direction of the Regional Water Supply Commission, CRD Water Services has been providing funding support to a project developed by Mr. Stan Orchard to eradicate American bullfrogs from a control corridor in the City of Langford and City of Colwood. This project is attempting to prevent further spread of bullfrogs to the west, prevent their establishment in the

Water Supply Area and adjacent Sooke Hills Wilderness Regional Park, and demonstrate the feasibility of eradication.

In 2005 and 2006, CRD Water Services and Regional Parks each made annual financial contributions of $15,000 to the bullfrog eradication program. This was increased to $20,000 per agency in 2007. In addition, CRD Water Services has devoted staff time to bullfrog monitoring in the Water Supply Area and adjacent Regional Park.

On June 26, 2008 CRD Water Services assembled a diverse group of biologists, invasive species specialists and land management agencies and local government representatives to discuss the management of American bullfrogs in the Capital Regional District. During the workshop it became apparent that even if eradication in the control corridor is proven feasible, preventing bullfrog colonization of the Water Supply Area will require a long term commitment of funds and staff resources because of the potential for humans to move bullfrogs to new water bodies in adjacent areas. As a result, workshop participants recommended a review of the available literature on the effects of American bullfrogs on public health, aquatic ecosystems, drinking water quality and water supply infrastructure. This information would be used to assist CRD Water Services in determining the risks associated with the establishment of American bullfrogs in the Greater Victoria Water Supply Area (GVWSA) and determine if the current management direction needed to be revised.

To implement this recommendation, CRD Water Services contacted the Centre for Coastal Health to request that the Centre carry out the literature review. Together, the agencies developed a number of questions to be addressed by the review:

1) To what extent might American bullfrogs impact the specific drinking water quality parameters identified by CRD Water in the GVWSA?

2) If bullfrogs are present in water supply reservoirs in other jurisdictions, have effects on water quality been identified?

3) Can American bullfrogs negatively affect water supply infrastructure?

    E.g., plugging water intake screens and pipes; causing soil erosion with the potential to damage infrastructure

4) Can invasive American bullfrogs affect aquatic ecosystems in the GVWSA in ways that affect drinking water quality?

    E.g., loss of biodiversity; habitat restructuring; emergence of novel pathogens; change in aquatic trophic structure; alteration of nutrient inputs or timing; change in terrestrial /

aquatic linkages (attraction of predators to a new food source and increase of contact of potential mammalian or avian disease vectors to reservoir water)

5) What is the risk to aquatic ecosystems (specifically by facilitating changes that may impact public health), drinking water quality and water supply infrastructure in the GVWSA if American bullfrogs were to become established?

A terms of reference was developed (Appendix 1) and the review was initiated.

This document presents the results and conclusions of this literature review and provides recommendations to guide CRD Water Services in bullfrog management.

1.2 Criteria for Assessment

It was necessary to identify and define indicators of change to aquatic ecosystems that best reflect the impacts that might result from the establishment of American bullfrogs in the GVWSA. A number of criteria were identified for the assessment of the potential effects that American bullfrogs may have in the Greater Victoria Water Supply Area (see also Appendix 1). The following list was developed in part from a full list of indicators that are routinely monitored and measured by the CRD Water Services, which is available online at <http://www.crd.bc.ca/water/waterquality/reports.htm>. Additional indicators of change were developed through internal consultations with the participants of this review.

A brief introduction to these assessment criteria, with an explanation of why they were chosen for study, is provided in Section 1.3: Water Quality Parameters.

1.2.1 GVWSA Measured Drinking Water Quality Indices

a. Microbial Parameters
    i. Coliform bacteria
    ii. Heterotrophic bacteria
    iii. Bacterial toxins
    iv. Parasitic protozoa

b. Physical Parameters
    i. Odour
    ii. Taste
    iii. Alkalinity
    iv. Hardness
    v. Total solids
    vi. Turbidity

vii. Water temperature
viii. pH

c. Non-Metallic Inorganic Chemicals
   i. Nitrogen
   ii. Phosphate

1.2.2 Additional Water Safety Criteria

a) Multi-cellular parasites (nematodes, cestaodes, trematodes)
b) Algal blooms
c) Fungi
d) Bacterial Toxins
e) Viruses

1.2.3 Effects on Water Supply Infrastructure

a) Direct Impacts (plugging intake screens / pipes)
b) Indirect Impacts (damage to intakes due to soil erosion or habitat destruction)

1.2.4 Effects on Aquatic Ecosystems

a) Habitat restructuring
b) Alteration of nutrient inputs or timing
c) Changes in primary production
d) Changes to aquatic trophic structures (phytoplankton, zooplankton, invertebrates)
e) Changes to riparian and terrestrial trophic structures (new or increased fish, bird and mammalian contact at margins of lakes, ponds and wetlands)
f) Introduction of novel pathogens
g) Loss of biodiversity (effects on native amphibians)

1.3 Water Quality Paramaters

1.3.1 Microbial Parameters

Water utilities routinely sample for and monitor levels of a select number of microbiological indicators for water safety and quality. CRD Water Services has a complete microbiology monitoring program in place with an in-house microbiology lab capable of quickly identifying increases in fecal coliform, *Escherichia coli*, *Aeromonas* species, *Enterococci* species and plate counts of heterotrophic bacteria. This program also routinely monitors for the protozoan parasites *Giardia* and *Cryptosporidium*. Furthermore, CRD monitors for the cyanobacterial toxins Anatoxin a and Microcystin, which although not human pathogens, do pose a public health risk.

Changes in these values might imply a change in microbial aquatic organism numbers or community structure, and thus provide a useful benchmark for monitoring the aquatic ecosystem.

The CRD currently uses a three-step process that involves ultraviolet (UV) disinfection, followed by the addition of free chlorine and then ammonia in a process called chloramination, to disinfect drinking water prior to distribution for human consumption. It is not the intent of this review to assess the efficacy of this process for water quality and safety.

1.3.2 Physical Parameters

Most public feedback and comments are in the area of taste, odour and appearance (colour, turbidity) that are governed largely by the phytoplankton of the reservoirs, and the presence or absence of cyanobacterial toxins produced as a result of phytoplankton blooms. The numbers and species present in the phytoplankton community affect the taste, odour and treatment processes used by water utilities.

Water clarity or turbidity is a well accepted primary indicator in all water supply reservoirs.

Decreases in water clarity can be due to increases in either:

- Inorganic water-borne particles (generally small or colloidal soil particles) that result from watershed or shoreline disturbance
- Organic particles (algal cells), generally as a result of increased nutrient supply

Increases in turbidity may be an indication of:

- Increased pathogen concentration since almost all viruses, bacteria and protozoan pathogens are attached to particles in the water
- Higher numbers of algal cells in the supply water, which can be a warning signal for drinking water quality problems such as taste, odour and colour
- Increases in algal cell populations, which reflects changes in aquatic food chains (such as a decrease in zooplankton grazing pressure)

CRD Water maintains continuous turbidity monitoring equipment at locations where water enters the treatment and distribution system from the supply reservoirs. If bullfrog colonization events sufficiently alter water clarity, turbidity, taste and odour, these changes should be measurable by currently accepted water quality monitoring protocols for physical parameters.

1.3.3 Non-Metallic Inorganic Chemicals

CRD Water Services monitors a wide range of metallic and non-metallic water chemistry parameters that are indicators of or might have an effect on water quality. General categories of water chemistry analyses include general anions and cations (e.g. calcium, magnesium, sulfates, inorganic carbon), organic carbon, metals (iron, manganese, zinc, lead copper cadmium mercury), and nutrients (nitrogen phosphorus) as well as anthropogenic chemicals such as pesticides, herbicides and petrochemicals. A full list is available online at <http://www.crd.bc.ca/water/waterquality/reports.htm>.

For this review, we limited the chemical parameters to include just nitrogen and phosphate. Increased nitrogen and phosphate nutrient supply to the reservoirs has the potential to increase phytoplankton numbers and taste, odour, colour or disinfection by-product precursors. We felt that these indicators best represent the health and integrity of the aquatic ecosystem, and would likely be the first chemical parameters to be impacted by large-scale biological processes (such as the successful colonization of an invasive species).

1.3.4 Additional Water Safety Criteria

We identified a number of important public health criteria that are not routinely monitored for by CRD Water Services. These included parasites and pathogens for which bullfrogs could either be hosts, reservoirs, or effective carriers, and included large multi-cellular parasites, fungi and viruses. Hypothesized public health impacts from successful bullfrog colonization include: changes in phytoplankton populations as a result of bullfrog larvae grazing pressures, which could release certain algal populations resulting in toxic algal blooms; or, massive die-offs of adult bullfrogs, which could facilitate or enhance bacterial toxin outbreaks such as botulism Type E. Although not supported by published papers, these changes were considered because of their biological plausibility.

1.3.5 Effects on Aquatic Ecosystems

This list of ecological indicators was developed through consultation with members of CRD Water Services and the authors of this report.

Any change in the community structure of the supply reservoir biological community could theoretically alter the water quality. Although invasive American bullfrogs have been implicated in biodiversity loss, it is feasible that they may facilitate changes to both the aquatic and terrestrial trophic structures even when there is no loss of biodiversity.

Changes at any level of the trophic structure can result in effects on other parts of the trophic structure (through a so called “trophic cascade”): the possibility that bullfrog introduction will facilitate some change in the aquatic or terrestrial trophic structure that might affect drinking water quality – specifically phytoplankton numbers or a shift in species composition – will be addressed in this review.

Much like the cane toads in Australia, invasive bullfrogs have very few natural predators. For the purposes of this report, however, we hypothesized that large bullfrog populations could facilitate a change in the patterns in movement of mammalian and / or bird predators to spend more time at the water. This could conceivably result in an increase in the amount of fecal material, and thus the enteric pathogen load, deposited at or near the water edge.

2 METHODOLOGY

Our investigations were divided into three parts:

1) Review of the Ecology and Distribution of American Bullfrogs on Vancouver Island

2) Effects of Bullfrogs on Water Quality Criteria and Infrastructure

3) Effects of Bullfrogs on Aquatic Ecosystems

Part 1 – A review of the ecology and distribution of American bullfrogs on Vancouver Island was conducted to contextualize the issues faced by CRD Water Services with respect to invasive bullfrog populations at the local level.

Part 2 – Examination of the effects on water quality and infrastructure focussed on the possibility that bullfrogs might carry with them bacteria, viruses, fungi or protozoan pathogens that could have some consequences to human health through the influx and spread of waterborne microbial pathogens. We briefly reviewed the potential for negative impacts on water supply infrastructure in this section. Although we did not limit our search of relevant material to western North America, North American studies and findings were emphasized in our discussion.

Part 3 – This review of the effects on aquatic ecosystems examined the ecological consequences and changes to the endemic native biological and chemical organic and inorganic elements of aquatic ecosystems that have occurred in other locations due to bullfrog introductions, and that could affect water safety or quality. The geographic emphasis on this aspect was on western North America, as that would be most relevant to the Greater Victoria Water Supply Area.

When information specific to American bullfrogs was absent or unavailable, we extended our search to include the effects of other invasive amphibian species on parameters of concern.

2.1 Literature Review

Peer-reviewed and grey literature on bullfrogs was located using several internet databases including Wiley Interscience, Springerlink, Science direct, JStor System, Web of Science and CSA Illumina (biological sciences). Google and Google Scholar were both used as internet search engines, and Google Earth was used as a source of elevation data for examining how this factor may relate to bullfrog dispersal.

A varied list of approximately 30 keywords was used either individually or in combination to locate the literature. Keywords contained both common and scientific names for the American bullfrog, pathogen (bacteria, virus, fungus, protozoa and parasites) names, pathogen descriptors, common terms used to describe water sources and water parameters, and general words that relate to human health. Once a few key papers had been identified, reference lists from those papers were combed for additional articles. Upwards of 50 websites were visited in order to gain additional insight on bullfrog ecology and behaviour.

Through the search described above, 106 references were identified as relevant to bullfrog impacts on ecosystem health and 70 as relevant to bullfrogs and human health. An additional 15 references deemed relevant to this review were placed in an ‘other’ category. All articles were reviewed and assessed for their relevance to both the review-specific questions and assessment criteria previously outlined (Section 1.1 and Section 1.2, respectively), and for their scientific strength for causality vs. association.

A list of bacteria isolated or described from varied frog species (see Table 2 and Appendix 2) was tabulated from the references, and then categorized by relative frequency of associated human infection and transmission routes. We used microbiology textbooks and online sources (Centre for Disease Control, Public Health Agency of Canada and focused searches on the internet databases listed above) to rank the bacteria species by relative public health importance into the categories ‘Reportable’, ‘Known pathogen’, ‘Opportunistic’, ‘Nosocomial’ and ‘Rare.’

‘Reportable’ pathogens are those that are considered to be of significant importance to human health. Typically, these are highly contagious or communicable pathogens for which suspected occurrences and/or laboratory confirmed cases are reported to local Health Units by physicians and medical laboratories.

Bacteria classified as ‘Known pathogen’ are not significant enough to be reportable, but can be contagious and communicable. These bacteria are frequently isolated from and implicated in respiratory or gastro-intestinal diseases of humans. Many of these bacteria are commonly found on healthy humans and in the environment around us.

Although bacteria classified as ‘Opportunistic’ and ‘Nosocomial’ can cause disease in humans, transmission to humans usually only occurs under very specific conditions: that is to say, because of a weak immune system the individual is unable to fight off an infection that would otherwise not result in any disease (opportunistic), or the individual acquires an infection while being treated for another condition at a hospital or clinical setting (nosocomial).

Bacteria that may be opportunistic or nosocomial under favourable conditions, but for which there are limited case reports of human infection, were classified as ‘Rare’. This designation also includes bacteria that have never been reported or isolated from humans.

2.2 Interviews

We contacted six experts in invasive American bullfrog biology and amphibian disease issues. Two have extensive knowledge on amphibian diseases and invasive amphibian species. The third has intimate local knowledge on bullfrogs in Victoria. The three remaining individuals have published numerous scientific papers and opinions with regards to invasive American bullfrogs in the peer-reviewed community. Each individual was asked the same basic set of questions, as well as a set of specific questions to address their unique knowledge or experience. A full list of interviewees and their affiliations can be found in Appendix 4.

In the fall of 2008, we contacted and interviewed city water managers from ten municipal districts on Vancouver Island, and thirteen additional water districts on both the East and West coasts of North America. Each district was asked a set of questions in regards to public access to the watershed, the presence of bullfrogs in or adjacent to the watershed, and water treatment protocols. A full list of water managers, with a summary of their response to the questions asked, is available in Appendix 5.

2.3 Risk Assessment

For the purpose of this literature review, risk is proportional to the likelihood (or probability) of an impact by American bullfrogs on one or more of water quality, water supply infrastructure and aquatic ecosystems, and the magnitude of these impacts on the CRD watershed. To evaluate risk, we developed a list of potential impacts (previously outlined in Section 1.2: Criteria for Assessment), then sought peer-reviewed and grey literature to better define the role of American bullfrogs on the magnitude and probability of these impacts.

We assumed that there would be variable degrees of uncertainty in the magnitude and probability of the impacts, based on the quantity and quality of the available literature, the inherent variability of complex biological ecosystems, and in errors associated with our habitat and population modeling. Uncertainty was dealt with in one of three ways: 1) comparing and contrasting the collective global experience of bullfrog colonization events against experiences from the American bullfrog's native range, 2) professional judgement from public health experts and respected herpetologists, and 3) overestimating rather than underestimating parameters, especially when modelling habitat preference and population sizes.

3 RESULTS AND DISCUSSION

Due to active relocation for frog farming purposes, American bullfrogs (*Rana catesbeiana)* are now established in western North America, South America, Europe, Japan and the Caribbean (Govindarajulu *et al.*, 2006).

A number of authors discuss bullfrogs as an invasive species (Ficetola *et al.*, 2007; Reinhardt *et al.*, 2003) and provide relevant information on life history, feeding, physiology, population trends and habitat preferences (Altig *et al.*, 2007; Beebee, 1995; Boone *et al.*, 2004; Boone *et al.*, 2008; Boyer & Grue, 1995; Emlen, 1968; Kruse & Francis, 1977; Maret *et al.*, 2006; Nie *et al.*, 1999; Pounds, 2001; Power, 1990; Pryor, 2003; Seale, 1980; Sterner, 1986; Travis *et al.*, 1985; Trenham *et al.*, 2003; Wake, 1991).

3.1 Distribution of American Bullfrogs on Vancouver Island

To aid in the understanding of how bullfrogs might impact the GVWSA should they become established, it is important to know something about their distribution on Vancouver Island and the areas adjacent to the Water Supply Area, and their ecology and diet.

American bullfrogs have been reported in over 100 ponds and wetlands on Vancouver Island, half of which are in the Capital Regional District (Figure 1 and Appendix 3). Although bullfrogs have not been found within the boundaries of the GVWSA, occurrences have been reported in Humpback Reservoir and Amy's Pond, both of which are less than 3 km to the south of Japan Gulch Reservoir in the Goldstream Water Supply Area (Figure 2).

Figure 1: Known bullfrog populations on Vancouver Island. Map courtesy of Trystan Willmott and BC Ministry of Environment (MOE). In our discussions with water managers (see Appendix 5), we were informed that bullfrogs are present in Campbell River. This information was not made available to the MOE in time for the finalization of this map.

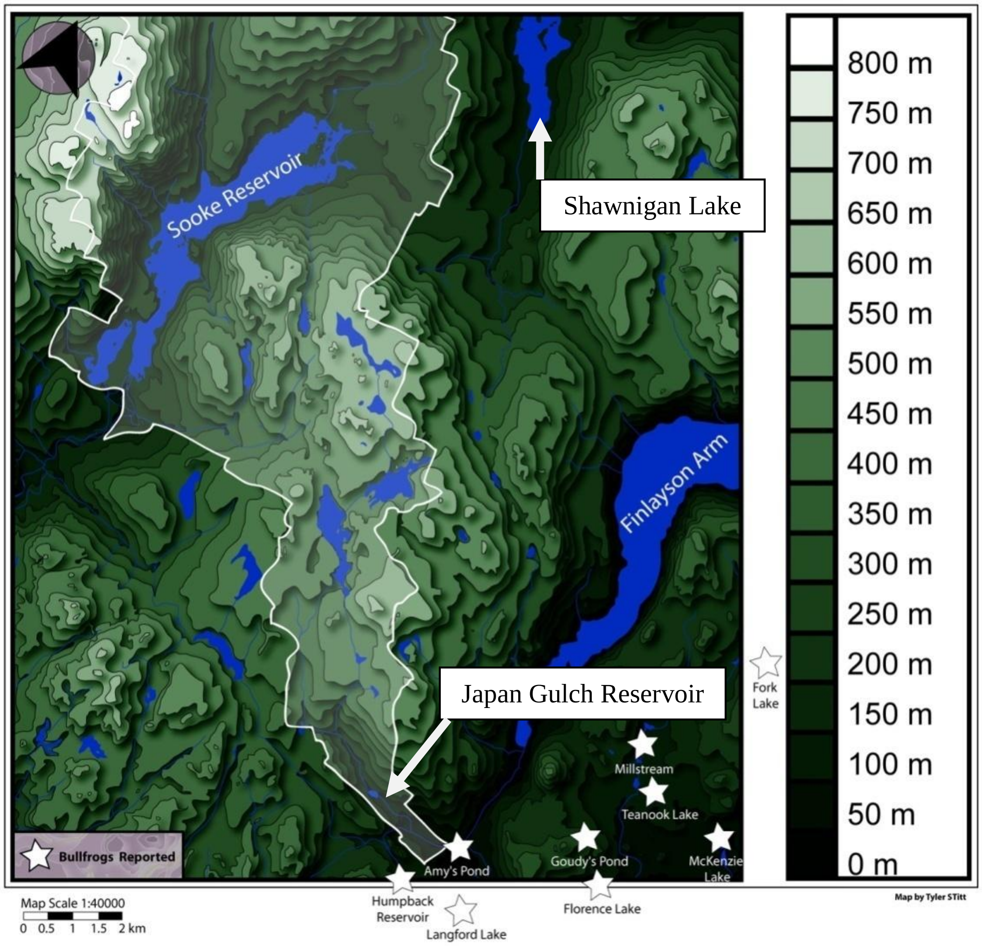

Figure 2: Map of the Greater Victoria Water Supply Area and adjacent areas. Stars indicate water bodies where American bullfrogs have been found. Japan Gulch Reservoir is the closest water supply reservoir to these bullfrog occurrences.

3.2 Bullfrog Ecology and Diet

American bullfrogs are large temperate fresh-water frog species that prefer warm and sunny permanent ponds, marshes or slow river backwaters, although adults will forage in streams and temporary ponds (Corkran & Thoms, 1996). Typically, in the Greater Victoria area, American bullfrogs emerge from hibernation in late April or early May, breed in June, and enter winter hibernation in November (Govindarajulu *et al.*, 2006). Adult females lay up to 20,000 eggs in floating mats on the water surface, and the hatched tadpoles live in the warm shallows and amongst dense aquatic shoreline vegetation (Corkran & Thoms, 1996).

Tadpoles usually metamorphose two years after hatching (Corkran & Thoms, 1996); however, Govindarajulu *et al.* (2005) found that bullfrog tadpoles in British Columbia can increase their development rates under favourable temperature conditions or when tadpole population densities are low, with metamorphosis occurring as early as one year after hatching. Depending on the length of the growing season, bullfrogs reach sexual maturity 1-5 years after metamorphosis. In Ontario, where the growing season is short, bullfrog females become sexually mature approximately five years after metamorphosis (NatureServe, 2008).

Tadpoles consume suspended matter, organic debris, algae, plant matter and small aquatic invertebrates (NatureServe, 2008). The carnivorous and insectivorous froglets and adults tend to ambush hunt from aquatic weeds and shoreline vegetation. Data from two independent graduate-level studies on Vancouver Island suggest that juvenile bullfrogs consume mostly insects and gastropods (Table 1).

The Yellowpoint study (Sturhahn, 2000) showed a much higher insect prey composition in adult intestines compared to Govindarajulu's study in Victoria (2006), which showed that 44% of the identifiable gut content was made up of amphibians (Table 1). There are reports of bullfrogs eating small rodents and birds, but these appear to be one-off and relatively unusual occurrences. A study from New Mexico identified by total percent-volume the composition of bullfrog diet to be newly metamorphosed Rana (87%) species (presumed to be predominantly other bullfrogs), aquatic snails (5%), adult and larval aquatic Coleoptera (3% beetle species), adult and larval Odonata (3% dragonfly and damselfly species) and unidentifiable or other material (2%) (Stuart, 1993). Stuart (1993) found that in highly dense American bullfrog populations, smaller con-specifics are an important food item, and this may account for the apparent discrepancy between Stuart and Sturhahn's findings with regard to the proportion of amphibians in the diet. Cannibalism among bullfrogs has been noted elsewhere as well (Altig *et al.*, 2007; Govindarajulu *et al.*, 2006).

Table 1: Diet composition analysis, displayed as a percentage of the total identifiable content for all the bullfrogs for that age class, of American bullfrogs collected from ponds and wetlands in Yellowpoint and Victoria, Vancouver Island.

Prey Taxa		**Life Stage**	
		Juvenile n = 12	**Adult** n = 26
Scientific Name	*Common Examples*	**Prevalence (%)**	
Gastropoda	Snails and slugs	33.3	7.7
Bivalvia	Freshwater mussels	0	19.2
Insecta	Aquatic larva	8.3	11.5
Insecta	Aquatic adults	50	30.8
Insecta	Terrestrial larvae	0	11.5
Insecta	Terrestrial adults	58.3	61.5
Arachnida	Spiders	33.3	3.8
Amphibia	Frogs and salamanders	0	19.2
Osteichthyes	Fish	16.7	7.7
Aves	Birds	0	7.7
Mammalia	Mammals	0	11.5

(Sturhahn, 2000)
Study location: Cedar/Yellowpoint, British Columbia

Prey Taxa		**Life Stage**		
		Metamorph n = 42	**Juvenile** n = 40	**Adult** n = 68
Scientific Name	*Common Examples*	**Prevalence (%)**		
Hymenoptera	Wasps and bees	43	63	Data N/A
Coleoptera	Beetles	28	40	Data N/A
Aphidoidea	Aphids	23	13	Data N/A
Odonata	Dragonfly & damselfly	0	25	Data N/A
Crustacea	Crayfish and shrimp	0	6	6
Amphibia	Frogs and salamanders	0	<1	44
Osteichthyes	Fish	0	0	0
Reptilia	Snakes and lizards	0	0	<6
Aves	Birds	0	0	<6
Mammalia	Mammals	0	<1	<6

(Govindarajulu *et al.*, 2006)
Study Location: Victoria, British Columbia

3.3 Nature and Magnitude of Possible Water Quality Hazards

3.3.1 Microbial Parameters

Of the thirteen published peer-reviewed studies that isolated and classified bacteria in amphibian intestinal tracts, only four studies involved American bullfrogs (Carr *et al.*, 1976; Ferreira *et al.*, 2006; Gray *et al.*, 2007; Mauel *et al.*, 2002) and only three were conducted in a temperate geographical location (Bartlett *et al.*, 1977; Clark *et al.*, 1982; Hird *et al.*, 1983). Because the other studies were conducted in tropical or subtropical regions (see the reference list in Appendix 2), any conclusions drawn from those studies are unlikely to be representative of the British Columbia situation.

The study by Mauel *et al*. (2002) was done in response to a red-leg outbreak in captive bred bullfrogs. These authors concluded that the clinical signs associated with red-leg disease, which is traditionally attributed to *Aeromonas hydrophila*, may result from infection with a number of different bacterial species. Ferreira *et al*. presents an outbreak of *Mycobacterium marinus* in a commercial bullfrog breeding farm in Rio de Janeiro, Brazil (2006). Both Gray *et al*. (2007) and Carr *et al*. (1976) used wild-caught tadpoles raised in aquarium settings, although Gray *et al*. inoculated bullfrogs to determine their suitability as hosts for *Escherichia coli*. All four of these papers describe cross-sectional point-in-time studies involving captive bullfrogs. Nevertheless, the four studies found six species of bacteria that are either known human pathogens or have pathogenic strains (*Escherichia coli, Shigella, Staphylococcus, Streptococcus, Enterobacter, and Klebsiella* – see Table 2). Four of the bacterial species that were isolated from bullfrogs have only been associated with infections that occur as a result of medical interventions in a hospital or clinical setting (the scientific term is 'Nosocomial'), usually as a result of long term care that involved urinary or intravenous catheterization. We classified one bacterial species as 'Opportunistic' because it is commonly found in the environment and only those with weak immunity, for example an HIV patient or a patient who has had an organ transplant, are susceptible to infection. The bacterial isolates from amphibian species other than the American bullfrogs, and the subject of the other nine research papers, can be found in Appendix 2. **None-the-less, we were unable to find any peer-reviewed papers that studied the role of amphibians, including bullfrogs, as biological sources of bacterial infections in people.**

Table 2 presents the primary route of human infection for the pathogens found in these studies, however, a more complete discussion of the bacteria classified as 'known pathogen' is given here. Gray *et al*. (2007) orally inoculated bullfrog tadpoles and metamorphs with the enterovirulent *E coli* 0157:H7; 54% of the 24 metamorphs tested positive after 14 days. *E coli* was isolated from water samples in 5 of the metamorph tanks, although the authors noted that only one of these bullfrogs had a positive culture from intestinal samples. Carr *et al.* (1976) cultured *E coli* (the particular strain and pathogenicity was not stated) from 4 out of 20 wild-caught American bullfrog tadpoles that had been raised in aquaria through to metamorphosis then subjected to hibernation-like conditions. By comparison, Carr *et al*. isolated Enterobacter species from 15 of the 20 bullfrogs, a much higher prevalence rate compared to *E. coli* (1976).

Table 2: Bacteria isolated from American bullfrog (*Rana catesbeiana*) intestinal tracts and organ samples. Definitions for human health risk are as follows: Known pathogen – Pathogenic strains do occur in humans, although they are often normal flora of skin, urinary tract or gastrointestinal tract, and frequently opportunistic; Nosocomial - current clinical knowledge and published literature suggests it is primarily hospital-aquired; Opportunistic - normal flora and/or environmental contaminant that requires direct innoculation in an immunocompromised individual; Rare - unusual cause of infection and/or a few isolated case reports are available in the literature; Unknown - no indication of pathogenicity to humans and/or has not been isolated from clinically ill patients.

Reference	Pathogen (spp name)	Human Health Risk	Route of Transmission
1 2	*Escherichia coli**	Known pathogen	Fecal-oral; opportunistic normal flora; consumption of contaminated food
1	*Shigella sp.**	Known pathogen	Fecal-oral; unchlorinated wading pools; interactive water fountains; unprotected sex/STI; consumption of contaminated food
1	*Staphylococcus sp.*	Known pathogen	Opportunistic normal flora
1	*Streptococcus sp.*	Known pathogen	Opportunistic normal flora
1	*Enterobacter sp.**	Known pathogen (prevalence appears to be increasing)	Fecal-oral; direct contact through mucous memebranes; nosocomial; opportunistic normal flora; opportunistic environmental contaminant
1	*Klebsiella sp.*	Known pathogen (species dependant)	Opportunistic environmental contaminant; immunocompromised; nosocomial
1	*Acinetobacter sp.*	Nosocomial	Penetrating wound; immunocompromised
3	*Chryseobacterium indolgenes*	Nosocomial	Penetrating wound; indwelling catheters
1	*Flavobacterium*	Nosocomial	see *Chryseobacterium indolgenes*
1	*Staphylococcus epidermidis*	Nosocomial	Nosocomial; indwelling catheter
1 3	*Aeromonas hydrophila**	Opportunistic	Fecal-oral; environmental / waterborne; ingestion of contaminated fish or reptiles
1	*Achromobacter sp.*	Rare	Penetrating wound; immunocompromised
1	*Bacillus cereus*	Rare	Fecal-oral
1	*Bacillus megaterium*	Rare	Penetrating wound; immunocompromised
1	*Bacillus sphaericus*	Rare	Penetrating wound; immunocompromised
3	*Chryseobacterium meningosepticum*	Rare	Opportunistic environmental contaminant
1 3	*Citrobacter freundii**	Rare	Fecal-oral; opportunistic normal flora; direct contact with infected person; nosocomial; immunocompromised

Reference	Pathogen	Human Health Risk	Route of Transmission
1	*Micrococcus sp.*	Rare	Immunocompromised
4	*Mycobacterium marinum*	Rare	Trauma; penetrating wound
1	*Pleisomonas shigelloides**	Rare	Opportunistic environmental contaminant; immunocompromised; opportunistic normal flora; zoonotic; penetrating wound
1	*Pseudomonas aeruginosa*	Rare	Nosocomial
1	*Pseudomonas alcaligenes*	Rare	Opportunistic environmental contaminant
1	*Pseudomonas fluorescens*	Rare	see *Pseudomonas alcaligenes*
1	*Pseudomonas putida*	Rare	see *Pseudomonas alcaligenes*
1 3	*Pseudomonas sp.*	Rare	Opportunistic environmental contaminant
1	*Serratia sp.*	Rare	Direct contact through mucous membranes; nosocomial; indwelling catheter; opportunistic environmental contaminant
3	*Streptococcus iniae*	Rare	Zoonotic; penetrating wound
3	*Edwardsiella tarda*	Rare	Penetrating wound, fecal oral
3	*Aeromonas sp.*	Unknown	see *Aeromonas hydrophila*
1	*Alcaligenes faecalis*	Unknown	
1	*Proteus mirabilis*	Unknown	Oppportunistic normal flora; opportunistic environmental contaminant; nosocomial
1	*Proteus morganii*	Unknown	see *Proteus morganii*
1	*Proteus rettgeri*	Unknown	see *Proteus morganii*
1	*Proteus vulgaris*	Unknown	see *Proteus morganii*
1	Unclassified gram negative bacteria	Unknown	Unknown

*Bacterial pathogens that can be transmitted in untreated drinking water

Reference	Authors	Geographic location of study
1	Carr et al, 1976	Louisiana, United States
2	Gray et al, 2007	Tennessee, United States
3	Mauel et al, 2002	Georgia, United States
4	Ferreira et al, 2006	Rio de Janeiro, Brazil

E. coli 0157:H7 is primarily associated with the intestinal tracts of domestic livestock, and most human infections are acquired through the consumption of raw or undercooked food rather than drinking water. These limited studies indicate that a proportion of bullfrogs can be suitable hosts for *E. coli* under experimental conditions, and that there is some evidence that they are capable of shedding it into the water. To pose a significant public health threat to human consumers, bullfrogs would need to be become infected, amplify the bacteria, and then shed large numbers of bacteria back into the reservoir. As with any epidemic, we would expect there to be a spike in bullfrog morbidity and mortality, and ending with some bullfrogs fully recovering and others becoming long-term carriers. Animals that are sick and dying will often become depressed and lethargic, and tend not to travel very far; they also become easy prey for predators. Assuming that *E. coli* is pathogenic to bullfrogs, individuals infected at a point source (e.g. a pond contaminated by cattle feces) would have a limited amount of time (the incubation period of the bacteria within the frog) in which to travel before succumbing to illness and either dying or recovering. There are no epidemiological studies of *E. coli* in bullfrogs, and so the duration of this incubation period, let alone the pathogenicity, is unknown. Furthermore, very large numbers of infected bullfrogs would need to successfully migrate to a new water body to pose a serious public health risk. *E. coli* could potentially be introduced into the reservoir via animals that recover or are infected but do not develop disease and continue to carry and shed *E.coli*. Large numbers of carriers would need to be shedding copious *E.coli* before levels would surpass CRD’s monitoring threshold. Fecal contamination of the water from a bird or mammalian source is much more likely to result in an *E. coli* outbreak, and therefore we feel that American bullfrogs will not result in a threat to public safety by facilitating increases in *E. coli* counts.

Shigella is a very common cause of gastrointestinal upset and diarrhea in humans, and is often associated with fecal-contaminated water and unsanitary handling of food. It is estimated, however, that a viable infectious dose can be as low as 10 bacteria, depending on the age and condition of the host (Walderhaug, 2007). Carr *et al*. (1976) isolated *Shigella* from one of 20 bullfrogs, and to date was the only report of Shigella in amphibians.

Both *Staphylococcus* and *Streptococcus* are opportunistic pathogens that normally inhabit skin and respiratory tracts. Some strains, for example *Staphylcoccus aureus* and Group A and Group D *Streptococcus*, can cause food poisoning when humans consume food that was undercooked or was left at room temperature for prolonged periods of time. Carr *et al*. (1976) cultured *Staphylococcus* from 3 bullfrogs, and *Streptococcus* from 6 bullfrogs. As with *Shigella*, these were the only reports of these bacteria ever being isolated from amphibians.

Klebsiella and *Enterobacter* are ubiquitous in the environment. Both of these bacteria can cause a variety of human diseases ranging from septicemia to urinary tract infections to soft tissue infections, but are most frequently associated with nosocomial infections and in immunocompromised individuals. Although *Klebsiella* was only isolated from one bullfrog, *Enterobacter* was cultured from 15 of 20 bullfrogs (Carr *et al.* 1976). *Enterobacter* is not known to cause disease in frogs, and these authors suggested that it might be normal flora of their intestinal tracts. Unfortunately, Carr *et al*. (1976) did not attempt to evaluate whether or not bullfrogs could shed any of the bacterial organisms that were cultured from them.

Mauel *at al*. (2002) were able to isolate a number of bacteria from clinically ill captively reared American bullfrogs during an outbreak of red leg disease. Red leg disease is a syndrome of cutaneous hyperemia and hemorrhage of the subcutaneous and skeletal muscles, often associated with septicemia. In particular, they isolated *Aeromonas hydrophila, Chryseobacterium (Flavobacterium) meningosepticum, Chryseobacterium (Flavobacterium) indolgenes, Edwardsiella tarda, Citrobacter freundii, Pseudomonas spp., and Streptococcus iniae*. Of these bacteria, Aeromonas has been described as an opportunistic human pathogen in a limited number of case reports (Davis *et al.*, 1978). Usual routes of infection by these opportunistic environmental pathogens include penetrating trauma and direct inoculation through the skin; they can be hospital acquired following medical intervention, and are more likely to result in illness in immunocompromised humans. They are not known to cause community outbreaks.

Of note is the observation that all the bacteria discussed in detail above can be isolated from the environment (terrestrial and aquatic) or from the gastro-intestinal tract of numerous species of wildlife, domestic animals and humans. These bacterial pathogens are not unique to bullfrogs.

Other pathogenic bacteria, including *Salmonella typhimurium, S. enteritidis*, Clostridia (see Appendix 2) and leptospirosis (Babudieri *et al.*, 1973; Everard *et al.*, 1988) have been isolated from amphibians other than American bullfrogs. However, these studies were conducted in tropical countries and care must be taken when applying their conclusions to temperate regions such as Vancouver Island, given substantial differences in environmental temperatures and conditions, and the proximity of human populations and fecal wastes to water bodies that contain amphibian populations.

3.3.2 **Bacterial Toxins**

We found no papers that associated bullfrogs with bacterial toxins that could affect human health or water quality indicators. Indeed, bullfrog tadpoles may selectively graze on algal species that produce toxins (see Section 3.3.9: Algal Blooms).

3.3.3 **Parasitic Protozoa**

We identified a single paper that suggested that fish, amphibians and reptiles might be able to spread *Cryptosporidium parvum* oocysts in the environment, but only after ingesting prey already infected with *C. parvum*. These authors used gastric intubation to put the infectious stage (oocysts) into the stomach of bluegill sunfish, poison-dart frogs, African clawed frogs, bearded dragon lizards and corn snakes. Animals were euthanized at 7 and 14 days post-infection, and sections of the intestines were analyzed microscopically (Graczyk *et al.*, 1996). Although histology for the replicating stage (sporozoites) was negative in all specimens, the authors detected oocysts in the feces of fish and frogs for up to 12 days post infection. This would suggest that poison-dart frogs and African clawed frogs may be able to ingest and defecate *C. parvum* oocysts, but that they are unsuitable hosts for parasite replication. This may also be true for American bullfrogs, but further studies are warranted. There are currently no published reports on the suitability of bullfrogs as hosts for *Giardia*.

3.3.4 Fungi

There has been one published case report of an incidental finding of the fungus *Adiaspiromycosis* in American bullfrog legs in South Carolina (Hill & Parnell, 1996). This soil-associated fungus is rarely associated with ectothermic animals such as amphibians, but has been reported in small mammals such as squirrels, mice, rats, beavers and racoons (Hill & Parnell, 1996). *Adiaspiromycosis* has been described as a rare causative agent of fungal lung infection in humans, but only after exposure to and inhalation of dust-borne spores (England & Hochholzer, 1993).

3.3.5 Viruses

It has traditionally been accepted that most viruses are host specific, as they are adapted to use the cellular mechanisms of its host to facilitate its own replication and spread within the host and to other hosts. Certain viruses can survive in the environment for prolonged periods of time (foot and mouth disease, a virus of cattle and sheep, can survive in the environment for up to 28 days under the right conditions; Government of Saskatchewan, Foot and Mouth Disease, http://www.agriculture.gov.sk.ca/Foot_And_Mouth_Disease), but for the most part viruses require direct contact with an infectious individual or contaminated object (fomite), or an intermediate host (vector) that can spread the virus between hosts. Furthermore, because of host specificity, many viruses will have optimal temperature ranges within which they are infectious. Amphibian viruses, therefore, are unlikely to be infectious to humans, and vice versa. Seemingly contradictory to this were the findings of references to a number of Russian studies that found that the Marsh Frog (*Rana ridibunda*) was not only a competent reservoir for West Nile Virus (WNv), but also a suitable target for *Culex pipiens*, one of the primary mosquito carriers of WNv (Kostiukov *et al*., 1985 in Hayes *et al.*, 2005; Kostiukov *et al*., 1986 in Hubálek & Halouzka, 2000). Klenk and Komar (2003) experimentally injected 24 North American bullfrogs with live WNv, but only two of the bullfrogs, killed at one and three days post exposure, had detectable virus in the blood (viremia). Although these authors postulated that bullfrogs could become infected, Hayes *et al*. (2005) concluded from the data presented in Klenk's and Komar's study that American bullfrogs were incompetent as amplifying hosts. Klenk and Komar further hypothesized that WNv might be spread from viremic bullfrogs to predators via the oral route (2003); however, the minimum dose required for oral infection of potential predators is unknown, and the role of amphibians in amplifying WNv has not been pursued any further.

Although human viruses such as Hepatitis A, Rotavirus and Norwalk Virus can be transmitted in water, there are no reports in the literature of amphibian involvement in their transmission. This literature review was unable to find any other peer reviewed or grey literature to suggest that American bullfrogs could harbour viruses that are pathogenic to humans.

3.3.6 Multi-cellular Parasites

Numerous parasitological surveys have been done to show that American bullfrogs, as well as other amphibian species, are definitive and intermediate hosts to a wide variety of multi-cellular nematode, trematode and cestode parasites (Andrews *et al.*, 1992; Hollis, 1972; Lemke *et al.*, 2008; Muzzall, 1991; Yoder & Gomez, 2007). **It is feasible that some of these parasites can affect humans, but based on our current understanding of parasite lifecycles, this would require the consumption of infectious stages (cysts) already present in frog leg muscle**. In general, encysted parasites are killed with proper cooking and food handling. Aquatic parasite life stages that cause human disease, such as Schistosoma (a causative agent of swimmer's itch), do not involve amphibians. Typically, these parasites develop in aquatic snails before they are released into the water, and need to penetrate the skin to cause infection in humans. This requires human contact with water.

Although a very large taxonomic list of parasite species has been catalogued from American bullfrogs throughout North America, data from studies done in other jurisdictions may not be applicable to Vancouver Island. Such a list was presented at the American Bullfrog Exploratory Meeting in Victoria on November 29, 2004 by Mr Stan Orchard (see Appendix 6). **However, the list presented in Appendix 6 is not specific to Vancouver Island**. In a relatively recent study from Vancouver Island, at least one of six different helminth parasite species were identified in 60% of the 48 wild-caught American bullfrogs (Sturhahn, 2000). Sturhahn found that the lung trematode *Haematoloechus longiplexus* was the most prevalent, followed by *Gorgodernia attenuate* and *Glypthelmins quieta*, trematodes of the urinary bladder and upper small intestine respectively (2000). This is a completely different composition from a survey conducted by Lemke *et al*. (2008) on wild-caught American bullfrogs purchased from a vendor in Massachusetts (see Table 3). The distribution of helminth parasites in anurans has previously been correlated to the prey availability and habitat used by the frog (Goater *et al*., 1987 and Aho, 1990 in Sturhahn, 2000); it can be expected, therefore, that parasite species compositions will vary even between different anuran species in the same water body. Furthermore, parasite species compositions & prevalence within and between water bodies will vary depending on:

- The host species (both definitive and intermediate) present in the water body
- Geographical location and environmental factors, including temperature and rainfall
- Habitat preferences and niches for each host species
- Prey availability and diet preferences
- Parasite lifecycle (i.e. transmission factors and intermediate hosts requirements)

Table 3 compares the parasites identified from American bullfrogs in Massachusetts to those found in American bullfrogs in Nanaimo, on Vancouver Island. The final host for these parasites are either the frogs themselves, or direct predators of frogs. Despite the fact that American bullfrogs are consumed by humans, and have been for many decades, **we were unable to find any references or case reports of bullfrog parasites causing human infection**.

Table 3: Inventory of parasites identified from American bullfrogs in Massachusetts and on Vancouver Island. Note that there is variability in both parasite species and genus.

	Species		Location	Intermediate host	Final host
Lemke et al., 2008 Massachusetts	*Clinostomum* spp.	Trematode	Musculature and fascia	Frogs and fish	Birds
	Gorgodera amplicava	Trematode	Urinary bladder	Clam and snail	Frogs infected by eating snails harboring metacercaria
	Haematolechus breviplexus	Trematode	Lungs	Snails (primary) and insects; odonates (secondary)	Frogs infected by eating infected insects
	Contracaecum spp.	Nematode	Stomach wall and coelom	Snails, fish, amphibians	Birds
	Cosmocercoides dukae	Nematode	Intestines, rectum	None	Molluscs
	Eustrongyloides spp.	Nematode	Intestine, muscle	Frogs and fish	Birds
Sturhahn, 2000 Nanaimo	*Haematoloechus longiplexu*	Trematode	Lungs	Snails (primary) and insects; odonates (secondary)	Frogs infected by eating infected insects
	Gorgoderina attenuate	Trematode	Urinary bladder	Snails (primary) and insects; odonates (secondary)	Frogs infected by eating infected insects
	Glypthelmins quieta	Trematode	Upper small intestine	Snails	Frogs
	Falcusta	Trematode	?	?	?
	Acanthocephalan		Intestine	Crustacean	Variable and host specific
	?	Nematode	?	?	?

'?' denotes a lack of published information on the lifecycle of that parasite

3.3.7 Physical Parameters

The CRD tests for a number of parameters that are thought to influence a consumer's perception of water quality and safety. These parameters include taste, alkalinity, hardness, total dissolved solids, turbidity and pH. We interviewed a number of municipal water managers and herpetologists to determine if bullfrogs had, or were ever implicated in, altering a water body's physical characteristics such that water quality and safety would be or would perceived to be negatively impacted. This question has apparently never been asked before, and no data currently exists to adequately support or refute this hypothesis. Of the 20 utility managers that we interviewed, nine indicated that bullfrogs had been found in their water supply and five of these manage utilities on the west coast where bullfrogs are invasive. Furthermore, none of these managers had recorded changes in water quality parameters, or taken steps to change their water treatment protocols (see Appendix 5). Where bullfrogs are present, five utilities use similar water treatment protocols to the GVWSA, with Campbell River being the most similar.

Even though invasive bullfrogs are now endemic in over 100 water bodies on Vancouver Island (see Figure 1) and in much of western North America, **we were unable to find any documented or published reports that discussed changes in physical or organic water quality parameters with the presence of invading American bullfrogs**. It may be that the American bullfrog colonized these wetlands before they were incorporated into municipal supplies, or that changes have gone unnoticed. Alternatively the successful colonization of new habitats by American bullfrogs has had negligible impacts on routinely measured water quality parameters.

3.3.8 Non-Metallic Inorganic Chemicals

Our original hypothesis that an established population of bullfrogs could significantly increase the supply of nitrogen and phosphorus in the water by eating algae and excreting soluble nutrients can not be substantiated. The collective experiences and observations reported from interviews with researchers and water managers on bullfrog effects in aquatic ecosystems, and specifically in drinking water supplies, in addition to a review of the literature, have shown no evidence that American bullfrogs alter nutrient supply or cycling.

Experience with nutrient flux by tadpoles (Seale, 1980) indicates that the overall effect is a loss of nitrogen from the aquatic system as tadpoles mature and move out of the water to the terrestrial environment. This transfer of aquatic nutrients to the terrestrial system by maturing frogs has been also noted by Dickman (1968) and Hoff *et al*. (1999), and indicates that, if anything, frogs represent a net loss of nutrients from an aquatic system.

3.3.9 Algal Blooms

We sought literature to examine the effect that an influx of very large numbers of herbivorous American bullfrog tadpoles might have on the composition of certain algae and plant species to determine the potential for toxic algal blooms within CRD's Water Supply Area. The hypothesis that American bullfrogs could place selective pressure on some algae species, thereby releasing constraints on other algal species and facilitating algal blooms, could not be substantiated from the peer reviewed and grey literature.

Nevertheless, tadpoles have a diverse role in aquatic ecosystems, and where population numbers are kept in check by food availability, predation and competition, they are an integral component of that ecosystem. The impacts that an invading population of American bullfrog tadpoles might exert on the vegetative strata of a water body is purely speculative given the complexity of the aquatic ecosystem and the lack of published data.

American bullfrog tadpoles are known to feed preferentially on periphyton and will also feed on phytoplankton, although at least one study has indicated that tadpoles may also consume and assimilate animal proteins into their diet (Schiessari 2004 in Altig *et al.*, 2007). In an early study by Seale (1980), increased tadpole biomass (of which American bullfrogs were one of a number of species present) in a pond ecosystem was associated with a decrease in filamentous blue green algae levels, leading Seale to conclude that tadpoles are largely regulatory consumers of phytoplankton. A study by Pryor (2003) examined four groups of bullfrog tadpoles that were fed different algal diets: two cyanobacteria (*Anabaena* and *Microcytis*) and two green algae (*Selenastrum* and *Ulothrix*). Surprisingly, the best growth was with *Anabaena*, which is generally thought to be a poor food source. Altig *et al*. (2007) caution, however, that what we can count, measure and identify in tadpole intestines may actually be misleading – we should expect that tadpoles will ingest a diversity of food items, only some of which they are able to fully digest and use for growth. Tadpole diets show temporal and spatial variability, and cannibalism and scavenging have been documented in a variety of tadpole species (Altig *et al.*, 2007).

Anabaena produces two different neurotoxins (Anatoxin-a and Anatoxin-a(s); although similarly named, they act on different pathways) and a hepatotoxin (Microcystin) (Crayton, 1993). Symptoms in mammals is largely dependant on the size of the animal and the amount of toxins ingested, but in severe cases can include tetanus-like paralysis with convulsions, excess salivation and tremors from the anatoxins and shock (due to a loss of blood), jaundice (indicating damage to the liver), nausea and vomiting from microcystin (Crayton, 1993). Human illness primarily occurs following recreational use (i.e. swimming, boating) that lead to accidental ingestion of water contaminated by cyanobacterial blooms. Symptoms can include stomach cramps, diarrhoea, fever, headaches and generalized joint pain and weakness; ingestion of contaminated water is infrequent, usually because the algal blooms alter the taste and odour of the water supply, but symptoms can be more severe and include gastroenteritis, liver damage and occasionally death (Health Canada, 2008).

Among Seale's other observed effects of increased tadpole biomass in Missouri ponds was an apparent reduction in the standing crop of suspended particles, reduced rates of primary production, an increase in dissolved versus particulate nitrogen and decreased proportions of chlorophyll *a* in the photosynthetic pigments of phytoplankton. Although the specific role of American bullfrogs were not isolated in comparison to the effects of other anurans, there was variability in temporal patterns of mating, egg deposition and hatching events across the different species studied (Seale, 1980). This would suggest that multiple anuran species act together to "manage" primary production throughout the growing season, which minimizes the potential for algal blooms in the wetlands. Seale did show that when metamorphosis removed tadpoles from an ecosystem, rates of primary production increased dramatically (1980); however, this review did not find evidence to support the hypothesis that changes in tadpole population and species structure could impact on primary production.

There was a general consensus among the interviewees that an invasion of bullfrogs into a new habitat would not directly change the composition of certain algae and plant species. The municipal water managers that we interviewed indicated that the presence or appearance of American bullfrogs has not been associated with impacts on water quality, which includes toxic algal blooms (Appendix 5). A general theme among the expert herpetologists consulted was that bullfrogs would have the largest impact on water bodies already modified by human activity, and that American bullfrog colonisations would cause minimal if any environmental changes (Appendix 4).

3.4 Impacts on Water Supply Infrastructure

We found no evidence to support the hypothesis that American bullfrogs have had, or could have, direct impacts on water supply infrastructure. We know that adult bullfrogs inhabit the shoreline of aquatic ecosystems, but their diving depth is currently unknown, or has not yet been reported in the literature. Without this information, we cannot accurately determine the true magnitude of the risk to the Sooke Reservoir water intake pipes. We can conjecture, however, that this risk is minimal given that:

- There is a lack of suitable bullfrog habitat in the near vicinity of the intake and dam
- The intake tower is separated from the shoreline by open water
- There are physical barriers (mesh screens) in place to prevent undesirable material from entering the water treatment plant.

Indirect impacts on water intake systems could conceivably occur as a result of ecosystem changes in algae, vegetation and silt/dissolved particles. However, we could not find any literature to suggest that bullfrog populations could cause the magnitude of change necessary in the ecosystem to facilitate damage to the water infrastructure.

We have identified Japan Gulch Reservoir as a potentially suitable habitat for American bullfrogs for several reasons:

- Close proximity to water bodies where bullfrogs have been found
- Presence of bullfrog aquatic and riparian habitat
- Well below the elevation ceiling of previously successful bullfrog colonisations on Vancouver Island

Water intakes at Japan Gulch Reservoir are located at 7 m below the water surface. Although bullfrog diving depths are unknown, it is unlikely that bullfrogs could enter the intake at Japan Gulch. Indeed we were unable to identify any reports of bullfrogs damaging or plugging water intakes.

Figure 1: Panorama of Sooke Reservoir intake and adjacent shoreline. Photo available from http://www.crd.bc.ca/water/watersupplyarea/reservoir2008.htm.

3.5 Impacts on Ecosystem Health

Section 1.2.4 presented a small list of potential effects on aquatic ecosystems. The first four topics (habitat restructuring, alteration of nutrient inputs or timing, changes in primary production, and changes to aquatic trophic structures) have overlap with water quality parameters, and were therefore discussed in previous sections of this report. This section will focus primarily on changes to riparian and terrestrial trophic structures, with an emphasis on the introduction of novel amphibian pathogens and impacts on biodiversity as a result of successful American bullfrog invasion.

More than 100 papers with titles or abstracts relevant to the topic area of bullfrogs and ecosystem health were identified, read and evaluated for information on ecological impacts that result from successful bullfrog colonisations. Papers published on the effects of bullfrogs in western North America provide some insight to what might happen in the CRD Water Supply Area (Adams, 1999; Adams, 2000; Adams *et al.*, 2003; Bury & Luckenbach, 1976; Chivers *et al.*, 2001; Cohen & Moyle, 2004; Dickman, 1968; Hayes & Jennings, 1986; Kupferberg, 1997; Kupferberg, 1997; Lawler *et al.*, 1999; McCamman & Chrisman, 2008; Moyle, 1973; Pearl *et al.*, 2004; Pearl *et al.*, 2005; Pearl *et al.*, 2005). These studies indicate that the most common observation with regards to invasive American bullfrogs is their potentially deleterious impacts on other amphibian species, for reasons that primarily involve predation, competition or disease introduction. Few other significant ecological effects as a result of bullfrog colonization are discussed, and there is no documentation of suspected or measured impacts on drinking water quality.

Local studies based out of the University of Victoria provide very specific and detailed information on the biology of the bullfrog in and around the Greater Victoria area (Garner *et al.*, 2006; Govindarajulu, 2004; Govindarajulu *et al.*, 2005). A number of considerations can be gleaned from these local studies:

- Bullfrogs prefer small productive ponds and lakes where there is heavy riparian terrestrial vegetation and good aquatic littoral vegetation. This vegetation provides cover from predators and habitat for food prey items.

- If there is an ecological effect of any kind, it is more likely to occur in the preferred habitat of the American bullfrog (small ponds and lakes and wetlands) than in larger lakes. These effects may not be transferred to downstream water supply reservoirs.

- No examples of major trophic disruption are described in the local studies, and certainly not in larger lakes or water reservoirs. We anticipate that had the researchers seen major trophic changes as a result of predation or competition by bullfrogs, they would have reported.

Although there is no standard accepted definition for lake and pond, they are usually differentiated on the basis of some arbitrary size (depth or surface area). For example, a ‘large’ pond is often less than 10 ha in surface area, and ‘small’ ponds under 1 ha (Kalff 2002), with a depth of up to 5 meters. This implies a lack of thermal stratification and light penetration to the bottom sediment, standing water year round, and the dominance of vascular aquatic plants. Wetlands are more difficult to define. The US Clean Water Act defines wetlands as "those areas that are inundated or saturated by surface or groundwater at a frequency and duration sufficient to support, and that under normal circumstances do support, a prevalence of vegetation typically adapted for life in saturated soil conditions. Wetlands generally include swamps, marshes, bogs and similar areas." Wetlands often have periods of time where there is no standing water.

3.5.1 Changes in Riparian and Terrestrial Trophic Structure

Most fish species native to western North America do not readily eat bullfrog tadpoles, because of taste or other repellent properties. One of the proposed reasons for the survival of the large bullfrog tadpoles is their rejection as a food item by fish (Kruse & Francis, 1977), and this seems to be a pattern for fish food preferences in both the bullfrog native range and in introduced areas. Certain fish species may have an indirect role in managing bullfrog populations through their direct impacts on invertebrate predator populations. The key role of dragonflies as the only effective predator of bullfrog tadpoles is thought to be important (Travis *et al.*, 1985; Werner & McPeek, 1994). In a recent experiment, it was shown that ponds containing bullfrog tadpoles as well as dragonfly larvae had very different outcomes if sunfish, which are predators of dragonfly larvae, are also present (Adams *et al.*, 2003). Adams reported that in ponds containing sunfish, bullfrog populations reached high densities, whereas in ponds without sunfish, tadpole numbers were much reduced due to dragonfly predation pressure (2003). The implication is that in the Greater Victoria Water Supply Area, bullfrog larval populations might be contained as long as there were no fish to prey on dragonfly larvae. Fisheries management could therefore be an important indirect controller of bullfrog populations.

In Section 4.1.2 below, we estimate expected population numbers for Sooke Reservoir. Our calculations indicate that bullfrog numbers would likely be too low in Sooke Reservoir to provide any additional attraction for the animals that already prey on native frog species, such as raccoons, herons, kingfishers, snakes and fish and therefore, no substantial increases in the risk of deposition of fecal material and mammalian-adapted pathogens into the reservoir. Although many of these same species are known to predate on juvenile and small American bullfrogs in British Columbia, large American bullfrogs have few predators (Govindarajulu & Dodd, 2008). It is unlikely that Sooke Reservoir would see a change in either composition or population size of mammalian and bird predator species.

3.5.2 Introduction of Novel Pathogens

We did not find any evidence in the peer reviewed and grey literature, or through interviews with knowledgeable herpetologists, to suggest that American bullfrogs could be a significant source of viruses, fungi or parasites that are capable of causing disease in humans.

Bullfrogs have been shown to carry *Batrachochytrium dendrobatidis*, the causative agent of chytridiomycosis (a disease of frogs), with very little negative impacts to themselves (Daszak *et al.*, 2004). However, new evidence suggests that bullfrogs may not be a vector of chytrid fungus. *B. dendrobatidis* has recently been found in soil samples from as far north as Alaska, Yukon and the NWT (regions still free of bullfrog), and the fungus has been found in native amphibians of the GVWSA (Govindarajulu, 2008). Although chytrid fungus has been cited as a major cause of amphibian declines worldwide (Cohen, 2001; Daszak, Tabor *et al.*, 2004; Daszak *et al.*, 2004; Longcore *et al.*, 1999), this fungus has not been associated with human illness.

Bullfrogs are a known host for tadpole edema virus (Daszak *et al.*, 1999). This virus has been isolated from diseased bullfrogs, and experimentally has been shown to cause up to 40% mortality in metamorphosing American bullfrogs and up to 100% in *Bufo marinus* toads (Daszak *et al.*, 1999). Tadpole edema virus causes pathological changes similar to those seen in the kidney, liver and intestinal tract of *Bufo marinus* caused by the ranaviruses frog virus 3, Bohle iridovirus, and Gutapo virus (Hyatt et al. 1998 in Daszak *et al.*, 1999). Ranaviruses do appear to be associated with amphibian and freshwater fish mortalities in North America and elsewhere (Daszak *et al.*, 2000), however, the risk to native amphibians and freshwater fishes in the CRD from ranaviruses as a result of American bullfrog introductions cannot be quantified. Bullfrogs in the Greater Victoria have never been tested for ranavirus, and die-offs in any amphibian species on Vancouver Island have not been reported. Whatever risk exists, however, is to other amphibians and not humans, as ranaviruses have only ever been isolated from ectothermic animals such as fish and amphibians (Pringle, 2002).

At this time, there have been no reported associations of American bullfrogs with botulism outbreaks in wetlands, or with any other bacterial or algal toxin.

3.5.3 Loss of Biodiversity (effects on native amphibians)

There are a large number of papers that document the effects of bullfrog invasions on native amphibians (Adams & Pearl, 2007; Clarkson & DeVos, 1986; Doubledee *et al.*, 2003; Hayes & Jennings, 1986; Hecnar & M'Closkey, 1997; Jennings & Hayes, 1985; Kiesecker & Blaustein, 1997; Kiesecker & Blaustein, 1998; Walston & Mullin, 2007; Werner & McPeek, 1994; Werner *et al.*, 1995; E. Wind. 2004; Wu *et al.*, 2005). Most researchers have noted that bullfrogs negatively affect other native frogs through competition and predation (Kiesecker & Blaustein, 1998; Kupferberg, 1997; Lawler *et al.*, 1999; Moyle, 1973; Schwalbe, 2008), although the introduction of bacteria, fungus, virus and parasites may also play a role (see section 3.5.2 above).

Some authors have contested the theory that American bullfrogs are solely responsible for the disappearance of native amphibian species, arguing that the confounder of human activity and fish predation is often overlooked and unaccounted for (Adams, 1999; Adams, 2000; Adams, 2008; Hayes & Jennings, 1986). In 1978, Green surmised that American bullfrog populations would be unlikely to cause the complete elimination of the native Northern Red-legged frog *Rana aurora* from Vancouver Island. Green observed healthy *R. aurora* populations in forest stream habitats near ponds with large bullfrog populations, and commented that the forest habitat preferred by the Red-legged frogs is generally unsuitable for American bullfrogs (1978). Pearl *et al.* (2004; 2005) have shown that American bullfrogs and Red-legged frogs (*R. aurora*) prefer different habitats, with the bullfrogs more likely to remain at or near the shoreline. These authors have also demonstrated that *R. aurora* had relatively better escape mobility than the aquatic Oregon Spotted frog (*R. pretiosa*), and was more likely to coexist with bullfrogs than the Oregon Spotted frog, which has seen marked population declines in areas populated by American bullfrogs.

We were unable to find any evidence to correlate changes in amphibian population dynamics, either through the loss of native species or the invasion of alien species, with negative changes to measurable water quality parameters.

4 RISK

Risk is proportional to the likelihood (or probability) of an impact by American bullfrogs on one or more of water quality, water supply infrastructure and aquatic ecosystems, and the magnitude of these impacts on the CRD watershed. In this section, we will first evaluate the risk of successful bullfrog colonization in the CRD watershed, on which we will base our discussion on the risks to water quality, water supply infrastructure and aquatic ecosystem health. Areas of uncertainty will be highlighted within the discussion.

4.1 Risk of Successful Bullfrog Colonization in the GVWSA

4.1.1 Likelihood of Successful Bullfrog Colonization

American bullfrogs are not well adapted to certain environments and terrains. Michael Adams (USGS, Oregon) suggested that in parts of Oregon and Washington, American bullfrogs have an elevation ceiling that is closer to sea level than in other parts of North America. Although Adams (2008) reports Puget Sound populations only in lowland areas, bullfrogs have been recorded in wetlands over 1800 meters above sea level in parts of California and Arizona (Schwalbe, 2008). Montana's Natural Heritage Program field guide for American bullfrogs states that they are present in ponds and rivers in the valleys, but have been unable to invade the colder, higher elevation waters of the Northwest (the website unfortunately does not qualify this statement with elevation data). The field manual 'The Amphibians of Oregon, Washington and British Columbia,' states that bullfrogs inhabit a range from between sea level and 760 meters, and occasionally up to 1500 m (Corkran & Thoms, 1996). We investigated the consistency of these comments with what has been observed on Vancouver Island by determining the elevations of all water bodies where bullfrogs have been sighted or confirmed to live (refer to Appendix 3. Data courtesy of P. Govindarajulu). The highest water body was Goudy's Pond (at 230 m above sea level) and Pease Lake (225 m), both of which are in Victoria. Sooke Reservoir, by comparison, is 186.75 meters above sea level at the high water mark, or within this hypothetical elevation ceiling (see figure 4).

There could be a number of reasons as to why bullfrogs have not been found at elevations above 250 m on Vancouver Island:

- Freshwater lakes, ponds, marshes and wetlands at higher elevations on Vancouver Island may be infrequently visited or modified by people as compared to lower elevation water bodies closer to towns and cities. An absence of bullfrogs could therefore either be real (i.e. bullfrogs have not colonized water bodies above 250 m) or perceived (i.e. there is an observation bias as a result of very low bullfrog population numbers, or peak bullfrog activity does not coincide with peak human activity in the same ecosystem).

- It is conceivable that bullfrogs have been sighted above 250 m, but have not yet been reported to and confirmed by the appropriate authorities (e.g. BC FrogWatch program).

- The terrain may be too steep or otherwise too difficult for bullfrogs to navigate between the lower aquatic ecosystems in the coastal plains and valleys to the higher more mountainous aquatic ecosystems. Dr Cecil Schwalbe (University of Arizona) has preliminary data to show that American bullfrogs can travel up to 11 km per day in valley bottoms, but have greater difficulties traversing steep gradients. Data from this study is expected to be analyzed and submitted for publication late in 2009.

- Vancouver Island is located in the northernmost extent of both native and introduced bullfrog distribution, which may imply a narrower acceptable thermocline for successful bullfrog colonization at higher latitudes. It is, therefore, conceivable that elevation has a greater influence on bullfrog biology, reproduction success and over-wintering survival on Vancouver Island than elsewhere in North America.

The elevation data for the CRD watershed was graphed out with the ceiling of 250 m (Figure 4). **Assuming elevation and steepness of terrain are obstacles to the expansion of bullfrogs, Japan Gulch and Sooke Reservoirs would seem to be areas of highest likelihood for invasion**. The other lakes and rivers appear to be outside of the elevation ceiling on Vancouver Island. Furthermore, Japan Gulch is within 3 km of water bodies with recorded bullfrog occurrences, and is connected via a network of streams to those water bodies. It is also possible that the smaller size of Japan Gulch reservoir makes it inherently more suitable for the rapid establishment of populations of American bullfrogs, as compared to the much larger and more remote Sooke Reservoir.

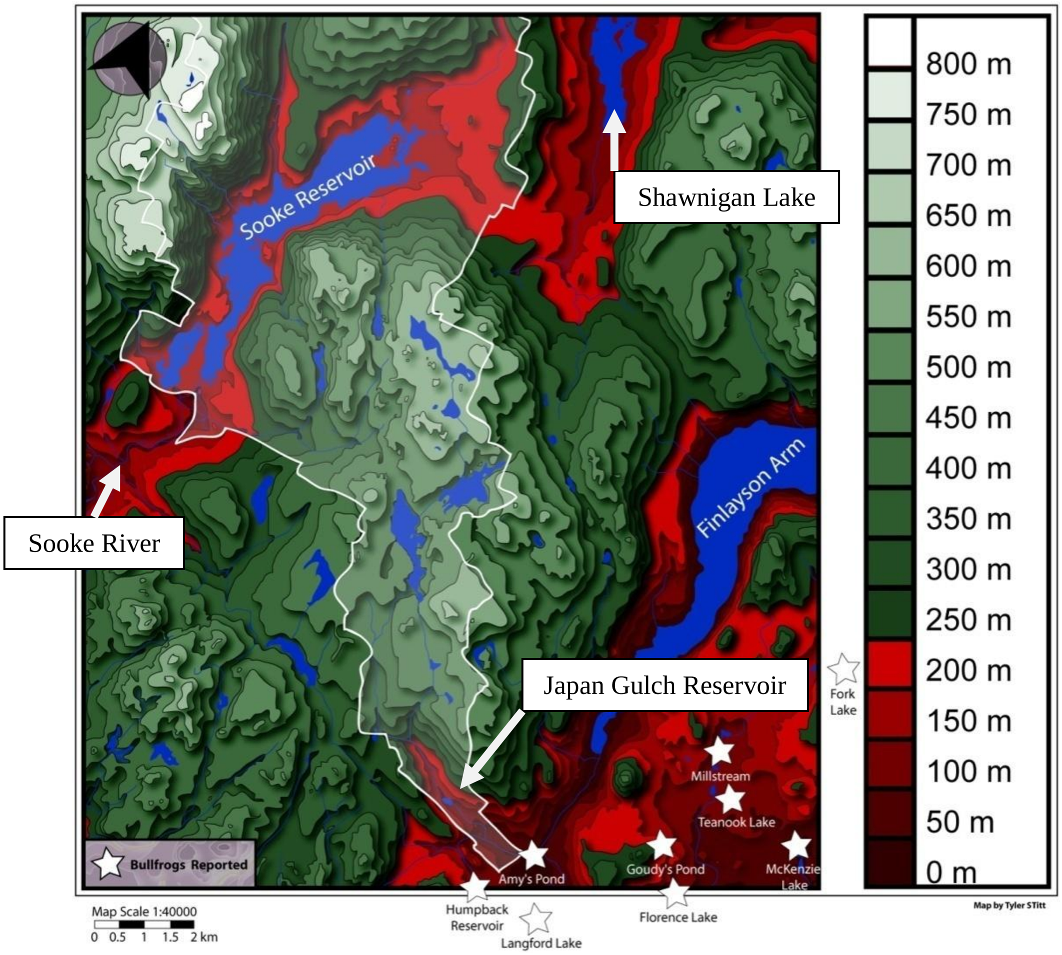

Figure 2: Current elevation ceiling for American bullfrogs on Vancouver Island. Note that Japan Gulch Reservoir and Sooke Reservoir are the only water supply reservoirs below 250 m elevation. This map also gives some idea of likely natural invasion routes – Japan Gulch via Amy's Pond or Humpback reservoir, and Sooke Reservoir via either Sooke River or Shawnigan Lake.

A number of factors may actually increase the likelihood of successful bullfrog colonization in the GVWSA. They include human-centric as well as unanticipated environmental factors. Human-centric factors are generally regarded as the primary method of bullfrog dispersal, and include the use and release of tadpoles as fishing bait, relocation of captive adult bullfrogs, and the migration of adult bullfrogs along man-made corridors (such as roads, ditches, and cleared land). Human-centric factors, however, can be mitigated through education and restricted access to the GVWSA, of which both are already practiced. Environmental factors include shorter and warmer winters, earlier start to spring, and increasing frequencies and quantities of heavy rainfall, which variably impact over-wintering success, reproductive success and migration distances. Environmental factors may occur under the umbrella of 'global warming,' and are difficult to predict with any certainty.

4.1.2 Magnitude of Successful Bullfrog Colonization

In this section we attempt to determine a 'worst-case scenario' quantitative assessment of a colonization event in Sooke Reservoir. We chose Sooke Reservoir because it is the primary source of drinking water for the Greater Victoria area, and at approximately 180 m above sea level, could theoretically support bullfrog populations. Our assumptions are as follows:

- The overall productivity of Sooke Reservoir will determine the number of bullfrogs that can be supported
  - Note that some factor other than food supply (for example predation or habitat) could be a controlling factor on bullfrog populations, but we are unable to account for this because of a general lack of information on this subject
- There is suitable habitat for both tadpoles and adults in Sooke reservoir
  - The wide gravel and rock beaches, along with the low biological productivity, of Sooke Reservoir are not typical of what is reported in the literature as preferred habitat for bullfrogs. Our bullfrog density estimates may be over-estimated as a result
- There are suitable food types in Sooke reservoir for bullfrog tadpoles, juveniles and adults
- Both the tadpole and adult stages of American bullfrogs reside within approximately 2 metres of the shore, and do not venture out into the open lake
- The draw-down of the reservoir does not change bullfrog habitat or food supply

There is no evidence from the literature to suggest that bullfrogs disrupt the movement patterns of energy and matter through an ecosystem (otherwise known as a 'trophic cascade'). However, absence of data does not guarantee absence of biological consequences. Although we evaluate adult and tadpole life stages separately for the purposes of this discussion, such an arbitrary separation likely does not exist in real life because of the complex and largely unknown food-web interactions that occur in aquatic ecosystems. We know that adult bullfrogs do not prey on phytoplankton or zooplankton directly, preferring instead to consume insects, freshwater mussels and other amphibians. Fish do not make up a large part of a frog's diet (fish comprised only 7.7% of the total identifiable gut content of adult bullfrogs in Sturhahn's study, and 0% in Govindarajulu's study – see Table 1), but collectively there is more experience with estimating fish biomass than there is with estimating insect, freshwater mussel or frog biomass. If bullfrogs were capable of preying on small fish and reducing their numbers, the likely consequence would be a release in grazing pressure on the zooplankton that the planktiverous fish prey on. This would result in better survival and higher numbers of zooplankton with subsequent decreases in phytoplankton until the zooplankton became ‘food limited’ and unable to support their growth rates. A reduction in phytoplankton numbers, from a drinking water supply perspective, would likely be seen as a positive consequence.

We present a very simple four layer trophic structure to visualize the relative size of these communities and, ultimately, the magnitude and role of the bullfrog community. Phytoplankton comprises the largest biomass in any aquatic ecosystem and is therefore represented as the bottom-most layer. Zooplankton represents the second layer and small planktiverous fish make up the third layer, with each layer dependent on the layer below it for its energy and growth needs (Figure 5). A fourth layer, which has marginal relevance to this discussion, would be the large top predator fish species such as rainbow trout and cutthroat trout. A coastal lake such as Sooke Reservoir may have five or more trophic levels, resulting in greater between-level losses of biomass and energy because of the additional levels and an overall smaller biological biomass at the level of the top predators. It is possible that the actual fish production of Sooke Reservoir is less than what we estimate with a theoretical four level model, given that coastal lakes cannot support the same amount of fish or bullfrog biomass (see figure 6) as an interior lake. We believe, therefore, that our estimates represent a very conservative high estimate of the bullfrog biomass that could be supported by Sooke Reservoir.

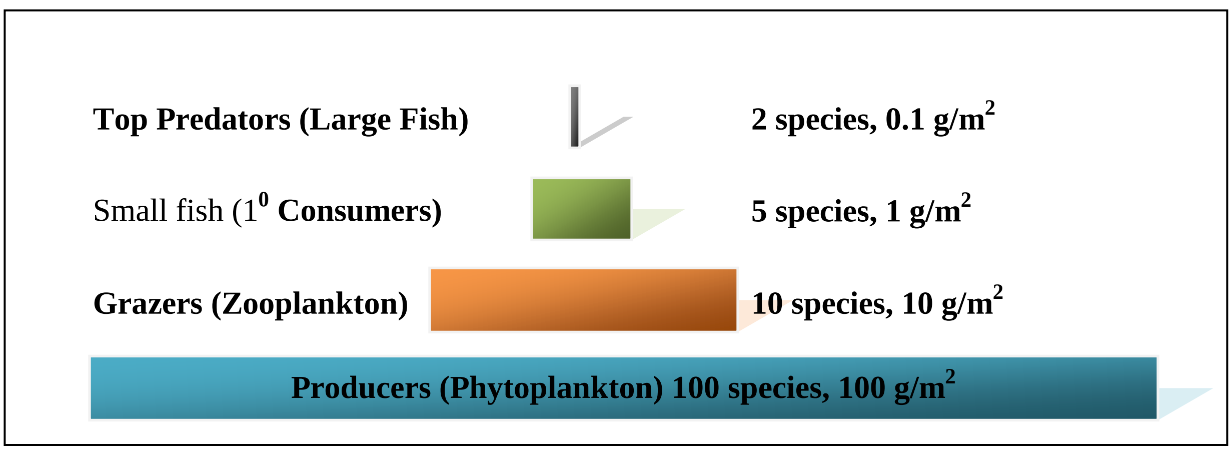

Figure 3: A hypothetical 4 layer trophic cascade for a moderately productive lake. An average number of species and the corresponding biomass (weight per area) are presented for each level to provide some concept of scale and structure.

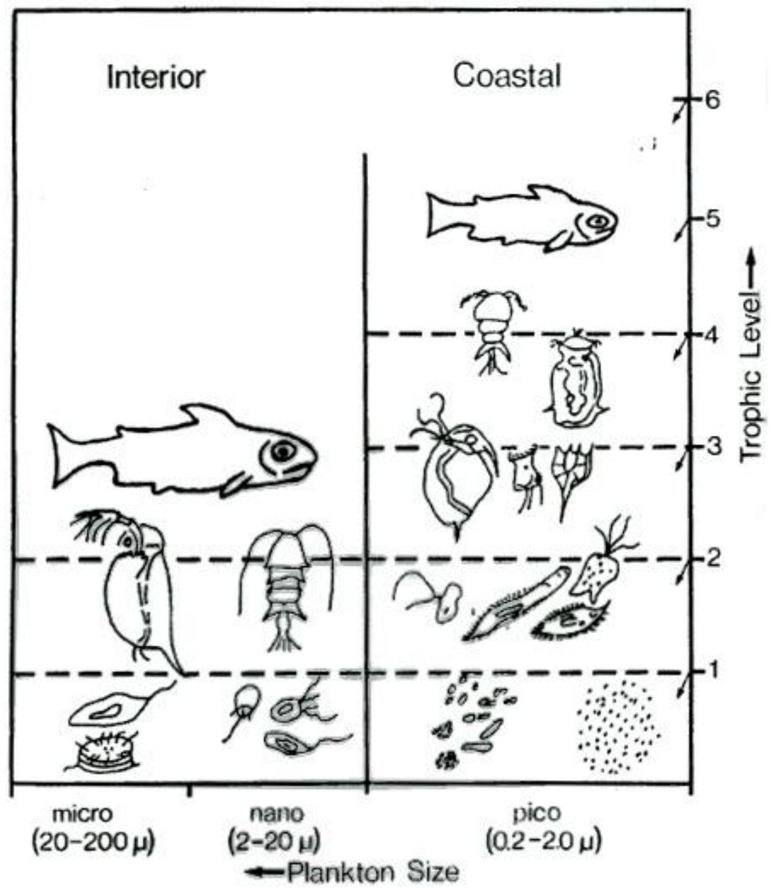

Figure 4: A visual representation of the differences between a nutrient poor coastal lake, and a nutrient rich interior lake (From J. G. Stockner&K. G. Porter. 1988)**. There is a difference of two orders of magnitude between a typical three level food chain and a five level food chain in the biomass that can be produced at the top levels of the pyramid, with the coastal lake being substantially less productive.**

Phosphorus is extremely important in wetland ecology, and must be taken into account before one can quantify the lake biological communities and trophic pyramid. Sooke Reservoir is strongly phosphorous limited and the biological productivity will be related to phosphorous. In the pre-inundation period the reservoir had a concentration of approximately 3.4 ug/L. With the present Sooke Reservoir water volume of 118x10^{6}m^3, this results in a phosphorous mass in the lake of 401 kg. In previous years, the concentration has been as high as 5.8 ug/L representing a mass of 684 kg.

4.1.2.1 *Estimate of Phytoplankon Biomass*

Chlorophyll *a* concentration provides a reasonable indicator of algal biomass and there are good data for Sooke Reservoir. An average summer chlorophyll concentration of 1.5 ug/L over the top 20 m of the reservoir (78x10^6 m^3) would represent 156 kg of chlorophyll *a*. The relationship between chlorophyll and biomass wet weight does vary but correlations exist for most lakes. Using the same model as Desortova (1981), we estimate the phytoplankton biomass (wet weight) equivalent from a chlorophyll concentration of 1.5 ug/L to be about 39,000 kg. Alternatively, if phosphorus typically represents 0.2 to 1% of the weight of phytoplankton tissue (Duarte, 1992), and the phosphorus mass in the top 20 m of the reservoir (265 kg at 3.4 ug/L Total Phosphorus) is almost entirely phytoplankton, then the phytoplankton biomass is about 45,000 kg at a P (phosphorus) content of 0.6%. Both estimates are very close, providing a rough verification of the techniques used.

4.1.2.2 *Estimate of Zooplankton Biomass*

From sampling data it appears that typical Sooke Reservoir zooplankton standing crops are 5-20 ug/L (dry weight). If an average biomass of 10 ug/L were used, this would represent a biomass of about 780 kg (dry weight) or about 7800 kg wet weight at a conversion of 10% (Pace & Orcutt, 1981) for the epilimnetic (warm surface layer) volume of the reservoir. This is in line with other reported estimates of zooplankton standing crop done as part of the federal government Lake Enhancement Program. Simpson *et al*. (1981) reported zooplankton standing crops of 6.7 mg/m^3 (ug/L) and 3 mg/m^3 for Woss and Nimkish lakes respectively on Vancouver Island. Similarly, Stockner *et al*. (1980) reported zooplankton standing crops for Great Central, Henderson and Kennedy lakes at 9 mg/m^3, 7.5 mg/m^3 and 5.4 mg/m^3, respectively. All are coastal oligotrophic lakes and provide a good comparison to Sooke Reservoir.

4.1.2.3 *Estimate of Fish Biomass*

Fish biomass is much more difficult to estimate, and so we have used two separate methods to verify our results. The first approach involves one of several techniques proposed and employed by fisheries biologists to estimate fish standing crop – many of these techniques also use limnological characteristics as the basis of their techniques. Fisheries biologists use a variety of other lake ecosystem metrics – from Total Dissolved Solids, Phosphorus and other aspects of lake characteristics – to estimate fish standing crop (biomass) and productivity. A large body of data exist for estimating fish biomass in lakes. At a very general level, based on summary papers of fish productivity in lakes, Sooke Reservoir is oligotrophic – bordering on ultraoligtrophic – and among the least productive lakes. Specific models have been used to estimate sockeye potential productivity in lakes, especially where the sockeye juveniles raised in the lake comprise a substantial portion of the fish biomass. From Koenings & Burkett (1987), Koenings & Kyle (1997), Hume *et al*. (1996), Bussanich *et al*. (2005) and Chapman & Hillman (2000) we derived estimates for a range of values then chose 5 kg/ha as a reasonable estimate for Sooke Reservoir, based on productive capacity derived from the published models. This value may be an over-estimate on our part, especially if fish productivity is limited more by spawning or rearing than by food supply. 5 kg/ha equates to approximately 2750 kg total fish biomass (based on a surface area of 550 ha). If we assume that juveniles and smaller fish species account for half of this, then 1400 kg of small fish biomass can be expected in the lake.

The second approach is to use the information in the trophic structure and relate this to potential fish standing crop. The phytoplankton and zooplankton certainly give, by being directly below the fish in the trophic structure, a general idea of potential fish productivity. If the zooplankton represents a biomass of 780 kg dry weight (DW), this then represents the available food supply (assuming fish are planktivorous and primarily dependent on zooplankton). Mass and energy is lost between trophic levels, and efficiency is assumed to be at most 15-20% (Lindemann, 1942). This equates to no more than 156 kg DW fish biomass (20% of 780 kg) in Sooke Reservoir. In Great Central Lake, LeBrasseur *et al*. (1978) concluded that the transfer of zooplankton biomass to sockeye biomass was on average 2%, but may have been as high as 10%. Based on the 10% estimate, the zooplankiverous fish biomass based on zooplankton biomass would therefore be 78 kg DW. Converting the above values to a wet weight using a conversion factor of 5 (Wetzel, 2001 p724), we calculate an equivalent of 390 to 780 kg of wet weight fish biomass (based on our previously calculated dry weights of 78 to 156 kg). This estimate compares reasonably to the sockeye productivity models estimate of 1200 kg discussed above.

4.1.2.4 *Predicted Biomass of American Bullfrogs*

All of the preceding was done to estimate how many adult bullfrogs might be supported by the productivity of Sooke Reservoir, assuming that they were dependent on small fish as their primary food source. Again using a 10% trophic transfer from our assumed biomass (say 1000 kg) of zooplankiverous fish, about 100 kg of bullfrogs would be supported if they were dependent on the production of small fish in the lake. At a weight of 1 kg each, this would represent at most 100 bullfrogs that could be supported in this scenario. Feeding pressure on the zooplanktivorous fish by bullfrogs may release predation pressure on the zooplankton, resulting in a temporary increase in zooplankton biomass and decrease in phytoplankton biomass. An alternative way to estimate frog biomass is to look at bullfrog densities in lakes that are similar to Sooke Reservoir. Fortunately, Govindarajulu (2004) gives an adult bullfrog density of $8.8 \times 10^{-4}/m^2$ (adults per ha / 1000 m^2) for Prior Lake, which is a moderately unproductive lake in the Greater Victoria area. If we assume that bullfrogs tend to remain within a metre of the shoreline, this would seem to indicate a density of 8.8 adult bullfrogs per 1000 metres. With a reservoir perimeter of approximately 35000 m (taking into account the shoreline of the islands), a maximum population carrying capacity is perhaps no more than about 300 bullfrogs in Sooke Reservoir, representing approximately 300 kg of biomass. This is unlikely to be large enough to cause any significant or measurable change.

Tadpoles are primarily herbivores, and feed on algae attached to rocks and other substrates in the shallow areas of lakes. They have also been reported to eat phytoplankton. The periphyton standing crop in a reservoir like Sooke is very low – probably in the range of 1-2 mg/m^2 chlorophyll *a* or at most 1 g /m^2 biomass (Wetzel, 2001), so the food supply would be extremely limited. Govindarajulu *et al*. (2005) indicate what seems to be fairly high tadpole densities in more productive ponds on the Saanich Peninsula – which are typically more than 10 times as productive (in terms of phosphorus concentration) than Sooke Reservoir. For Prior Lake, which is much more comparable, they give a density of 8.8 juveniles and adults per ha. Although they give no data for tadpole density, they do provide estimated survival rates. Calculating tadpole numbers from survival rates seems difficult as it requires a whole set of additional assumptions. Assuming that tadpoles in Sooke Reservoir reside primarily within a metre of shore and using the adult densities recorded for Prior Lake (8.8 kg/1000 m and 100 g each), there would be 88 tadpoles per 1000 metres of shoreline, equating to approximately 600 in the reservoir. This would represent a biomass of 308 kg, which is unlikely to be of any consequence in the overall biomass of the reservoir. A population of only a few hundred tadpoles would unlikely cause any measurable change to the ecological structure of a large reservoir, even if they consumed primarily zooplankton rather than algae (an assumption that could not be substantiated from the available literature). Nevertheless, if tadpoles consumed only zooplankton at a rate of 10% of their body weight per day, the amount consumed would only be 30 kg – a small fraction of the zooplankton population and far too small to be of any overall significance.

Even though these calculations are based on conjecture and assumptions, some of which are known to be implausible, this exercise is useful in one major way – **the likely biomass of American bullfrogs, should they successfully colonize Sooke Reservoir, can be expected to be extremely low.** Such a low biomass would contribute a comparatively small amount of the hazards described above and thus there would be no consequence to human health, given that many of the issues that we will discuss in the next section are density dependant.

4.2 Risks Associated with Bullfrog Colonization in the GVWSA

The available peer-reviewed and grey literature on bullfrog impacts on water quality, water supply infrastructure and aquatic ecosystems have been discussed previously. Overall, there was a noticeable lack of published research on bullfrog impacts on water quality, water supply infrastructure and ecosystem health, with the exception of bullfrog impacts on amphibian biodiversity and threatened species. To address the uncertainty associated with limited published information, we compared and contrasted the collective global experience of bullfrog colonization events against experiences from the American bullfrog's native range, and consulted with public health experts and knowledgeable herpetologists.

To assist in the interpretation of the magnitude of the risks to water quality, we will now briefly describe current water treatment and processing procedures by the CRD Water Services. When fully operational, UV disinfection will inactivate viable *Giardia* and *Cyrptosporidium* and chlorination will inactivate pathogenic bacteria and viruses. This system will not sterilize the water, however, and cannot inactivate large multicellular parasites such as trematodes (flukes) and nematodes (round worms). Partial failures of disinfection occur when any one component of water treatment becomes inoperable; for example, the UV process can be interrupted during power outages. Watershed protection is vital, therefore, as the health and integrity of the source water is considered one of the barriers to contamination in the event of a partial failure of the UV or chlorine systems.

CRD Water Services does not use filtration, but instead employs a system of high and low water-column intakes that can be preferentially closed depending on the stratification of particulate matter within the reservoir. Furthermore, CRD Water Services has the ability to draw water from two separate sources, Sooke Reservoir (which is the primary reservoir), and Goldstream Reservoir via Japan Gulch Reservoir during the winter. Typically, Goldstream Reservoir is only used in January and February, when the Kapoor Tunnel is shut down for maintenance and repairs.

4.2.1 Risks to Water Quality

The probability of all hazards being significant is low due to the anticipated small population size in Sooke Reservoir, and the CRD Water Services practice of diverting water from Goldstream Reservoir for human consumption only in the winter. As previously described, bullfrogs emerge from hibernation in April and May; with their metabolism greatly decreased due to hibernation, they are unlikely to shed any bacteria, pathogenic or otherwise. Global warming may alter bullfrog's emergence from hibernation, however, hypothesized impacts as a result of global warming are beyond the scope of this paper.

4.2.1.1 *Bacteria*

The information gathered suggests that Sooke Reservoir could not support the bullfrog densities required for a major bacterial contamination event, either from a massive die-off of bullfrogs or from fecal shedding. The uncertainty associated with this statement is considered negligible to very low. Japan Gulch Reservoir, however, appears to offer a better habitat in terms of the vegetation cover and proximity to already established bullfrog populations. The likelihood of a population of bullfrogs establishing in Japan Gulch Reservoir is moderate to high. Nevertheless, we expect that a fully functioning ultraviolet disinfection and chloramination process would be capable of inactivating any bacterial species or parasitic protozoans that bullfrogs could potentially introduce into the reservoir. Therefore we can say with high confidence that the potential magnitude of impacts that relate to water quality is very low.

4.2.1.2 *Other Pathogens*

There is a low to non-existent risk from parasites, virus or fungi because of the anticipated small population sizes, and the fact that these pathogens tend to be host adapted to amphibians and other ectothermic animals (reptiles and fish) and pose little to no risk to human health. We are confident in this conclusion, given that bullfrogs have been consumed by humans for generations with no apparent increase in human health risk to parasites, viruses or fungi.

4.2.1.3 *Water Palatability: Taste and Odour*

We did not find any peer-reviewed literature that describes bullfrog impacts on water taste and odour. This does not appear to be an issue among utility managers or the expert herpetologists that we consulted. The potential magnitude of impacts to taste and odour are considered low with a high confidence.

4.2.1.4 *Water Clarity*

The potential magnitude of impacts to water clarity is considered low with moderate certainty. The uncertainty associated with these potential impacts relates to our general lack of understanding on bullfrog tadpole diet. The available literature suggests that bullfrog tadpoles primarily consume algae. Furthermore, published literature and expert opinion have yet to associate toxic algal blooms with changes in tadpole composition and numbers.

4.2.1.5 *Turbidity*

Bullfrogs do not dig nests or excavate shoreline areas, and are not known to damage or disturb shoreline vegetation. We do not expect, based on our understanding of bullfrog behaviour, to see any disruption to shoreline vegetation that may lead to measurable increases in soil erosion or increases in reservoir water turbidity. This statement is made with high certainty.

4.2.2 Risks to Water Supply Infrastructure

The likelihood that adult or tadpole bullfrogs could be sucked against intake screens seems remote given that the water intakes in both Sooke Reservoir and Japan Gulch Reservoir are at sufficient depth in the water column to minimize the potential for floating objects to be drawn in. We make this statement with only moderate certainty given the lack of data on bullfrog and tadpole diving depths. However, the magnitude of this risk is considered very low given our predictions for bullfrog numbers in Sooke Reservoir. Water supply infrastructure at Japan Gulch Reservoir may be at higher risk given the likelihood of Japan Gulch Reservoir supporting a larger bullfrog population than Sooke Reservoir.

4.2.3 Risks to Aquatic Ecosystems

We did not find any peer-reviewed or grey literature to support the hypothesis that bullfrogs will alter primary production, introduce novel pathogens, or facilitate changes in trophic structures (in either aquatic, riparian or terrestrial environments) in ways that will alter water quality.

There is evidence on impacts to amphibian biodiversity, but the extent of the impacts that bullfrogs have on native species is contested; there is a large amount of uncertainty associated with this. However, the impacts that a loss of amphibian biodiversity, or even a replacement of a few native species with American bullfrogs, will have on water quality has not previously been studied. We believe the magnitude of impacts on water quality from a loss of biodiversity is low, but there is a great deal of uncertainty because of the limited information on this subject.

We can state with relatively high certainty that bullfrog tadpoles and adults have few predators, both in their natural habitats and in invaded habitats. As a result, American bullfrogs are unlikely to attract new predators to newly invaded habitats. The potential magnitude of impacts from increased bird and mammalian traffic to the reservoirs, therefore, appears to be negligible.

4.3 Summary of Risk and Uncertainty

In section 4.1.1 we showed that among all the water supply reservoirs within the GVWSA, Sooke Reservoir and Japan Gulch Reservoir have the highest likelihood of supporting bullfrog populations. We are confident about this conclusion, so long as human and environmental factors do not drastically alter bullfrog habitat preferences and ranges on Vancouver Island. In Section 4.1.2, we estimated the magnitude of successful bullfrog colonization in Sooke Reservoir to be low or very low. We made a number of assumptions and utilized multiple published estimation techniques to deliberately overestimate the likelihood and magnitude of the risk of colonization. Despite the large number of unknowns regarding invasive species in new habitats, which increases the uncertainty surrounding population modelling, **the overall likelihood that Sooke Reservoir will be overrun by American bullfrogs is low**.

It became evident in Section 4.2.1 that **the potential risk to water quality from successful American bullfrog colonization is low**, although this is dependant on a fully functioning ultraviolet disinfection and chloramination process and the accuracy of our assessment with regards to bullfrog population numbers. The greatest level of uncertainty is associated with bullfrog impacts on water clarity, primarily due to a lack of published information on this topic.

Section 4.2.2 indicates with moderate uncertainty that **the overall risk to water supply infrastructure is very low in Sooke Reservoir and only slightly higher in Japan Gulch Reservoir**, mostly as a result of expected population density differences between the two reservoirs. The uncertainty stems from a general lack of knowledge on bullfrog behaviour, including diving depths, below the water surface.

There is potentially a very large risk to native and endangered amphibian species from the successful colonization of American bullfrogs in the GVWSA, although the uncertainty of this statement is also quite large given the current debate in the scientific literature. This risk does not appear to negatively impact water quality or human safety, however, and seems to be more important to a discussion on wildlife conservation.

5 CONCLUSIONS

5.1 Impacts on Water Quality

We do not believe that the successful colonization of American bullfrogs into the GVWSA will pose any threat to drinking water quality parameters, as defined in this report. This statement is based on our literature review of bullfrog pathogens (bacteria, viruses, parasites and fungi) in conjunction with our population modeling results. We do not anticipate any changes to the physical characteristics, non-metallic inorganic chemical (phosphorus and nitrogen) characteristics, or algal populations of aquatic ecosystems, based on current knowledge from peer reviewed literature and expert opinion, as a result of bullfrog colonization.

5.2 Impacts on Water Supply Infrastructure

We do not believe that an invasion of American bullfrogs into the GVWSA will pose any threat to the water supply infrastructure. This statement is based on our population modeling results, the relative lack of appropriate bullfrog habitat on Sooke Reservoir, and the depth at which water is drawn from the water column. Furthermore, amphibians, including American bullfrogs, have not been reported in the literature as causing damage to water pipes and drains.

5.3 Impacts on Ecosystem Health

We do not anticipate negative impacts on ecosystem health as a result of successful bullfrog colonization, ***as defined in this report for the preservation of safe drinking water for human consumption***. There is no documentation of bullfrogs restructuring habitats, altering nutrient inputs or timing, or facilitating changes to trophic structures (whether it is aquatic, riparian or terrestrial), in ways that affect drinking water quality. However bullfrogs have been documented in the peer-reviewed literature to negatively affect biodiversity and to introduce new pathogens (e.g. chytrid fungus and ranavirus) that may impact native amphibians.

5.4 Final Statements

CRD Water Services has a suite of water quality indicators that are capable of detecting significant changes to water supply reservoirs if bullfrogs were to successfully colonize them. This is potentially very useful given that:

- The GVWSA has many years worth of water quality data
- American bullfrogs are not currently present in the GVWSA

- In the event of unforeseen circumstances, the CRD Water Services is in a position to measure changes to water quality. Measurable change as a result of bullfrog colonization could prove invaluable to utility managers in other jurisdictions.

A newspaper article in 2005 carried a story with this headline: “Invasive bullfrogs pose risk to water supply, expert says” (TC Feb 17 2005 B2). We found no current evidence to substantiate this claim for the Greater Victoria Water Supply Area.

Strictly on the basis of water quality, water supply infrastructure and ecosystem health, as defined in this report for the preservation of safe drinking water for human consumption, it is difficult for us to justify the current level of activity to prevent the invasion of bullfrogs into the Greater Victoria Water Supply Area.

Figure 5: Simplified summary of our conclusions. Magnitude of risk is not represented. Although we have concluded that the microbial parameters may change from current levels, we do not anticipate these changes to be measurable with current protocols. The risk associated with bacteria is dependant on American bullfrog density in the reservoir as well as the enteric population of local American bullfrogs.

Indicators of water supply health and integrity	Evidence that Bullfrogs will lead to undesirable changes in Indicators		Public Health Risk Associated with Greater Victoria's Drinking Water		
	None	Limited	Unknown	No	Yes
Community Structure					
Phytoplankton	X			X	
Zooplankton	X			X	
Fish Biomass	X			X	
Nutrient Supply	X			X	
Predator Movement Patterns	X			X	
Microbial Parameters					
Bacteria		X	X		
Fungus		X		X	
Virus	X			X	
Parasite		X		X	
Protozoa	X			X	
Physical Parameters					
Taste	X			X	
Alkalinity	X			X	
Hardness	X			X	
Total Dissolved Solids	X			X	
Turbidity	X			X	
pH	X			X	
Organic Parameters					
Algal Blooms		X		X	
Water Supply Infrastructure	X			X	

6 RECOMMENDATIONS

Given this risk conclusion, current bullfrog surveillance and eradication activities in the bullfrog control corridor to the south east of the CRD Water Supply Area are not necessary for the protection of drinking water quality in the GVWSA. This conclusion **does not take into account** the possible ecological impacts on native and endangered amphibian species. It also does not consider policy or opinions regarding management of invasive species in general.

Because of public concerns and prevailing uncertainties, we recommend the following to the CRD Water Services:

1. Consider the possible ecological impacts of bullfrogs on native and endangered amphibian species, and best practices for the management of invasive species when making final policy decisions on bullfrog control.

2. Periodically review current and newly published bullfrog-relevant literature. This report recognizes that bullfrog research is ongoing, and that changes in climate patterns over time may alter bullfrog ecology and behaviours and could affect water quality parameters.

3. Support (financial or in-kind) of local research on bullfrog ecology, habitat preference and migratory patterns relevant to the Greater Victoria Water Supply Area

Should the CRD Water Services decide to continue with the current policy of preventing bullfrog colonization of the GVWSA, prediction models for habitat suitability and migration would be invaluable towards concentrating surveillance and eradication to maximize effectiveness and decrease costs.

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8 APPENDIX 1: Terms of Reference

8.1 Context Information

1) Identify and define indicators of changes to aquatic ecosystems that result from the establishment of American bullfrogs in the Greater Victoria Water Supply Area.

2) Identify and define the specific drinking water quality parameters of concern to CRD Water in the Greater Victoria Water Supply Area (GVWSA).
    a. E.g. parasitic protozoa, bacteria, viruses or fungi.
    b. E.g. algal populations, water turbidity, soil erosion, pollution (soil, air, water), toxins.
    c. E.g. taste, odour or appearance of drinking water.

3) Review and determine threshold values at which point an intervention to protect human health, water supply infrastructure and/or water palatability will be considered by CRD Water.

8.2 Literature Review

1) Can invasive American bullfrogs affect aquatic ecosystems in the GVWSA?
    a. Is there evidence to suggest that invasion by American bullfrogs can alter the health and integrity of aquatic ecosystem?
    b. If there is evidence that bullfrogs can alter aquatic ecosystem balance, and how is the change facilitated?
        i. E.g. loss of biodiversity, habitat restructuring, and emergence of novel pathogens, change in aquatic trophic structure, alteration of nutrient inputs or timing, change in terrestrial / aquatic linkages (attraction of predators to a new food source and increase of contact of potential mammalian or avian disease vectors to reservoir water)
    b. The review will examine any literature on the effects of other amphibian species on these impacts that may also be relevant to American bullfrogs
    c. All articles will be reviewed and assessed to determine the scientific strength for causality vs. association.

2) To what extent can American bullfrogs impact the specific drinking water quality parameters identified by CRD Water in the Greater Victoria Water Supply Area (GVWSA)?
    a. Review and assess scientific literature and technical reports from public agencies and non-governmental organizations, supplemented as appropriate by interviews with people with expertise in the topics being reviewed, to determine if and how bullfrogs affect these parameters.
    b. The review will examine any literature on the effects of other amphibian species on these water quality parameters that may also be relevant to American bullfrogs.

c. All articles will be reviewed and assessed to determine the scientific strength for causality vs. association.

3) How could American bullfrogs affect water supply infrastructure?
   a. Determine potential direct impacts (e.g. plugging water intake screens and pipes) and indirect impacts (e.g. damage due to soil erosion or habitat destruction) of American bullfrogs on water supply infrastructure.
   b. The review will examine any literature on the effects of other amphibian species on infrastructure relevant to water supply.
   c. All articles will be reviewed and assessed to determine the scientific strength for causality vs. association.

4) An interpretation of the risk that American bullfrogs pose to aquatic ecosystems, drinking water quality and water supply infrastructure in the GVWSA will be provided.
   a. Literature review and assessment summary.

8.3 **Key Water Quality Parameters**

1) Drinking Water Quality Indices
   a. Microbial Parameters
      i. Coliform bacteria
      ii. Heterotrophic bacteria
      iii. Bacterial toxins
      iv. Parasitic protozoa
   b. Physical Parameters
      i. Odour
      ii. Taste
      iii. Alkalinity
      iv. Hardness
      v. Total solids
      vi. Turbidity
      vii. Water temperature
      viii. pH
   c. Non-Metallic Inorganic Chemicals
      i. Nitrogen
      ii. Phosphate

2) Additional Water Safety Criteria
   a. Multi-cellular parasites
   b. Algal blooms
   c. Fungi
   d. Bacterial Toxins
   e. Viruses

3) Water Supply Infrastructure
   a. Direct Impacts
      i. Plugging intake screens / pipes
   b. Indirect Impacts
      i. Damage to intakes due to soil erosion or habitat destruction

4) Aquatic Ecosystems
   a. Habitat restructuring
   b. Alteration of nutrient inputs or timing
   c. Changes in primary production
   d. Changes to aquatic trophic structures
   e. Changes to riparian and terrestrial trophic structures
   f. Introduction of novel pathogens
   g. Loss of biodiversity

9 APPENDIX 2: Bacteria Cultured from Amphibian Species

Complete table of all bacterial species isolated from amphibian species globally.

Reference	Pathogen	Source of Isolate	Human Health Risk	Route of Transmission
8 12	Salmonella enteriditis	Bufo marinus (cane toad) Bufo marinus (cane toad)	Reportable	Fecal oral
1 10	Salmonella typhimurium	Aquarium water & Frog spp. Toad spp.	Reportable	Fecal-oral
3	Salmonella sp.	Municipal water	Rare to Reportable (species dependant)	Consumption of contaminated food; direct contact; 'zoonotic'; fecal-oral
1	Bacteroides	Frog spp.	Known pathogen	Zoonotic; opportunistic normal flora; nosocomial; antibiotic or immunosuppressive therapy
1	Clostridium	Frog spp.	Known pathogen (species dependant)	Opportunistic environmental contaminant; penetrating wound; opportunistic normal flora
1 3 6	Enterobacter cloacae	Municipal water Rana pipiens (leopard frog) Aquarium water & Frog spp.	Known pathogen	see *Enterobacter sp.*
2	Enterobacter sp.	Rana catesbeiana (bullfrog)	Known pathogen (prevalence appears to be increasing)	Fecal-oral; direct contact through mucous memebranes; nosocomial; opportunistic normal flora; opportunistic environmental contaminant
2 3 4 6	Escherichia coli	Rana catesbeiana (bullfrog) Municipal water Rana pipiens (leopard frog) Tank water and Bullfrog	Known pathogen	Opportunistic normal flora
2	Klebsiella sp.	Rana catesbeiana (bullfrog)	Known pathogen (species dependant)	Opportunistic environmental contaminant; immunocompromised; nosocomial
1 3 6	Klebsiella pneumonia	Aquarium water & Frog spp. Municipal water Rana pipiens (leopard frog)	Known pathogen	Opportunistic normal flora
8 9 12	Salmonella anatum	Bufo marinus (cane toad) Frog spp. Bufo marinus (cane toad)	Known pathogen	Fecal-oral; consumption of contaminated food
1	Salmonella hadar	Aquarium water & Frog spp.	Known pathogen	Fecal-oral; consumption of contaminated food
2 3	Shigella sp.	Rana catesbeiana (bullfrog) Municipal water	Known pathogen	Fecal-oral route; unchlorinated wading pools; interactive water fountains; unprotected sex/STI; consumption of contaminated food
2	Staphylococcus sp.	Rana catesbeiana (bullfrog)	Known pathogen	Opportunistic normal flora
2	Streptococcus sp.	Rana catesbeiana (bullfrog)	Known pathogen	Opportunistic normal flora

Reference	Pathogen	Source of Isolate	Human Health Risk	Route of Transmission
1 2 3 6 7	Aeromonas hydrophila	Frog spp. Rana catesbeiana (bullfrog) Municipal water Rana pipiens (leopard frog) Rana catesbeiana (bullfrog)	Opportunistic	Fecal-oral; environmental / waterborne; ingestion of contaminated fish or reptiles
1	Fusobacterium spp.	Frog spp.	Opportunistic	Opportunistic normal flora; penetrating wound; animal bites; antibiotic or immunosuppressive therapy; zoonotic
1 6 12	Salmonella arizonae	Aquarium water Rana pipiens (leopard frog) Bufo marinus (cane toad)	Opportunistic	Opportunistic, usually in immunocompromised individuals
1 2	Acinetobacter sp.	Frog spp. Rana catesbeiana (bullfrog)	Nosocomial	Penetrating wound; immunocompromised
7	Chryseobacterium indolgenes	Rana catesbeiana (bullfrog)	Nosocomial	Penetrating wound; indwelling catheters
1 2	Flavobacterium	Frog spp. Rana catesbeiana (bullfrog)	Nosocomial	see *Chryseobacterium*
2	Staphylococcus epidermidis	Rana catesbeiana (bullfrog)	Nosocomial	Nosocomial; indwelling catheter
2	Achromobacter sp.	Rana catesbeiana (bullfrog)	Rare	Penetrating wound; immunocompromised
1	Alcaligenes sp.	Frog spp.	Rare	see *Achromobacter*
2	Bacillus cereus	Rana catesbeiana (bullfrog)	Rare	Fecal-oral
2	Bacillus megaterium	Rana catesbeiana (bullfrog)	Rare	Penetrating wound; immunocompromised
2	Bacillus sphaericus	Rana catesbeiana (bullfrog)	Rare	Penetrating wound; immunocompromised
5	Basidiobolus ranarum	African clawed frog	Rare	Opportunistic environmental contaminant
1	Chromobacterium violaceum	Frog spp.	Rare	Opportunistic environmental contaminant; isolated case reports suggest direct contact with stagnant water, penetrating wound, aspiration in near-drowning victims
7	Chryseobacterium meningosepticum	Rana catesbeiana (bullfrog)	Rare	Opportunistic environmental contaminant
6	Citrobacter amalonaticus	Rana pipiens (leopard frog)	Rare	Fecal-oral; opportunistic normal flora; direct contact with infected person; nosocomial; immunocompromised
1 3	Citrobacter diversus	Aquarium water & Frog spp. Municipal water	Rare	see *Citrobacter amalonaticus*
1 2 3 6 7	Citrobacter freundii	Aquarium water & Frog spp. Rana catesbeiana (bullfrog) Municipal water Rana pipiens (leopard frog) Rana catesbeiana (bullfrog	Rare	see *Citrobacter amalonaticus*
6	Citrobacter sp.	Rana pipiens (leopard frog)	Rare	see *Citrobacter amalonaticus*
3 9	Edwardsiella sp.	Municipal water Frog spp.	Rare	Penetrating wound; fecal-oral

Reference	Pathogen	Source of Isolate	Human Health Risk	Route of Transmission
1 6 7	Edwardsiella tarda	Aquarium water Rana pipiens (leopard frog) Rana catesbeiana (bullfrog)	Rare	Penetrating wound, fecal oral
3 6	Enterobacter aerogenes	Municipal water Rana pipiens (leopard frog)	Rare	see *Enterobacter sp.*
6	Hafnia alvei	Rana pipiens (leopard frog)	Rare	Opportunistic environmental contaminant; opportunistic normal flora
3 6	Klebsiella oxytoca	Municipal water Rana pipiens (leopard frog)	Rare	Nosocomial
2	Micrococcus sp.	Rana catesbeiana (bullfrog)	Rare	Immunocompromised
6	Morganella morganii	Rana pipiens (leopard frog)	Rare	Opportunistic environmental contaminant; nosocomial
13	Mycobacterium marinum	Rana catesbeiana (bullfrog)	Rare	Trauma; penetrating wound
2	Pleisomonas shigelloides	Rana catesbeiana (bullfrog)	Rare	Opportunistic environmental contaminant; immunocompromised; opportunistic normal flora; zoonotic; pentrating wound
1 3	Proteus sp.	Aquarium water & Frog spp. Rana pipiens (leopard frog)	Rare	Oppportunistic normal flora; opportunistic environmental contaminant; nosocomial
6	Providencia stuartii	Rana pipiens (leopard frog)	Rare	Nosocomial; indwelling catheter; opportunistic normal flora
1 2	Pseudomonas aeruginosa	Not stated Rana catesbeiana (bullfrog)	Rare	Nosocomial
2	Pseudomonas alcaligenes	Rana catesbeiana (bullfrog)	Rare	see *Pseudomonas sp.*
1 2	Pseudomonas fluorescens	Frog spp. Rana catesbeiana (bullfrog)	Rare	see *Pseudomonas sp.*
2	Pseudomonas putida	Rana catesbeiana (bullfrog)	Rare	see *Pseudomonas sp.*
2 7	Pseudomonas sp.	Rana catesbeiana (bullfrog) Rana catesbeiana (bullfrog)	Rare	Opportunistic environmental contaminant
8	Salmonella Aberdeen	Bufo marinus (cane toad)	Rare	Fecal-oral; consumption of contaminated food
1	Salmonella bovis-morbificans	Aquarium water	Rare	Fecal-oral; consumption of contaminated food
8	Salmonella chester	Bufo marinus (cane toad)	Rare	Fecal-oral; consumption of contaminated food
10 11	Salmonella newport	Toad spp. Bufo marinus (cane toad)	Rare	Fecal-oral; consumption of contaminated food
8 12	Salmonella oranianburg	Bufo marinus (cane toad) Bufo marinus (cane toad)	Rare	Fecal-oral; consumption of contaminated food
11 12	Salmonella panama	Bufo marinus (cane toad) Bufo marinus (cane toad)	Rare	Opportunistic environmental contaminant; consumption of contaminated food
9	Salmonella poona	Frog spp.	Rare	Consumption of contaminated food; immunocompromised
11	Salmonella rubislaw	Bufo marinus (cane toad)	Rare	Case report: direct contact with infected reptiles

Reference	Pathogen	Source of Isolate	Human Health Risk	Route of Transmission
11 12	Salmonella san diego	Bufo marinus (cane toad) Bufo marinus (cane toad)	Rare	see *Salmonella sp.*
1	Salmonella saint-paul	Aquarium water	Rare (but can cause outbreaks)	Fecal-oral
12	Salmonella thompson	Bufo marinus (cane toad)	Rare	Consumption of contaminated food
8	Salmonella virchow	Bufo marinus (cane toad)	Rare	Consumption of contaminated feed
10	Salmonella weltevreden	Toad spp.	Rare	Consumption of contaminated food
1	Salmonella worthington	Aquarium water	Rare	Nosocomial
6	Serratia fonticola	Rana pipiens (leopard frog)	Rare	Nosocomial; opportunistic environmental contaminant
6	Serratia liquefaciens	Rana pipiens (leopard frog)	Rare	Nosocomial
6	Serratia marcescens	Rana pipiens (leopard frog)	Rare	Nosocomial
6	Serratia odorifera	Rana pipiens (leopard frog)	Rare	Nosocomial
6	Serratia plymuthica	Rana pipiens (leopard frog)	Rare	Nosocomial
2 3 6	Serratia sp.	Rana catesbeiana (bullfrog) Municipal water Rana pipiens (leopard frog)	Rare	Direct contact through mucous membranes; nosocomial; indwelling catheter; opportunistic environmental contaminant
7	Streptococcus iniae	Rana catesbeiana (bullfrog)	Rare	Zoonotic; penetrating wound
1	Vibrio sp.	Frog spp.	Rare	Consumption of contaminated seafood; open wound exposure to saltwater
6	Yersinia enterocolitica	Rana pipiens (leopard frog)	Rare	Zoonotic; fecal-oral; consumption of contaminated food
6	Yersinia intermedia	Rana pipiens (leopard frog)	Rare	Unknown
1	Edwardsiella hafniae	Aquarium water	Unknown	
7	Aeromonas sp.	Rana catesbeiana (bullfrog)	Unknown	see *Aeromonas hydrophila*
2	Alcaligenes faecalis	Rana catesbeiana (bullfrog)	Unknown	
3 9	Arizona sp.	Municipal water Frog spp.	Unknown	see *Salmonella sp.*
3 6	Enterobacter agglomerans	Municipal water Rana pipiens (leopard frog)	Unknown	see *Enterobacter sp.*
3	Enterobacter hajkiae	Municipal water	Unknown	
6	Enterobacter sakazakii	Rana pipiens (leopard frog)	Unknown	see *Enterobacter sp.*
3 6	Klebsiella ozaenae	Municipal water Rana pipiens (leopard frog)	Unknown	
2	Proteus mirabilis	Rana catesbeiana (bullfrog)	Unknown	see *Proteus sp.*
2	Proteus morganii	Rana catesbeiana (bullfrog)	Unknown	see *Proteus sp.*
2	Proteus rettgeri	Rana catesbeiana (bullfrog)	Unknown	see *Proteus sp.*
2 6	Proteus vulgaris	Rana catesbeiana (bullfrog) Municipal water	Unknown	see *Proteus sp.*
6	Providencia alcalifaciens	Rana pipiens (leopard frog)	Unknown	Fecal-oral (possible agent of traveller's diarrhea)
6	Providencia rettgeri	Municipal water	Unknown	Opportunistic normal flora
3	Providencia sp.	Rana pipiens (leopard frog)	Unknown	

Reference	Pathogen	Source of Isolate	Human Health Risk	Route of Transmission
11	Salmonella abaetetuba	Bufo marinus (cane toad)	Unknown	see *Salmonella sp.*
10	Salmonella bareilly	Toad spp.	Unknown	Unknown
9	Salmonella brijbhumi	Frog spp.	Unknown	Unknown
9 10	Salmonella goverdhan	Frog spp. Toad spp.	Unknown	Unknown
8 9	Salmonella hvittindoss	Bufo marinus (cane toad) Frog spp.	Unknown	Unknown
11	Salmonella kaapstad	Bufo marinus (cane toad)	Unknown	Unknown
8	Salmonella lansing	Bufo marinus (cane toad)	Unknown	Unknown
9	Salmonella london	Frog spp.	Unknown	Unknown
8	Salmonella mgulani	Bufo marinus (cane toad)	Unknown	Unknown
9	Salmonella newbrunswick	Frog spp.	Unknown	Unknown
9 10	Salmonella Richmond	Frog spp. Toad spp.	Unknown	Unknown
2	Unclassified gram negative bacteria	Rana catesbeiana (bullfrog)	Unknown	Unknown
6	Yersinia ruckeri	Rana pipiens (leopard frog)	Unknown	Unknown
3	Yersinia sp.	Municipal water	Unknown	Unknown

Reference	Authors	Geographic location of study
1	Bartlett et al, 1977	British Columbia, Canada
2	Carr et al, 1976	Louisiana, United States
3	Clark et al, 1982	Ontario, Canada
4	Gray et al, 2007	Tennessee, United States
5	Groff et al, 1991	California, United States
6	Hird et al, 1983	Minnesota, United States
7	Mauel et al, 2002	Georgia, United States
8	O'Shea and Speare, 1990	Queensland, Australia
9	Sharma et al, 1974	Hissar, India
10	Singh, 1979	Pantnagar, India
11	Bool and Kampelmacher, 1958	Surinam
12	Kourany et al, 1970	Republic of Panama
13	Ferreira et al, 2006	Rio de Janeiro, Brazil

10 APPENDIX 3: Bullfrog Populations on Vancouver Island

Reported and confirmed bullfrog habitats as of summer of 2007, with elevation data.

LOCATION	Town	Elevation (m)
1370 Kurtis Cr	Cedar	7
1945 Shasta Rd	Cedar	13
Quennell Lk	Cedar	32
Fuller Lk	Chemainus	55
Richards Lk	Cinnabar Valley	24
Richardson Lk	Cinnabar Valley	24
Cobble Hill	Cobble Hill	95
n/a	Cumberland	170
6508 Wicks Rd	Duncan	41
6600 Lakes Rd	Duncan	40
6883 Lakes Rd	Duncan	57
Quamichan Lk	Duncan	25
Queen Margaret's School	Duncan	35
Somenos Lake	Duncan	10
1215 Grafton Ave	Errington	117
Beth's Pond	Errington	106
n/a	Errington	124
Fork Lk	Highlands	210
645 Rason Rd	Langford	95
Florence Lk	Langford	82
Glen Lk	Langford	66
Goudy's Pond	Langford	230
Langford Lk	Langford	66
7401 Aulds Rd	Lantzville	93
n/a	Lasqueti Island	132
Cherri's Pond	Merville	68
n/a	Merville	58
4335 Lindholm	Metchosin	25
Happy Valley Rd	Metchosin	52
n/a	Metchosin	55
n/a	Mill Bay	76
Kristina's Pond	n/a	33
Spencer Pond	n/a	86
#113-5685 Edgewater Lane	Nanaimo	133
2011 Brothers Road	Nanaimo	25
2245 Ashlee Rd	Nanaimo	120
3645 Dix Road	Nanaimo	127
688 Montague Rd	Nanaimo	71
Brannen Lk	Nanaimo	76
Broadmoor Pl	Nanaimo	185

LOCATION	Town	Elevation (m)
Buttertubs Marsh	Nanaimo	64
Cathers Lk	Nanaimo	100
Goose Poo Pond, Rutherford Rd	Nanaimo	115
Green LK	Nanaimo	107
McGregor Marsh, Rutherford Rd	Nanaimo	63
McGregor Marsh, Rutherford Rd	Nanaimo	175
McGregor Marsh, Rutherford Rd	Nanaimo	154
Westwood Lake	Nanaimo	164
Westwood Lk	Nanaimo	165
1598 Beaver Creek Wharf Rd	Nanoose Bay	15
2180 Kaye Rd	Nanoose Bay	47
n/a	Nanoose Bay	7
French Creek	Parksville	22
2331 Otter Bay Rd	Pender Island	29
McCoy Lk	Port Alberni	28
Sprout Lake	Port Alberni	35
Jordan River Watershed	Port Renfrew	0
Eaglecrest Golf Course	Qualicum Beach	53
n/a	Qualicum Beach	33
Pheasant Glen Golf Course	Qualicum Beach	56
1129 Labrador	Saanich	36
300-4396 West Saanich Rd	Saanich	62
4661 Boulderwood Dr	Saanich	105
5000 Old West Saanich Rd	Saanich	78
5142 Old West Saanich Rd	Saanich	116
5757 West Saanich Rd	Saanich	98
6420 Anndon Place	Saanich	95
6525 Central Saanich Rd	Saanich	81
Adam Kerr Park	Saanich	54
Blenkinsop Lk	Saanich	28
Island View Beach Park	Saanich	4
Island View Golf Course	Saanich	36
Kilarney Lk	Saanich	107
Layritz Park	Saanich	79
Prospect Lk	Saanich	48
Saanich Historical Artefact Society	Saanich	20
2940 Lamont Rd	Saanichton	35
3145 Livesay Rd	Saanichton	31
Sandhill Creek	Saanichton	58
1663 Whiffin Spit Rd	Sooke	11
n/a	Texada Island	69
n/a	Thetis Lake	63
1649 Millstream Rd	Victoria	183
243 Stevens Rd	Victoria	100

LOCATION	Town	Elevation (m)
255 Hectare Rd	Victoria	68
3162 Munns Rd	Victoria	84
402 Viaduct Ave W	Victoria	32
4373 Prospect Lake Rd	Victoria	63
671 Millstream	Victoria	179
821 Finlayson Arm Rd	Victoria	166
987 Glenview Pl	Victoria	84
Caldecote Rd	Victoria	93
Eagles Lk	Victoria	98
Elk Lake Dr	Victoria	96
Humpback Reservoir	Victoria	114
Kensington Rd	Victoria	37
Old East Rd	Victoria	33
Pease Lk	Victoria	225
Ross Durrance Rd	Victoria	205
Royal Oak Golf Course	Victoria	66
Teanook Lake	Victoria	108
Mckenzie Lk	View Royal	62
Black Creek	Yellow Point	54
Graham Rd	Yellow Point	65
n/a	Yellow Point	57
Nanaimo Airport	Yellow Point	37
Max Elevation		**230**
Min Elevation		**0**
Avg Elevation		**77**
Mean Elevation		**64**

Waterbodies in the CRD Watershed, Bullfrogs NOT present	Elevation (m)
Cabin Pond	339
Japan Gulch Reservoir	131
Goldstream Reservoir	456
Lubbe Reservoir	479
Butchard Reservoir	543
Sooke Reservoir	186
Deception Reservoir	180

11 APPENDIX 4: Interviews with Leading Experts

Name	Affiliations
Adams, M.	• USGS Amphibian Research and Monitoring Initiative • USGS Forest and Rangeland Ecosystem Science Centre
Govindarajulu, P.	• BC Ministry of Environment
Jones, T.	• Arizona Fish and Game
Schock, D.	• Postdoctoral Research Fellow, Faculty of Veterinary Medicine, University of Calgary • Previously was a Curator of Amphibians, Detroit Zoo
Schwalbe, C.	• Research Ecologist, USGS Sonoran Desert Research Station • Faculty, Wildlife and Fisheries Resources Program, University of Arizona
Speare, R.	• Professor Director, Anton Breinl Centre for Public Health and Tropical Medicine, James Cook University

From our discussions with the above mentioned individuals, we identified the following four broad themes:

1) It is **very** unlikely that invading American bullfrog populations will have direct impacts on human health

2) Prevention of bullfrog colonization in certain water bodies on strictly human health grounds may be a hard argument to justify

3) The severity of ecosystem impacts will be situational dependant

4) With the exception of bullfrog effects on amphibian biodiversity, ecosystem impacts will largely be speculation-based

The following list, taken directly from our interview notes, qualifies the above themes:
1) There are no published associations between American bullfrogs and human health
2) Bullfrog bacteria, parasite or viral pathogens are likely to be already present in the environment
3) Chytrid fungus, a known problem for amphibians, does not infect humans
4) Bullfrogs are not known to cause or facilitate changes to their physical surroundings, including water quality. However, no one has specifically looked at this
5) Bullfrogs tend not to alter the trophic structure of intact/natural habitats
6) Impacts on native species compositions, particularly insects and other amphibians, is more pronounced in disturbed and modified habitats
7) Ecosystem impacts, if they occur at all, will either be density dependant (i.e. extremely high bullfrog populations may cause environmental degradation and biodiversity loss) or due to man-made changes (bullfrogs appear to be highly successful at exploiting permanent water bodies)

8) Bullfrogs are temperate low-land species, and are unlikely to successfully colonize high-land ecosystems
9) Bullfrogs are generalist and opportunistic predators and are known to eat anything they can fit in their mouth. However, reports of bullfrogs consuming birds and snakes are the exception rather than norm
10) The most common food item of bullfrogs are other bullfrogs
11) It is very unlikely that bullfrogs would impact water supply infrastructure
12) Bullfrogs can be expected to continue to spread, largely as a result of habitat change and human behaviours
13) If a permanent waterbody is within the migratory and ideal thermal ranges, it will be invaded and colonized by American Bullfrogs
14) Human activities and structures (e.g. roads and waterways) will greatly assist bullfrog colonization of new water bodies
15) Successful eradication has only really been accomplished by finding the source population, installing fences to prevent migration, then completely draining the waterbody

12 APPENDIX 5: Interviews with Municipal Water Suppliers

Response summary of the twenty-two North American municipal water suppliers that were interviewed by telephone in December, 2008.

Province State	Municipality	Reservoir Type	Interview questions: 1. Are American bullfrogs Present or Absent?	1a. If present: Any impacts on water parameters?	1b. If present: Any changes in treatment protocols?	2. Current water treatment protocols	3. Current water intake filtration	4. Reservoir elevation	5. Public access	Notes
British Columbia	Nanaimo	Lake	Absent	n/a	n/a	Chlorine gas injected	2 inch coarse screen	Jump lake: 414 m South Forks: 271 m Number 1 reservoir: 107 m	Closed to public	
	Duncan	Aquifer	Absent	n/a	n/a	n/a	n/a	n/a	n/a	North Cowichan apparently has a bullfrog problem
	Parksville	n/a	Absent	n/a	n/a	Chlorination	Perforated pipes at river bottom covered in gravel	Dam: 830 m River intake: 5 m	Closed to public	
	Qualicum	Aquifer	Absent	n/a	n/a	n/a	n/a	n/a	n/a	
	Courtney & Comox)	n/a	Absent	n/a	n/a	Chlorination	Water obtained from BC hydro station on the river	131 m	Public access	
	Cumberland & Union Bay	n/a	Absent	n/a	n/a	Chlorine gas injected	Ellens lake: 3/4 inch filter 2 inch filter on all other intakes	Ellens Lake Henderson Dam: 609m Second Dam Hamilton Dam Stevens Dam: 683 m	Public access	
	Campbell River	Lake	Present	No	No	UV radiation; Chlorination	Water obtained from BC hydro pen stocks	John Heart water reservoir: 138 m	Limited public access	
	Port Alberni	Lake	Absent	n/a	n/a	Chlorination	Spill way or Valve & Weir system	Bambridge: 180 m China Creek: 203 m Lizard lake: 833 m	n/a	
Washington	Kennewick	Lake	Present	No data available	No	Membrane filtration system	Bar and mesh screen, 2 microns	Columbia River intake: 113 m	Public access	
	Spokane	Aquifer	Absent	n/a	n/a	n/a	n/a	n/a	n/a	

Province State	Municipality	Reservoir Type	Interview questions: 1. Are American bullfrogs Present or Absent?	1a. If present: Any impacts on water parameters?	1b. If present: Any changes in treatment protocols?	2. Current water treatment protocols	3. Current water intake filtration	4. Reservoir elevation	5. Public access	Notes
Oregon	Portland	Lake	Absent	n/a	n/a	Chlorination	1/4 inch screen	Reservoir 1: 340 m Reservoir 2: 286 m	Closed to public	American bullfrog tadpoles have been caught downstream of the intake
	Salem	Lake	Present	No	No; Follow EPA standards	Chlorination; Filtration	Screen with an airburst system; follow NOAH guidelines for screen size	River intake: 166 m	Closed to public	American bullfrogs have been endemic for many years; unaware of any changes to water parameters as a result
	Baker City	Lake	Present	No	No	Chlorination	1/4 inch screen	Goodrich Reservoir: 2300 m	Closed; by permit only	American bullfrogs are present at lower elevations, wetlands at higher elevations may be too cold for them
California	Redding	Lake	Present	No	No; Follow guidelines set by California legislature and Federal EPA	Chlorination	n/a	n/a	Closed to public	American bullfrogs are present in lakes above and around primary reservoirs, although water temperature is very cold; Bullfrogs are endemic in California
Ontario	Peterborough	Lake	Present	No	No	Chlorination	3 inch screen	216 m	Public access	Do have issues with odour and taste, but this has been attributed to the algae in the lake and not the bullfrogs
	Guelph	Aquifer	Absent	n/a	n/a	n/a	n/a	n/a	n/a	
	Sarnia	Lake	Present (assumed)	No	No; Follow the Canadian drinking water guidelines	Chlorination	2 inch screen; intake is 60 ft below water surface	Lake Huron: 192 m	Public access	
Nova Scotia	Cape Breton Regional Municipality	n/a	Present	No	No	Precipitation; Oxidation; Chlorination	Triangle mesh screen, has 1/4 inch sides by 1/2 inch bottom	66 m	Limited public access	Water intakes at the centre of the lakes; have had a frog die off in a feeder lake, but the frogs washed up on shore and did not come into contact with the primary reservoir intake
	Truro	Lake	Absent	n/a	n/a	Precipitation; Filtration; Chlorination	1 inch mesh screen	Leper Brook reservoir: 100 m	Limited public access	
	Halifax	Lake	Present (assumed)	No	No; Follow the Canadian drinking water guidelines	Precipitation; Oxidation; Chlorination	Rotating 1/4 inch auto mesh screen with three channels	120 m	n/a	

13 APPENDIX 6: American Bullfrog Parasites

The following parasite list was presented by Stan Orchard at the American Bullfrog Exploratory Meeting of November 29, 2004.

Tapeworms
- Ophiotaenia magna
- Proteocephalus magnus

Trematodes
- Glyphelmins quieta
- Glyphelmins subtropica
- Gorgodera minima
- Gorgodera attenduata
- Haematoloechus breviplexus
- Haematoloechus floedae
- Megalodiscus temperatus

Nematodes
- Cosmocercoides dukae
- Foleyella Americana
- Rhabdias ranae
- Falcaustra catesbeianae
- Oxysomatium americanum

Acanthocephala
- Centrorhynchus larva

Anthropoda – Crustacea – Copepoda