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<https://doi.org/10.15760/etd.6221>

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THESIS APPROVAL

The abstract and thesis of Catherine Mary Callison for the Master of Science in Biology were presented July 18, 2001, and accepted by the thesis committee and the department.

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ABSTRACT

An abstract of the thesis of Catherine Mary Callison for the Master of Science in Biology presented July 18, 2001.

Title: Factors Influencing Oviposition Among Pond-Breeding Amphibians: Exotic Vegetation, Oviposition Braces, and Cover.

I examined oviposition in four pond-breeding amphibians (northwestern salamander [*Ambystoma gracile*], long-toed salamander [*Ambystoma macrodactylum*], Pacific treefrog [*Hyla regilla*], northern red-legged frog [*Rana aurora aurora*]), at Burlington Bottoms, a lowland riverine site in northwestern Oregon, to determine whether differential use of native versus exotic plant substrates occurs. I found differential use in all four species, but use was inconsistent with the hypothesis that selection for native plants (or selection against exotic plants) was occurring. If selection was occurring, the pattern implied that reed canarygrass (*Phalaris arundinacea*), a widespread exotic in this lowland system, was sometimes favored. However, inconsistent use of reed canarygrass led me to examine the alternative that substrate strength (measured as density, diameter, and mass), rather than plant status, might be the basis of selection. Species used for oviposition differed in strength, but eggs were not consistently laid on braces based on my strength measures. Failure to find support for this hypothesis led to examination of a third hypothesis for two

species (red-legged frog and northwestern salamander), that cover characteristics of the oviposition brace or nearby vegetation might influence egg mass location.

Analysis of structural complexity of species used as a brace (as percent cover within 15 cm of the egg mass) did reveal a pattern consistent with complexity, but that was species-specific. Red-legged frogs selected braces with significantly more near-space cover; northwestern salamanders selected braces with sparser cover.

Comparison of marginal shrub and tree cover to the number of red-legged frog egg masses revealed that ponds with $< 50\%$ shrub/tree cover had few (< 5) masses.

Ponds with $\geq 50\%$ shrub/tree cover had many (≥ 10) masses. More complex vegetation may provide greater protection for egg masses or ovipositing red-legged frogs but further investigation is clearly warranted. Elucidating northwestern salamander cover requirements will require examining more occupied ponds. In particular, more refined examination, using experimental manipulation, is needed to verify the cover relationships revealed in this study. Meanwhile, managers should treat cover as important in red-legged frog oviposition life history, paying special attention to minimizing loss of marginal shrub and tree cover.

FACTORS INFLUENCING OVIPOSITION AMONG
POND-BREEDING AMPHIBIANS: EXOTIC VEGETATION, OVIPOSITION
BRACES, AND COVER

by

CATHERINE MARY CALLISON

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

MASTER OF SCIENCE
in
BIOLOGY

Portland State University
2001

Dedication

In memory of my father, Richard Worth Coldren (1927-2000), and my brother, Peter Worth Coldren (1960-2000):

Two of my first teachers and supporters who gave me the courage to try new things and helped me believe in myself.

Acknowledgements

This project highlighted the collaborative nature of science. I would like to thank Dr. Marc P. Hayes for helping develop this study and sharing so much of his expertise. Dr. Richard B. Forbes gave endless encouragement, advice, and help. The others on my thesis committee, Dr. Stanley S. Hillman, Dr. Michael T. Murphy, and Dr. Ric Vrana also spent many hours editing and giving good advice.

This project would not have been possible at all without Sue G. Beilke, wildlife biologist and project site manager for the Oregon Department of Fish and Wildlife, and her willingness to provide time, equipment, expertise, and volunteers.

The Oregon Zoo Foundation provided funds to purchase equipment. Additional equipment was borrowed from the PSU Biology, Environmental science, and Geology departments.

The amount of data collected required much assistance, and I will be forever grateful to the cheerful willingness of all my field assistants: Sarra L. Moller, Richard Y. Primmer, Anita M. Christenson, Denis M. O'Brien, Joe Maxey, Karen S. Sexton, Lisa D. Karst, Kyle Spinks, Adam R. Gallom, Rachel Sanchez, Shonene A. Scott, and Diana Gordon and her students from St. Mary's High School.

To all those people who helped me learn how to use Arcview software, understand GPS, and get my analysis done! Brady Callahan - ODFW, Sarra and Chris Moller, Shonene A. Scott, Lilian Herron, and Dr. Ric Vrana.

Thanks to Char C. Corkran for permission to use her photographs for some of the amphibians and egg masses.

The encouragement and love of my family kept me going throughout the whole process. They also gave formatting and editing help. Last, but certainly not least, my husband Brad.

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I. Introduction

Numerous recent reports of amphibian declines have increased awareness of the importance of this group and of environmental conditions to which they appear linked. Increased awareness has led to greater scrutiny of the status of amphibians globally (Blaustein and Wake 1990, Blaustein *et al.* 1994). Commercial harvesting, competition from exotic species, habitat loss, pathogenic fungi, pollution, ultraviolet radiation, and an interaction of one or more of these factors have all been suggested as causal in amphibian declines (Jennings and Hayes 1985; Pechmann *et al.* 1991; Sjogren 1991; Blaustein *et al.* 1994, 1995; Corn 1994; Berger *et al.* 1998, Lawler *et al.* 1999, Bosch *et al.* 2001). The factor deemed most important, however, frequently varies depending on the geographic area and amphibian species in question and may sometimes be confounded with several other factors. Careful field experiments and clear hypotheses can help eliminate confusion in determining the causal factors for each species. Studies on the decline of western ranid frog species provide examples of how these factors can be confused (Hayes and Jennings 1986). For instance, recent work suggests that declines of native species in the northwest as a result of apparent competition from introduced bullfrogs (*Rana catesbeiana*) may in fact be a result of negative impacts of humans on habitats that favor bullfrogs over native species (Kiesecker *et al.* 2001), an alternative that Hayes and Jennings (1986) originally emphasized as a fertile area for research.

Habitat loss is the one factor that is consistently touted as an important contributor to the decline of amphibians (Sjogren 1991, Delis 1996, Marsh and Pearman 1997, Kiesecker 1998). Habitat loss can however, vary in degree and form from total loss of a habitat to more subtle changes in hydrology and vegetation. Pond-breeding amphibians often attach eggs to emergent vegetation. One potentially important, but subtle type of habitat change that could affect amphibian reproduction is the introduction of invasive exotic plants. While many studies have examined the effects of invasive exotic plants on the success and distribution of native plants (Macdonald and Frame 1988, Smith 1990, Fernberg and Wehr 1995, Yavitt and Williams 1996, Stohlgren *et al.* 1999), few have addressed the influence of exotic plants on animals and other non-plant elements in ecosystems (Waring and Tremble 1992, Trammell and Butler 1995, Ramos 1996, Chown 1997, Stromayer 1998). Still fewer studies have identified what specific features of vegetation may be important to amphibians (Wiens 1970, Resetarits and Wilbur 1989, Figiel and Semlitsch 1995, Holomuzki 1995, Richter and Azous 1995, Kiesecker and Blaustein 1997).

Studies on the influence of exotic species on amphibians have frequently focused on animals (Hayes and Jennings 1986, Kiesecker 1998, Lawler *et al.* 1999). In particular, the influence of exotic plants on amphibians has not been directly studied (Adams 1999). My study was designed to help fill this gap by examining a guild of four pond-breeding amphibians in northwestern Oregon. I

examined oviposition to determine whether selectivity in oviposition substrate might occur where both native and exotic plants were available. I also examined the not necessarily exclusive alternative hypotheses of whether structural features affecting oviposition substrate strength or cover complexity might affect oviposition site choice.

The four pond-breeding amphibian species that I studied included northwestern salamander (*Ambystoma gracile*, abbreviated AMGR hereafter in tables and graphs), long-toed salamander (*Ambystoma macrodactylum*, AMMA), Pacific treefrog (*Hyla regilla*, HYRE), and northern red-legged frog (*Rana aurora aurora*, RAAU). Selection of these species was based largely on their abundance at my target study site and their use of oviposition braces that would permit tests of the aforementioned hypotheses. Review of the literature on reproduction and oviposition for these four amphibians revealed substantial data on their breeding habits and development but almost nothing on the factors involved in selection of oviposition sites.

Positioning of egg masses and timing of oviposition as well as the quantity and quality of zygotes are important to survival, and in most amphibians are species specific (Beebee 1996). Factors that can influence placement of eggs and the shape of the egg cluster are predation rate and predator behavior, rate of water flow, desiccation, low temperatures, UV radiation and acidic water (Stebbins and Cohen 1995, Beebee 1996). Only a few studies have looked at the survivorship of

aquatic eggs to hatching. Licht (1974) found a variation in egg survivorship from 2.6 % in the newt, *Triturus vulgaris*, to 91% in *Rana aurora aurora*. A correlation between embryonic temperature sensitivities and environmental temperature during the breeding season has been found in other studies of anuran eggs (Moore 1939, Licht 1971). Hatching success and larval survival of *Rana aurora* and *Hyla regilla* was unaffected by ambient UV radiation, but embryonic and larval life stages of these anurans were sensitive to enhanced UV-b, suggesting potential vulnerability to increases in solar UV-b in the future (Ovaska *et al.* 1997). Egg mass location, therefore, may influence exposure of embryos to UV-b.

Northern red-legged frog embryos have a thermal tolerance of 4-21°C and adults do not oviposit until waters are at least 6-7°C (Licht 1971). Licht also found that the embryos could withstand short-term cold exposure as low as 1°C. Submerging their eggs protects the red-legged frog embryos from thermal extremes. Spawning at night also may allow the embryos to progress to developmental stages that are able to tolerate higher daytime temperatures. Eggs that are in globular masses are better insulated and less likely to freeze (Zweifel 1968, Zweifel 1977).

Attachment of eggs to stems or leaves by ambystomatid salamanders in mid-water also prevents eggs from sinking into the mud on the bottom with a lower oxygen supply (Duellman and Trueb 1994). Other experiments on newts showed that in *Triturus* and *Notophthalmus*, the female has a cloacal mechanism for

detecting oxygen production and eggs are placed only on young leaves with higher oxygen production (Winpenny 1951).

Various studies have also shown that vulnerability of amphibian embryos to predation appears to strongly influence selection of oviposition sites. Resetarits and Wilbur (1989) found that female treefrogs (*Hyla chrysoscelis*) from the eastern US and southeastern Canada) tended to avoid pond sites where significant threats of predation and intraspecific larval competition existed. Kats and Sih (1992) studied the streamside salamander (*Ambystoma barbouri*) (eastern US) and found they select egg-laying sites along streams that reduce exposure of the young larvae to predatory fish. Egg-laying sites of larger, presumably more experienced male bullfrogs (*Rana catesbeiana*) have been found to have significantly lower larval mortality than the sites of smaller males (Howard 1978). Grubb (1972) studied the differential predation of the mosquitofish (*Gambusia affinis*) on the eggs of seven species of central Texas anurans to ascertain whether permanent water predators may influence breeding site selection. He found differences in the size and texture of the jelly envelopes between permanent and temporary water breeders, with temporary water breeders having smaller eggs and greater attacks by the fish. Differential vulnerability of the eggs to aquatic predators may be a factor that at least contributes to preferences among certain species for temporary pools as breeding sites (Grubb 1972).

In this research, I first focused on examining the previously unaddressed hypothesis that native amphibians exhibit distinctive patterns of oviposition based on vegetation status (native versus exotic). I then re-examined the oviposition substrate data to test the hypothesis whether the strength of the substrate might be responsible for differences in oviposition. . Lastly, I re-examined the substrate data to determine whether cover, either provided by the oviposition substrate or by egg masses being position close to cover, might influence oviposition. Each of these hypotheses was addressed by testing of a null hypothesis of no difference in oviposition patterns between substrates having different statuses, strengths, or cover characteristics.

Besides simply addressing factors influencing oviposition site selection, this study was intended to provide data to help develop basic recommendations for properly managing habitat that amphibians use for reproduction. In particular, I hoped to gain insights that would allow developing preliminary recommendations on how to address the influence of exotic plants, preferred oviposition substrates, and cover conditions for the amphibian species I studied.

II. Study Species Natural History

Northern red-legged frog

The northern red-legged frog (*Rana aurora aurora*, Figure 1) is distributed west of the Cascade Range in forests and lowlands from northwestern California to southwestern British Columbia below 1,097 m [3,600 ft] elevation. They breed

during a relatively cool seasonal interval, typically early February through March, when water temperatures are between 4-10° C (Brown 1975). They use still-water habitats, including ponds, lake edges or slow-moving backwaters along streams for reproduction (Nussbaum *et al.* 1983). Males typically call from under water (Hayes and Miyamoto 1984) and adults move to upland areas with mesic substrates soon after breeding (Storm 1960, Licht 1969).

Northern red-legged frogs have large, relatively loose egg masses that, when fully hydrated, range from the size of a grapefruit to a cantaloupe (600-2,000 eggs, Figure 1) and are typically attached to an emergent vegetation brace (Licht 1969, Licht 1971). Egg masses are usually found in water 0.5-2.0 m deep (Corkran and Thoms 1996). The eggs are black above and white below and lie within a relatively large jelly capsule. During early development, Licht (1971) found that egg mass temperature was significantly higher (by about 3.6 °C) than that of the surrounding water.

Temperature is the major factor influencing developmental rate of egg masses (Storm 1960). He reported that the time from laying to hatching averaged 35-49 days. During development, a growth of green alga often develops within the capsule; oxygen production by this alga tends to promote floating of egg masses as the embryos near hatching (Storm 1960). Recently hatched larvae will remain close to the egg mass for a few days either resting on the bottom or hanging on to plants or debris with their adhesive (chin) glands (Brown 1975).

Northwestern salamander

The northwestern salamander (*Ambystoma gracile*, Figure 2) is a species similar to northern red-legged frog in it ranges largely west of the Cascades (Nussbaum *et al.* 1983). However, it occurs further north into British Columbia (Green and Campbell 1984).

They live in moist forests or partly wooded areas from sea level up to 2,000 m [6,550 ft] and breed in ponds, lakes, and slow backwater areas of streams. The breeding season varies depending on altitude and latitude. In the southern coastal parts of its range, the northwestern salamander may breed as early as late January or February but in northern high-elevation lakes which may have ice until July, breeding may begin as late as August (Nussbaum *et al.* 1983). Males are reported to arrive at the breeding pond first and remain at the pond until the end of the breeding interval. In contrast, females leave soon after depositing their eggs. Length of the breeding season ranges from less than one week to nearly two months depending on the onset of warm spring weather and also, perhaps the proportion of terrestrial versus paedomorphic adults (reproductive adults that retain external larval features) in the breeding population (Eagleson 1976). Egg masses are laid in water and typically attached to relatively thin stems or sticks (0.4-0.8 cm in diameter), 0.5-2.0 m below the water surface. The egg mass has a very firm jelly with a smooth or wavy surface due to an extra layer of jelly around the entire mass.

The size of the mass ranges from that of a small orange to a small grapefruit, and contains 40 to 270 tan and cream-colored eggs (Figure 2).

The embryonic period for northwestern salamanders is 30 to 60 days depending on egg size and temperature (Brown 1976). Slower embryonic development within *A. gracile* egg masses allows enough time for significant intracapsular algal growth. This pattern of algal growth appears to benefit embryonic survival (Marco and Blaustein 2000). Hatchlings are ca. 14-15 mm total length (TL). Larvae usually do not metamorphose until the second year when they are about 50 mm snout-vent length (SVL) (Licht 1975). Larvae can also become paedogenetic in the second or third year (Eagleson 1976). The development of paedomorphic individuals does not seem to be related to food abundance but habitat stability (aquatic habitat permanence) in this facultatively paedomorphic species (Licht 1992). The larvae feed on many diverse aquatic invertebrates but are highly dependent on microcrustacean zooplankters at smaller body sizes (Licht 1975). Aquatic beetle larvae and fish (e.g., trout) are among the main predators of larvae (Tyler *et al.* 1998).

Pacific treefrog

The Pacific treefrog (*Hyla regilla*, Figure 3) is the most common, widespread frog in western North America (and the Pacific Northwest), occurring wherever still-water provides reproductive sites, ranging from sea level to high

elevations (Nussbaum *et al.* 1983). The species is terrestrial during the non-breeding season. Pacific treefrogs will utilize almost any small, stillwater body, which can place them at risk when small ponds dry too quickly (Nussbaum *et al.* 1983). Pacific treefrogs move to aquatic breeding sites from November (coastal) to July (mountains) after warm ($>10^{\circ}\text{C}$) winter rains (Nussbaum *et al.* 1983). Males “chorus” mostly at night once in the water and tend to call in groups where one is the leader (Awbrey 1978). Females are attracted to the loud choruses and often select the group leader as a mate (Perrill 1984). Males use short single-note calls, which appear to function largely to space the males within the pond, but have a specific advertisement call that attracts females (Brenowitz and Rose 1999).

The eggs are laid in packets of 9-70 eggs (Figure 3) attached to submerged vegetation or laid on bottom of shallow pools (Corkran and Thoms 1996). Individual eggs are relatively small (about 0.7 - 1.3 mm in diameter), brown above and a dirty yellow below with two jelly envelopes (Nussbaum *et al.* 1983). Eggs hatch in three to five weeks in western Oregon. Tadpoles are about 6-8 mm TL at hatching (Corkran and Thoms 1996).

Long-toed Salamander

The long-toed salamander (*Ambystoma macrodactylum*, Figure 4) is the smallest member of the genus *Ambystoma* in the Pacific Northwest. They use a wide variety of habitat types including semi-arid sagebrush deserts, dry woodlands,

humid forests, alpine meadows, and other intermediate habitats from sea level to ca. 3000 m [9,800 ft] elevation (Nussbaum *et al.* 1983). Terrestrial individuals remain mainly below ground during the extreme weather conditions (i.e., freezing or hot and dry), and then emerge in early spring to breed near ponds and lakes. The breeding season varies, depending on the microclimate of the habitat (Howard and Wallace 1985). In mild coastal climates, like the Willamette Valley, the breeding season starts as early as October and may last until April. In areas with harsher climates, the breeding migration will not start until late February to early April, depending on the local conditions. The breeding season can be as short as three weeks (Verrell and Pelton 1996).

Egg laying methods vary from single eggs attached to vegetation in shallow water up to packets of 5-100 eggs (Figure 4) in shallow to deep water, attached to vegetation or under the surface of a log (Leonard and Richter 1994). Eggs are sometimes placed loosely on the bottom. The size of the individual eggs ranges from 2.0 to 2.5 mm in diameter and the eggs are dark (black or brown) on top and white on the bottom. Clutch size of an individual female ranges from 85 – 411 eggs and increases with female body size (Howard and Wallace 1985). Typically, larvae reach maturity in one season, but in some high elevation habitats they may take two seasons (Fukumoto and Herrero 1998).

Figure 1 – Northern red-legged frog
(Photo: C. M. Callison 2000)

Egg mass of northern red-legged frog
(Photo: C. M. Callison 2000)

Figure 2 – Northwestern salamander
(Photo: C. M. Callison 1999)

Egg mass of northwestern salamander
(Photo: C. M. Callison 2000)

Figure 3 – Pacific treefrog
(Photo: C.C. Corkran)

Egg mass of Pacific treefrog
(Photo: C.C. Corkran)

Figure 4 – Long-toed salamander
(Photo: C.C. Corkran)

Egg mass of long-toed salamander
(Photo: C.C. Corkran)

III. Materials and Methods

Study Area

I conducted this study at Burlington Bottoms, a riverine site along the Multnomah Channel near the confluence of the Columbia and Willamette Rivers. Under the management of the Oregon Department of Fish and Wildlife (ODFW), Burlington Bottoms is a mitigation site for Bonneville Power Administration (BPA) dam-associated modifications of the Lower Columbia River region. This site consisted of a complex array of ponds, lakes, and sloughs, intermixed with lowland riparian forest (Appendix A).

Many factors, human-linked and otherwise, have influenced the hydrology of this site. Annual spring freshets (high water events) of the Columbia and Willamette Rivers regularly inundate parts of the site. Further, a series of culverts carry runoff from uplands and nearby Highway 30 to the west. Since the site is now a protected wetland, the main factors affecting onsite hydrology are freshwater inflows from the elevated landscape across the highway, and the tidal influence of the Columbia. In addition, beaver activity onsite (and perhaps, to some degree, offsite) modulates freshwater inflows. Some of the topography and water features are the result of excavation and fill. The elevation of the study ponds, which are located on the northern portion of Burlington Bottoms, are all below 4 m [12 ft] above mean sea level (see Pacific 1993; specifically maps in hydrology report for Burlington Bottoms). Soils are Rafton and Sauvie silt loams typical of lowland

riverine areas subjected to repeated flooding. Both soils are considered poorly drained and poorly suited to most human uses but can be valuable wildlife habitat. Seasonal air temperatures vary from highs above 18°C (65° F) in summer to lows reaching 1.7°C (35°F) in winter. Freezing is infrequent, usually occurring in the interval November-February. The mean annual precipitation for the area averages 94 cm (37 in) with most precipitation falling as rain from November through March. Wind is seasonally largely bidirectional, coming primarily from the NW during winter and the SE during summer. Low levels of wind (Beaufort scale values 1-4) are frequent, but strong wind is rare. The wind is calmest between the spring and fall months. Weather data were extracted from summaries of the interval 1971-2000 for the Portland International Airport Data Station (National Climatic Data Center Website: <http://www4.ncdc.noaa.gov/>).

Human activity over the last 60 years has markedly influenced this site. In particular, maintenance of railroad equipment, livestock grazing, and building of shantytown housing and access roads has occurred. From 1936 through the early 1960s, a log depot and maintenance facility existed in the southern portion of the area. Cattle grazing occurred from 1961 through 1991 (Pacific 1993). Another important change during this century has been the alteration of seasonal hydrology, which was a consequence of the dam-building era on the Columbia River between the 1930s and 1970s. The most prominent alteration of seasonal hydrology has been the decrease in frequency of large magnitude of spring freshets, which have

both decreased levels of annual inundation and the load of water-borne sediments that are deposited seasonally. Both are important in the pattern of renewal of off-channel riverine habitats (e.g., oxbows, sloughs), of which my study ponds are all a part.

Study Sites

I selected seven study ponds based on their known amphibian use and their different proportions of native versus exotic aquatic vegetation. I selected the latter to ensure a range of variation in native versus exotic vegetation in order to determine whether that variation might affect the pattern of use by native amphibians. Monitoring, mostly for amphibian egg masses, had been done on these ponds since 1996. This study complemented and augmented previous surveys through examination of microhabitat characteristics of egg mass locations. Previous surveys lacked opportunity to examine microhabitat characteristics because of time and funding constraints.

During the droughty interval when I did my fieldwork (1999-2000), all seven ponds were largely individual units, and except for Kidney, all ultimately dried in 2000 (Table 1). Seasonal freshets in previous years have inundated Burlington Bottoms so it was not possible to distinguish individual ponds during high water (S. Beilke, M. Hayes, *pers. comm.*), but not in 2000. During 2000 high water conditions, which peaked in February, aquatic connections to other water

bodies were restricted to beaver channels between each of three of the ponds and an adjacent permanent, fish-occupied slough (Table 2; see also Appendix K). Some activity I saw in these ponds (e.g., great blue heron [*Ardea herodias*] in Kidney Pond) may result from these aquatic connections, allowing fish access. Besides the above-noted beaver channels, old or fresh beaver sign as the result of bark removal, chewed stumps, felled trees, and terrestrial pathways were recorded at five of the seven ponds. Most beaver sign was old; most chewed stumps or felled trees were Pacific willow (*Salix lucida* var. *lasiandra*); and Pancreas Pond, with its array of old willow stumps, had most of the beaver sign. Evidence of grazing (e.g., clipped herbaceous vegetation) was other animal sign that was prominent during study of these ponds. Some grazing occurred in all ponds, but it was dramatic in Kidney Pond, in which I made regular observations of at least five pairs of mallards, and some sign of muskrat and nutria was seen. Muskrat activity was also frequent in Pancreas Pond.

Table 1 – Study Pond Characteristics, 2000

Pond	Maxima ^a		Hydroperiod		Dominant Vegetation
	Surface Area (m ²)	Depth (m)	Status	Dry by	
Butterfly	482 ^b	0.5	Temporary	Late June	Reed canarygrass
Kidney	2,940	0.4	Permanent	-	Wapato
Muskrat	525	0.6	Temporary	July	Reed canarygrass; Water pepper
Oak	74,400	0.8	Temporary	Late August	Reed canarygrass; Water pepper
Pancreas	1,550	0.6	Temporary	Late June	Water pepper; Reed canarygrass
Sedge	2,790	0.4	Temporary	June	Reed canarygrass
Spirea	336	0.5	Temporary	June	Reed canarygrass

^a Surface area and depth maxima occurred during February 2000. Surface area was estimated using GIS and pond maps (see Methods Section on Mapping, step 4). Depth was measured using a gauge placed near the lowest elevation point in each pond.

^b Butterfly was a bi-lobed pond; the surface area is indicated for the north lobe, which was the only part of this pond with significant water during the amphibian reproductive season in 2000.

Study ponds varied over two orders of magnitude in their maximum surface area in 2000, but their maximum depth varied only two-fold and even the deepest pond (Oak) was relatively shallow (Table 1). Although herbaceous vegetation diversity partly guided pond selection, every pond had at least some reed canarygrass, an exotic that dominates much of the lowland riverine landscape in this region. A native aquatic, water pepper (*Polygonum hydropiperoides*) was a prominent element in three ponds, and another native aquatic, wapato, was dominant in Kidney Pond. Details of the herbaceous species composition of each of the seven ponds are provided in Table 3. All seven ponds lie in a mosaic of

bottomland riverine forest, the canopy of which was located at varying distances from the ponds (Table 2). Forest composition varied, but was largely dominated by a composite of black cottonwood (*Populus balsamifera* var. *trichocarpa*), Oregon ash (*Fraxinus latifolia*), and Pacific willow.

Table 2 – Other Features of Study Ponds

Pond	Structural	Vegetation	Illustrations (Figure #)
Butterfly	Two pond lobes	No tree or shrub vegetation on edge; nearest trees and shrubs > 20 m distant	5
Kidney	Beaver channel connection to slough; extremely silty	Woody vegetation on three sides; herbaceous vegetation heavily grazed and seasonal dieback	6
Muskrat		Ash and willow surrounded	7
Oak	Beaver channel connection to slough	Northwest margin with ash and willow; some dead or live willow in pond; hardhack and reed canarygrass on south/west margins	8
Pancreas	Beaver channel connection to slough; many stumps	Limited marginal woody vegetation	9
Sedge	Near slough, but no aquatic connection	North margin with ash and willow	10
Spirea		Lone willow in mid-pond; hardhack margin	11

^a Nearest tree and shrub vegetation was > 20 m away.

Figure 5 – Butterfly Pond, February 2000
E to W (Photo: C. M. Callison 2000)

Figure 6 – Kidney Pond, September 1999
E to W (Photo: C. M. Callison 1999)

Kidney Pond, February 2000
SE to SW (Photo: C. M. Callison 2000)

Figure 7 – Muskrat Pond, September 1999
SW to NW (Photo: C. M. Callison 1999)

Muskrat Pond, March 2000
SE to SW (Photo: C. M. Callison 2000)

Figure 8 – Oak Pond, September 1999
West lobe to NE (Photo: C. M. Callison 1999)

Oak Pond, March 2000
NW lobe to SE (Photo: C. M. Callison 2000)

Figure 9 – Pancreas Pond, September 1999
NE to SE (Photo: C. M. Callison 1999)

Pancreas Pond, February 2000
NE to SE (Photo: C. M. Callison 2000)

Figure 10 – Sedge Pond, February 2000
SE to NW (Photo: C. M. Callison 2000)

Figure 11 – Spirea Pond, February 2000
SW to NE (Photo: C. M. Callison 2000)

Vegetation Surveys

Within Ponds

I surveyed the vegetation in each pond in the interval 10 September to 1 October 1999, when the ponds were dry (Appendix B). Parallel line transects with points 3.0 m apart (Sutherland 1996) were used to determine vegetation species present in each pond. The first point on each transect was at the start of the transect and plant species within 30 cm circle surrounding that point were recorded along with the status of each species (exotic or native). The direction and starting locations of transects varied according to the size and shape of the pond in order to cover the entire pond area (Appendix C). The vegetation transects were not redone during the winter to minimize disturbance of the egg masses by entering the ponds as little as possible. However, I recorded changes in pond vegetation that might alter the availability of oviposition braces. Only two ponds experienced visible change in vegetation during the winter; the vegetation composition in these ponds was re-assessed to reflect this change. As only the north lobe of Butterfly retained significant water during the amphibian- breeding season in 2000 and it was the only area of Butterfly used by amphibians, I redid the vegetation analysis to show only the north lobe.

Around Ponds

I scored marginal vegetation around the pond (within ca. 2 m of the shoreline) into three structural categories (herbaceous, shrub, and tree) at single

points 3 m apart. Only the species of trees and shrubs were recorded during these surveys. Surveys were conducted in March 2000 during the mapping of pond water levels (Appendix D).

Animal Surveys

Amphibian Egg Masses

I surveyed egg masses in the interval 19 January 2000 to 31 March 2000.

The dates and survey effort for each pond is shown in Appendix E. I took a series of measurements and observations on the egg masses or packets of the four different amphibians the first day they were discovered. Egg masses of northern red-legged frog and northwestern salamander were sampled in all seven ponds. As their numbers were large, I sub-sampled the egg packets of long-toed salamander and Pacific treefrog only in Muskrat and Butterfly Ponds. The survey protocol and sheet for collection of egg mass or packet data are shown in Appendix F and G. These were developed from several established protocols for this site and others used in the Northwest (Olson *et al.* 1997). I located the egg masses by doing visual systematic surveys starting at one end of the pond and covering the entire pond. Once an egg mass was located, I flagged it and assigned it a number (each mass numbered consecutively by species as it was located). I recorded each egg mass, its height in the water column, its distance from the nearest shoreline, and measure the percentage of vegetation cover with a 15-cm radius circle around the egg mass. These variables were taken to help characterize the oviposition substrate structure,

amphibian egg mass or packet position, and potential differences within and among ponds.

The vegetation substrates on which the egg masses or packets were deposited were also scored for three variables: exotic versus native; the oviposition substrate species represented; and a diameter of the stem or leaf of the substrate. I also took samples of the plant that were used as oviposition substrates back to the laboratory to be dried and weighed. Density was determined by calculating a cylindrical volume from a set length (10 cm) and measured diameter. These samples were used to determine the strength index of the substrates. The various masses, diameters and densities for each species were first compared to determine if they were significantly different from one another. They were then ranked in each measurement category from largest to smallest. These rankings were then totaled for each species to determine an overall ranking that was used as the index of strength.

I selected Muskrat and Butterfly Ponds to obtain a representative sample of Pacific treefrog and long-toed salamander egg packets based on the favorable habitat characteristics of these ponds for these two species. When surveying these two ponds, as many egg packets were marked as three people could find walking in the same direction spaced 3 m apart. The ponds were divided into 10 m² quadrats covering the entire pond area, and the estimated number of egg packets in each quadrat was totaled for each species.

A subsample of 25 egg packets from each of the Pacific treefrog and the long-toed salamander was taken in each of Muskrat and Butterfly Ponds. In order to ensure that the sampled packets in each pond were representative, I first calculated the percentage of each species per quadrat from the total estimated number of egg packets determined from initial walk-through. This percentage was used to estimate the number packets (out of 25) that would be selected from each quadrat. If the sampled number was less than one, I added one to a neighboring quadrat. A north-south transect line ran through the center of each quadrat. I randomly selected points along the transect and measured the egg mass nearest each point in any direction. If two egg masses were the same distance from the randomly selected point, or if two egg masses were one on top of the other, both were measured.

Fish Surveys

Fish species composition was determined to understand whether fish presence might influence egg mass location or pond choice. Fish surveys were conducted from the 2 February to the 28 April 2001. The dates and times each pond was sampled are shown in Appendix H. I sampled ponds with minnow traps ($\frac{1}{4}$ in mesh [6 mm] with 2 in [5.1 cm] openings) to determine the presence and species composition of fish in the ponds. I placed unbaited traps at three random locations in each pond for a 24-hour sampling interval. Because I had few traps, I could not sample all ponds simultaneously; so I sampled two ponds per night with

three traps each. Butterfly Pond was not sampled for fish due to its high visibility in the water column, small size, and its low water levels prevented effective use of minnow traps. In addition to trapping, I recorded any visual observations of fish in the ponds.

Invertebrate Sampling

Six out of seven of the ponds were sampled for invertebrates. Spirea Pond was not sampled because it dried up before the survey could be conducted. Butterfly and Muskrat Ponds were sampled on 27 March 2000. I sampled Kidney, Oak, Pancreas, and Sedge Ponds on 4 April 2000. Invertebrates were sampled using a 900- μ m mesh sweep net. Six samples were taken in each pond (three in dense emergent vegetation, three in open water) (Sutherland 1996). Three random locations in each area (vegetated or open water) were selected from a transect through the center of the area. The direction of each transect in each pond is shown in Appendix C. Each sample was taken by doing three sweeps moving back and forth through the entire water column to standardize sampling. After a sample was collected in the net, its contents were emptied into a plastic tub. The net was then rinsed twice with two 900-ml containers of water. Contents of each sample were placed into labeled plastic bags, preserved in 70% ethanol, and brought back to the laboratory for identification and quantification. Fish and tadpoles were counted and released prior to putting the samples in plastic bags.

Water Quality Measurements

For each pond, repeated measurements of water temperature, dissolved oxygen, pH, and turbidity were taken to determine potential differences within and among the ponds. These measurements were used to determine whether oviposition patterns might be more closely tied to a simple physical difference within or among the ponds than to the focal hypotheses being tested.

I used a H₂O[®] Water Quality Multiprobe [Hydrolab Corporation – software v2.2 1995] to gather all water quality measurements. All ponds except Spirea and Butterfly (declining water levels prevented me from obtaining measurements as the ponds approached drying) were sampled on three different days (late February, early April, and late April) at about the same time of day (within 2 hours) to avoid diel confounds. I measured water depth, temperature, pH, dissolved oxygen [DO], and turbidity at 10 locations throughout the pond. The ten measurements were taken at 5-7 minute intervals and spaced evenly around the pond. I took each of the three days' measurements at the same locations within the ponds. The logistics of transporting the Hydrolab around to all the selected ponds proved to be the most difficult part in determining the time of day to sample. Evaluation of turbidity was difficult in Kidney pond because my movements easily disturbed its soft substrate. To minimize this problem, I took turbidity readings after standing in one place for 5 minutes and then recorded for 5 more minutes.

One HOBO data logger was also placed in each pond at the beginning of the oviposition period (4 February 2000) to record water temperatures continuously. Each data logger was placed in the deepest part of the pond and recorded hourly water temperatures. Temperatures were recorded until 29 May 2000.

Mapping

I mapped each pond's vegetation in order to evaluate the cover hypothesis. The first step in mapping was to place permanent stakes at the edges of the pond that were used as reference points from which the egg mass locations and pond margins were measured. Some of these were also used as starting points for vegetation transects. I labeled each stake according to its location around the pond to the nearest compass or joint direction, sometimes using pond features as part of the description (i.e. NE, SE, NW Lobe NE corner, etc.) The boundary of typical full pool and the location of the egg masses within the pond were originally measured in polar coordinates (radial distance and angle from a base point). Later, I re-measured the reference and perimeter boundary points with a Trimble GeoExplorer II GPS unit. Ultimately, I used only the GPS points, but I include the steps for each method for comparison. Measurements were obtained as follows:

1. I measured a series of points around the pond to obtain the full pool boundary. For each point, one person (A) would stand at a stake with a compass and the end of a tape measure. Another person (B) would walk to the point to be measured with the other end of a tape measure, and I recorded the distance and the degrees off North from the stake to that point. Points were taken at least every 3 m (10 ft), but if unique features existed, such as trees or stumps along the pond margin, they were also measured.
2. The polar coordinates for each egg mass were measured from a pole no more than 30 m (100 ft) away, using two people in the same manner as the boundary measurements.
3. Boundary and egg mass measurements were taken no more than 30 m (100 ft) from any given pole in order to reduce error in compass readings. Once the whole pond was measured, each point had to be recalculated using geometric formula so that every point and egg mass had a bearing and distance from one reference point.
4. The last step was to measure the poles and boundaries using a GPS receiver (Trimble GeoExplorer II). The accuracy of this unit was within 1 m after differential correction. The GPS data was used to help create GIS spatial data layers of the ponds (Appendices C and K). The bearing and distance data, along with the GPS data, were compiled with an aerial

photograph to create GIS “shape file” polygons of the ponds. The locations of egg masses were also geocoded as coordinate points and so could be mapped in relation to their location in each pond. I also calculated the surface area of each pond with these data. I then converted my measurements of egg mass locations to Universal Transverse Mercator (UTM) coordinates using a coordinate geography extension tool in the ESRI Arcview 3.2 GIS program.

Analysis

I tested for selection/avoidance of native and nonnative plant species for oviposition using data from all species. To perform the tests, I grouped the oviposition substrates into native or nonnative and compared them to the percentage of either native or nonnative substrates available in each pond. Substrate availability was based on the percentage of the fall vegetation comprised of native and exotic species (Table 3). Expected and observed use was compared using Chi-square tests. I used a rejection criterion of $P \leq 0.05$ for all tests performed. Species having fewer than 10 egg masses per pond were not analyzed.

I then examined substrate use in relation to relative substrate strength. After doing the Analysis of Variance (ANOVA) to determine if each species was significantly different for each measurement type, the species were ranked highest to lowest for each measurement. For example, if Pacific willow had the highest

density it would be ranked 1, the next highest would be a 2, etc. The rankings from each measurement type were then totaled to give an overall relative strength index ranking for each species. The higher the rank, the stronger they were considered to be.

The next step in evaluating the substrate strength hypothesis was to compare each individual species used for oviposition to all the other species available in the pond using a Chi-square test to determine if that species was used significantly more often than would be expected from its percent availability. I then compared this to the strength index to see if the brace species used were stronger than the brace species available.

Lastly, I addressed the cover hypothesis, which I tested in several ways. First, I compared the vegetation cover around the pond to the egg mass locations in the pond. After I created polygons of the ponds and converted the egg mass locations to points within those polygons, I divided the edge of the polygon into sections according to their vegetation type (assigned from field notes). Each pond was analyzed using a topographical analysis (Near command) from ArcInfo (ESRI Corp) software that measured the nearest vegetation edge type to each egg mass. The total percentage of each vegetation type per pond was determined from these data. The final step was to compare the percent vegetation cover types available around the pond margin to the percentage of egg masses found nearest each using a Chi-square analysis.

I performed a correlation analysis of the vegetation within and around the pond margin only for the northern red-legged frog because northwestern salamander was represented in too few ponds. Numbers of red-legged frog egg masses was compared to the percent of vegetation in each of the three cover types (tree, shrub, herbaceous).

Distributions of the percentages of cover estimates taken around egg masses were compared between the northern red-legged frog and northwestern salamander egg masses.

Vegetation used versus available for oviposition was compared using a ranking of structural complexity with a Chi-square analysis for northern red-legged frogs and northwestern salamanders.

Table 3 – Pond vegetation, Fall 1999
Plant species codes are in Appendix H.

Plant Species	Status ¹	Percentage of Total Points Sampled Fall 1999						
		Butterfly	Kidney	Muskra t	Oak	Pancreas	Sedge	Spirea
BICE	N	0.0	0.5	0.0	7.7	0.0	0.0	0.0
CAAP	N	0.0	0.0	0.0	2.2	1.5	3.9	0.0
CAOB	N	41.3	0.0	0.0	0.0	2.3	1.4	27.7
ELAC	N	2.2	1.0	0.0	0.0	0.0	0.0	0.0
ELOV	N	0.0	4.5	0.0	3.7	0.0	1.4	0.0
ELPA	N	3.9	2.0	0.0	0.0	4.6	7.0	3.6
EQAR	N	0.0	0.0	0.0	0.4	0.0	0.0	0.0
GAsp	N	0.0	0.0	0.0	0.0	0.0	0.0	3.6
JUAC	N	0.0	0.0	0.0	0.0	0.0	0.4	0.0
PACA	N	2.8	0.0	0.0	0.0	0.0	0.0	1.2
POsp	N	1.1	29.2	13.9	17.7	25.9	24.0	0.0
SGLA	N	0.0	51.8	0.0	0.0	1.5	0.0	0.0
SALA	N	0.0	0.0	0.1	2.6	0.4	0.0	0.1
SPDO	N	0.0	0.0	0.0	0.0	1.9	1.1	9.6
SPEM	N	0.0	0.0	0.0	0.0	9.5	0.0	0.0
FEAR	I	1.1	0.0	0.0	0.0	0.0	0.0	0.0
LUPA	I	0.0	2.0	0.0	0.0	14.4	0.0	0.0
PHAR	I	47.5	9.0	86.1	64.9	38.0	60.6	54.2
SODO	I	0.0	0.0	0.0	0.7	0.0	0.4	0.0
Totals		100.0	100.0	100.0	100.0	100.0	100.0	100.0

¹ N = native, I = Introduced, nonnative

IV. Results

Pond Vegetation Composition

Nineteen plant species were found within the seven ponds (Appendix I gives the four-letter codes corresponding to the scientific and common names of the plant species discussed used hereafter). The ponds varied from a low of three species (Muskrat) to a high of 10 (Pancreas). Only four nonnative species were found among the seven ponds but reed canarygrass was by far the most frequent (Figure 12). Vegetation structure changed significantly in Kidney and Oak ponds during the winter (Table 3). Kidney pond changed markedly because of a reduction in wapato (Fig. 6). The cause of this reduction is unclear, but seasonal dieback and the 10 pairs of grazing mallard ducks (observed almost every day I collected data) were probably responsible. In Kidney Pond, scoring of winter vegetation composition revealed that **POsp** had increased 47% (from 29% during the September 1999 survey to 76% during the February 2000 survey). Similarly, **PHAR** increased 15% over the same period (9% to 24%). In Oak pond, increases in were observed in **POsp** (18% to 19%), and **PHAR** (65% to 68%).

Amphibian Species Composition

The first eggs of the 2000 amphibian oviposition season at my study ponds were detected on 19 January in Oak Pond at a water temperature of 4.1°C. These

eggs consisted of egg packets of long-toed salamander. I made the first observation of northern red-legged frog egg masses on 3 February in Oak pond in water that was 6.9°C. Pacific treefrog egg packets were not observed until 11 February in Butterfly pond at a water temperature of 6.3°C. I also first observed egg masses of the northwestern salamander on 11 February at Kidney pond in 6.4°C water. I surveyed ponds only once or twice a week so these dates do not necessarily reflect the first day the egg masses or packets may have been deposited for each species. The numbers of northern red-legged frog egg masses varied from a low of 2 (Butterfly Pond) to a high of 133 (Oak Pond) (Figure 13). Northwestern salamander egg masses were observed in six of the seven ponds with none found in Butterfly Pond up to 23 found in Pancreas Pond (Figure 13). Egg packets for long-toed salamanders and Pacific treefrogs were observed in all ponds and were the most frequent species encountered in all ponds.

Table 3 shows the average temperatures and range of water temperatures in each pond during the four-month sampling interval. I observed a progressive increase in mean water temperature from February (6.4°C; range: 4.3-9.2°C) through May (16.5°C; range: 12.6-23.5°C).

Figure 12 – Vegetation composition and oviposition use per pond of the four amphibians
Plant species codes are in Appendix H.

Figure 12 – Vegetation composition and oviposition use per pond of the four amphibians (continued) Plant species codes are in Appendix H.

Muskrat Pond Vegetation Composition vs. Oviposition Use

Oak Pond Vegetation Composition vs. Oviposition Use

Figure 12 – Vegetation composition and oviposition use per pond of the four amphibians (continued) Plant species codes are in Appendix H.

Pancreas Pond Vegetation Composition vs. Oviposition Use

Sedge Pond Vegetation Composition vs. Oviposition Use

Figure 12 – Vegetation composition and oviposition use per pond of the four amphibians (continued) Plant species codes are in Appendix H.

Figure 13 – Amphibian Egg Mass Count Per Pond

Rana aurora and *Ambystoma gracile* are actual counts. A subsample of 25 egg packets were taken for each of *Hyla regilla* and *Ambystoma macrodactylum* in Butterfly and Muskrat Ponds although they were observed in all seven ponds and were the most abundant species in all ponds. Oak was the largest pond in surface area with the largest number of egg masses. Pancreas and Sedge were third and fourth largest in area but had greater numbers of RAAU and AMGR combined than Kidney, which was second in surface area. Kidney also had very little available vegetation in the center of the pond.

Table 4 – Pond Water Temperatures, February-April 2000

Temperatures were recorded hourly. Spirea and Butterfly Ponds almost dried at the end of March and temperature ranges were calculated excluding the interval when the data loggers were exposed to air.

	HOBO Data Logger Water Temperatures (Celsius)											
	February			March			April			May		
	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min
Butterfly	9.18	6.76	4.34	12.23	9.04	5.84	20.65	15.06	9.47	17.45	15.71	13.98
Kidney	8.52	7.18	5.84	13.98	10.73	7.48	19.94	15.64	11.34	20.36	17.41	14.45
Muskat	6.07	5.40	4.73	10.40	8.47	6.54	19.22	14.66	10.09	23.50	18.05	12.59
Oak	7.21	6.39	5.57	10.02	8.72	7.41	15.79	14.14	12.48	19.18	17.34	15.50
Pancreas	6.99	6.29	5.58	10.85	9.40	7.96	15.50	13.91	12.32	17.30	15.84	14.38
Sedge	7.04	6.28	5.52	10.28	8.75	7.22	16.18	13.28	10.38	19.70	16.33	12.96
Spirea	8.07	6.81	5.55	14.44	9.46	4.48	20.91	14.70	8.50	16.96	14.91	12.86

Exotic Vegetation Hypothesis

Based on Fall 1999 surveys, ponds varied over two-fold in the percentage of native vegetation cover. Ranked in descending order of percentage of native vegetation (in parentheses), the ponds were Kidney (88.9%), Muskrat (86.1%), Butterfly (51.4%), Pancreas (47.5%), Spirea (44.5%), Sedge (39.6%), and Oak (33.4%) (Figs. 14 - 17).

Analysis of oviposition substrate use by all of the amphibians (Table 5) showed a heavier than expected use of nonnative vegetation in over half the analyses and none showed a heavier than expected use of native vegetation (Table 6).

Of the five ponds in which RAAU was common, three (Muskrat, Sedge, and Oak) showed significantly greater use of nonnative vegetation (Figure 14). Pancreas and Spirea Ponds failed to reveal significant differences between native and nonnative substrate use.

Only one of two ponds analyzed for AMGR vegetation use, Kidney, showed significantly greater use of nonnative PHAR (60%) than would be expected, but this difference was not large.

Analysis of AMMA and HYPE vegetation brace use in Butterfly and Muskrat Ponds revealed significant use of nonnative braces in only Butterfly Pond (100%).

Both HYPE and AMMA used only PHAR in Muskrat Pond also but it was not statistically significant.

Table 5 – Amphibian Egg Mass Numbers, 2000 Reproductive Season

Ponds with less than 10 egg masses were not analyzed. These are all egg masses or packets that were attached to vegetation and not floating near vegetation.

Pond	Number of Egg Masses or Packets (n)			
	Raau	Amgr	Hyre	Amma
Butterfly	2	0	25	25
Kidney	3	15	0	0
Muskrat	20	1	25	25
Oak	124	3	0	0
Pancreas	18	23	0	0
Sedge	36	5	0	0
Spirea	10	1	0	0

Table 6 – Use versus availability of native versus nonnative substrates for each amphibian species, 2000 reproductive season

Species	Pond	Chi-Square	Sig
RAAU	Muskrat	47.5	*
	Oak	56.1	*
	Pancreas	3.4	
	Sedge	16.8	*
	Spirea	0.4	
AMGR	Kidney	8.5	*
	Pancreas	0	
HYPE	Butterfly	27.1	*
	Muskrat	4.8	
AMMA	Butterfly	16	*
	Muskrat	4.8	

Figure 14 – Native versus exotic substrate use for the northern red-legged frog
Percent of total egg masses found in native and nonnative categories was calculated and compared to the percent of vegetation available in each category. Ponds are shown alphabetically. Only Oak, Sedge and Muskrat Ponds revealed significant differences in used versus available vegetation.

Figure 14 – Native versus exotic substrate use for the northern red-legged frog (continued)

Percent of total egg masses found in native and nonnative categories was calculated and compared to the percent of vegetation available in each category. Ponds are shown alphabetically. Only Oak, Sedge and Muskrat Ponds revealed significant differences in used versus available vegetation.

Figure 14 – Native versus exotic substrate use for the northern red-legged frog (continued)

Percent of total egg masses found in native and nonnative categories was calculated and compared to the percent of vegetation available in each category. Ponds are shown alphabetically. Only Oak, Sedge and Muskrat Ponds revealed significant differences in used versus available vegetation.

Figure 14 – Native versus exotic substrate use for the northern red-legged frog (continued)

Percent of total egg masses found in native and nonnative categories was calculated and compared to the percent of vegetation available in each category. Ponds are shown alphabetically. Only Oak, Sedge and Muskrat Ponds revealed significant differences in used versus available vegetation.

Figure 15 – Native versus exotic substrate use for the northwestern salamander
Only two ponds had more than 10 egg masses and were available for analysis. Only Kidney Pond showed slightly more significant use of nonnative reed canarygrass than would be expected.

Figure 15 – Native versus exotic substrate use for the northwestern salamander (continued)

Only two ponds had greater than 10 egg masses and could be analyzed using Chi-square.

Kidney Pond showed slightly more significant use of nonnative reed canarygrass than would be expected.

Figure 16 – Native versus exotic substrate use for the long-toed salamander
Of the two ponds sampled for this species, only Butterfly pond revealed significant use of nonnative vegetation.

Figure 17 – Native versus exotic substrate use in the Pacific treefrog
Of the two ponds sampled for this species, only Butterfly pond revealed significant use of nonnative vegetation

Substrate Strength Hypothesis

Only five of the 19 plant species available in the study ponds were used for oviposition. My analysis of "strength" of the oviposition substrate revealed that each species differed significantly in mass, density, and diameter (Tables 7 and 8). In descending order of strength, the plants used for oviposition included Pacific willow (SALA), hardhack (SPDO), sedge (CAOB), water pepper (POsp.), and reed canarygrass (PHAR).

In three of the five ponds with adequate numbers of egg masses to analyze (Pancreas, Sedge, Oak), the northern red-legged frogs used the weaker reed canarygrass (PHAR) significantly more than would be expected based on its availability (Table 9). Only in Muskrat pond was the stronger substrate, water pepper (POsp.), used significantly more than expected (Table 10).

The northwestern salamander oviposited on only one species, PHAR, significantly more than expected in one out of three ponds (Kidney) analyzed (Sedge and Pancreas) (Table 11). Again, this was not the strongest species available.

Pacific treefrogs (Table 12) and long-toed salamanders (Table 13) laid eggs on PHAR significantly more than would be expected in Butterfly Pond. The long-toed salamanders also used sedge (CAOB – the third-ranked species in strength) in Butterfly Pond, but less than would be expected. Slightly more use of PHAR was

found in Muskrat Pond for both Pacific treefrog and long-toed salamanders (Table 9).

Tables 10-13 also show the substrate species ranks according to relative structural complexity. Most of the plants used for oviposition were substrates with higher complexity ranking, but use differences existed among amphibians. Northern red-legged frogs utilized the greatest variety of vegetation braces of the four species using single stems, single leaves, multiple stems, multiple leaves, and a combination of stems and leaves (Appendix J). Only six northern red-legged egg masses were found unattached and floating on the surface of the water. These floaters may have been knocked off by wave action, by an animal moving through the water, or simply detached as the eggs neared hatching. Of the 45 northwestern salamander egg masses found, 41 were on single stems, 1 was on two stems, and 3 were on multiple leaves (Appendix J). Forty-six of the 50 total small egg packets of long-toed salamanders and Pacific treefrogs sampled in Butterfly and Muskrat Ponds were found on either a single leaf or stem. The other four egg packets were found on multiple leaves or multiple smaller diameter stems but none were found on more than two braces (Appendix J).

Table 7 – Variation in oviposition substrate mass, density, and diameter

Variable	Stem	N	MEAN (g)	STD DEV	STD ERROR	95% CONFIDENCE INTERVAL FOR MEAN	MIN	MAX
MASS	PHAR	34	0.0615	0.0499	0.0086	0.0441 - 0.0789	0.01	0.20
	CAOB	10	0.1900	0.0963	0.0304	0.1211 - 0.2589	0.07	0.32
	SPDO	26	0.3296	0.1646	0.0423	0.2631 - 0.3961	0.05	0.73
	SALA	14	0.6914	0.5892	0.1575	0.3512 - 1.0316	0.10	1.94
	POSP	36	0.1258	0.5577	0.0093	0.1070 - 0.1447	0.03	0.27
	TOTAL	120	0.2231	0.2909	0.0266	0.1705 - 0.2757	0.01	1.94
DENSITY	PHAR	34	0.2118	0.8885	0.0152	0.1808 - 0.2428	0.10	0.51
	CAOB	10	0.1130	0.04644	0.0147	0.0798 - 0.1462	0.05	0.20
	SPDO	26	0.5238	0.09716	0.0191	0.4846 - 0.5631	0.34	0.72
	SALA	14	0.4950	0.1861	0.0497	0.3876 - 0.6024	0.25	0.99
	POSP	36	0.6269	0.2254	0.0376	0.5507 - 0.7032	0.17	1.08
	TOTAL	120	0.4288	0.2434	0.0222	0.3847 - 0.4728	0.05	1.08
DIAM	PHAR	34	0.1835	0.0716	0.0123	0.1585 - 0.2085	0.08	0.38
	CAOB	10	0.4600	0.1506	0.0476	0.3523 - 0.5677	0.20	0.70
	SPDO	26	0.2731	0.0827	0.0162	0.2397 - 0.3065	0.10	0.40
	SALA	14	0.4000	0.1922	0.0514	0.2891 - 0.5109	0.20	0.90
	POSP	36	0.1778	0.0540	0.0090	0.1595 - 0.1961	0.10	0.30
	TOTAL	120	0.2495	0.1370	0.0125	0.2247 - 0.2743	0.08	0.90

Table 8 – Comparison of oviposition substrate variation

ANOVA was used to compare the three measures used in the strength index within and among ponds.

Variable	Comparison	Sum of Squares	Degrees of freedom (df)	Mean Square	F	Significance Level
Mass	Between Ponds	4.604	4	1.151	24.224	< 0.0005
	Within Ponds	5.465	115	0.048		
	Total	10.069	119			
Density	Between Ponds	4.308	4	1.077	45.146	< 0.0005
	Within Ponds	2.744	115	0.024		
	Total	7.052	119			
Diameter	Between Ponds	1.108	4	0.277	28.267	< 0.0005
	Within Ponds	1.127	115	0.010		
	Total	2.235	119			

Table 9 – Use versus availability of specific oviposition substrates for each amphibian species, 2000 reproductive season

Comparison of the observed use of each substrate species to its expected use had the eggs been randomly placed. The expected value was calculated from the percentage of each substrate in the pond multiplied by the total number of egg masses. I adjusted the rejection criterion conservatively based on the number of tests for each species using Sidak's multiplicative inequality ($\alpha' = 1 - (1 - \alpha)^{1/n}$). Degrees of freedom based on how many substrate species were actually used. Substrate species not used were grouped into a collective category called "other" for each test.

Pond	Species	Observed Values		Critical χ^2	Degrees of freedom
		χ^2	Probability		
Butterfly	HYRE	27.64	< 0.005	5.025	1
	AMMA	16.64	< 0.005	7.378	2
Kidney	AMGR	2.21	0.9 > p < 0.1	5.025	1
Muskrat	RAAU	52.51	< 0.005	9.210	2
	HYRE	4.21	0.05 > p < 0.025	5.025	1
	AMMA	4.21	0.05 > p < 0.025	5.025	1
Oak	RAAU	52.24	< 0.005	9.210	2
Pancreas	RAAU	11.51	0.01 > p < 0.005	11.344	3
	AMGR	12.81	< 0.005	7.378	2
Sedge	RAAU	17.94	< 0.005	11.344	3
Spirea	RAAU	24.35	< 0.005	13.277	4

Table 10 – RAAU use of substrates ranked by strength and complexity

Species	Strength Ranking	Structural Complexity	Substrate Species	Number of Ponds Analyzed	Usage more than expected	Usage less than expected	Random Use
RAAU	5	1	Reed Canarygrass	5	3	0	2
	2	2	Hardhack	3	1	2	0
	3	3	Sedge	4	1	1	2
	4	4	Waterpepper	4	1	2	1
	1	5	Willow	4	1	2	1

Table 11 – AMGR use of substrates ranked by strength and complexity

Species	Strength Ranking	Structural Complexity	Substrate Species	Number of Ponds Analyzed	Usage more than expected	Usage less than expected	Random Use
AMGR	5	1	Reed Canarygrass	3	1	0	2
	2	2	Hardhack	2	0	1	1
	3	3	Sedge	2	1	1	0
	4	4	Waterpepper	3	1	0	2
	1	5	Willow	1	0	1	0

Table 12 – HYRE use of substrates ranked by strength and complexity

Species	Strength Ranking	Structural Complexity	Substrate Species	Number of Ponds Analyzed	Usage more than expected	Usage less than expected	Random Use
HYRE	5	1	Reed Canarygrass	2	2	0	0
	2	2	Hardhack	0	0	0	0
	3	3	Sedge	1	0	1	0
	4	4	Waterpepper	1	0	1	0
	1	5	Willow	1	0	1	0

Table 13 – AMMA use of substrates ranked by strength and complexity

Species	Strength Ranking	Structural Complexity	Substrate Species	Number of Ponds Analyzed	Usage more than expected	Usage less than expected	Random Use
AMMA	5	1	Reed Canarygrass	2	2	0	0
	2	2	Hardhack	0	0	0	0
	3	3	Sedge	1	0	1	0
	4	4	Waterpepper	1	0	1	0
	1	5	Willow	1	0	1	0

Cover Hypothesis

Mapping analysis revealed variation in vegetation cover types around pond margins and amphibian egg mass distribution (Appendix K shows individual pond maps). Tree cover around each pond ranged from 0% (Butterfly) to 76% (Pancreas)(Figure 18). Shrub cover ranged from 0% (Butterfly and Kidney) to 48% (Spirea), whereas herbaceous vegetated margins ranged from 13% (Spirea) to 100% (Butterfly) (Figure 18). In the nearest edge analysis of the distribution of northern red-legged frog egg masses, 0 – 89% of the egg masses were nearest tree cover, 0 – 80% were nearest shrubs, and 0 – 100% were nearest herbaceous cover (Figure 18). Comparison the percent of each cover type available to the nearest egg mass distribution percentages showed no significant differences (Table 14). Nearest edge analysis for northwestern salamander in Kidney, Pancreas and Sedge Ponds showed egg masses associated with tree cover 9 – 100%, shrub cover 0 – 40%, and herbaceous cover 0 – 91% (Figure 18). Similarly, no significant relationship was found between the distribution of the northwestern salamander eggs and available cover types.

Vegetation cover immediately around northern red-legged frogs and northwestern salamanders was also examined (Table 15). Vegetation cover around northern red-legged egg masses ranged from 10% (Pancreas) to 90% (Spirea). Northwestern salamander egg masses were found in vegetation cover ranging from

5% (Kidney) to 60% (Sedge). Percent cover of vegetation around egg masses with reed canarygrass averaged 56%, whereas similar cover around egg masses with hardhack was 77%, 43% for sedge, 48% for willow, and 23% for water pepper (Table 15). The distribution of vegetation cover within 15 cm of the egg masses was significantly different between northern red-legged frog and northwestern salamander (Figure 19).

The correlation analysis between the number of egg masses and the percentage of the three cover types and cover type combinations within and around the ponds showed no significant relationship (Table 16). However, when the marginal vegetation category analyzed as $< 50\%$ tree and shrub cover versus $\geq 50\%$ tree and shrub cover was compared to a high ($n \geq 10$) versus low ($n < 10$) number of northern red-legged frog egg masses, all ponds with few egg masses ($n = 2$) were in the lower tree and shrub cover category, whereas all ponds with many egg masses ($n = 10$) were in the high tree and shrub cover category. This result is significant (Fisher's exact test: $df = 1$, $\chi^2 = 7.00$, $p = 0.0082$). The parallel analysis done with within-pond vegetation did not show a significant result. Too few ponds had northwestern salamander present to do a parallel analysis for that species.

Figure 18 – Oviposition versus cover around pond edges for the northern red-legged frog and northwestern salamander
In the two subsequent graphs, “Eggs” on the x axis represent the percentages of pond margin vegetation in each structural category closest to egg masses; “Edge” represents the actual percentages of pond margin vegetation in each structural category.

Table 14 – Egg mass distribution versus pond edge cover, 2000 reproductive season

The following two tables describe the Chi-square analysis comparing the percentages of pond margin vegetation associated with egg masses versus pond margin vegetation available. Both of the latter are depicted in Figure 18.

RAAU			
Pond	Vegetation Category	Chi-square Value	Significance
Muskrat	Herb	0.06	
	Shrub	0.29	
	Tree	0.30	
Oak	Herb	0.36	
	Shrub	1.13	
	Tree	1.70	
Pancreas	Herb	0.92	
	Shrub	0.64	
	Tree	0.44	
Sedge	Herb	2.56	
	Shrub	0.78	
	Tree	1.10	
Spirea	Herb	1.34	
	Shrub	2.10	
	Tree	0.88	

AMGR			
Pond	Vegetation Category	Chi-Square	Significance
Kidney	Herb	1.73	
	Shrub	0.00	
	Tree	2.62	
Pancreas	Herb	4.10	*
	Shrub	0.67	
	Tree	1.50	
Sedge	Herb	0.01	
	Shrub	0.86	
	Tree	0.38	

Table 15 – Percentage of aquatic vegetation cover around egg masses, 2000 reproductive season

Percent vegetation refers to cover within 15 cm of individual egg masses.

Pond	Amphibian Species	Sample Size	Substrate	Percent Vegetation		
				Range	Mean	Mode
Kidney	AMGR	9	PHAR	< 5 - 50	19	5
	AMGR	6	PO.sp	5 - 90	20	5
	RAAU	2	PHAR	5 - 25	15	*
Muskrat	RAAU	7	PHAR	30 - 50	41	80
	RAAU	14	PO.sp	20 - 90	53	50
Oak	AMGR	3	PHAR	75 - 90	83	*
	RAAU	108	PHAR	10 - 95	62	80
	RAAU	7	SALA	20 - 75	50	20
Pancreas	AMGR	12	PHAR	10 - 80	32	10
	AMGR	7	PO.sp	5 - 20	11	10
	RAAU	1	CAOB	< 5	*	*
	RAAU	8	PHAR	15 - 60	34	20
	RAAU	4	PO.sp	< 5 -10	9	10
Sedge	AMGR	5	PHAR	40 - 60	56	60
	RAAU	1	CAOB	80	*	*
	RAAU	32	PHAR	10 - 90	55	50
Spirea	RAAU	7	PHAR	30 - 90	72	90
	RAAU	4	SPDO	60 - 90	77	*

*Indicates too few values existed to generate mode

Figure 19 – Variation in percent cover around oviposition sites for the northern red-legged frog and northwestern salamander.

Analysis of the means revealed significant differences in the mean percent cover found around the egg masses of each species ($z = -4.955$, $df = 1$, $p < 0.0001$).

Table 16 – Relationship of number of northern red-legged frogs egg masses and vegetation cover, 2000 reproductive season

	Comparison	Spearman's ρ Correlation Coefficient	Significance Level
Vegetation in Pond	Herb	-0.523	0.229
	Shrub	0.111	0.812
	Tree	0.433	0.331
	Tree/Shrub	0.523	0.201
	Herb/shrub	-0.433	0.331
Vegetation Margin	Herb	-0.500	0.253
	Shrub	0.595	0.159
	Tree	0.393	0.383
	Tree/Shrub	0.500	0.221
	Herb/Shrub	-0.393	0.383

Within Pond Percent Cover (From Pond Vegetation Avail Data)					
Pond	RAAU (n)	Herb	Tree	Shrub	sum
Butterfly	2	100.0	0	0	100.0
Kidney	3	100.0	0	0	100.0
Muskrat	20	99.9	0	0.1	100.0
Oak	118	97.0	3	0	100.0
Pancreas	19	97.7	0.4	1.9	100.0
Sedge	36	98.9	0	1.1	100.0
Spirea	10	90.3	0.1	9.6	100.0
Pond Margin Percent Cover (From Mapping Analysis)					
Pond	RAAU (n)	Herb	Tree	Shrub	sum
Butterfly	2	100.0	0	0	100.0
Kidney	3	60.0	40	0	100.0
Muskrat	20	23.0	51	26	100.0
Oak	118	18.0	46	36	100.0
Pancreas	19	21.0	76	3	100.0
Sedge	36	42.0	37	21	100.0
Spirea	10	14.0	38	48	100.0

Invertebrate and Fish Species Composition

Details of invertebrate sweep net surveys and the minnow trapping are provided in Appendices L and M. Some water column invertebrates (e.g., fairy shrimp and mayflies) were positively correlated with the abundance of northwestern salamander egg masses (Appendix L), so invertebrate abundance could explain the greater numbers of northwestern salamander egg masses in some study ponds. Microcrustacean invertebrates are known to be a key food source for young ambystomatid salamanders (Anderson 1967, Efford and Mathias 1969), and may be limiting northwestern salamander use of some ponds.

Since few fish were observed or sampled (Appendix M) and the lowest egg mass numbers were not from ponds in which the fish were found, I had no evidence that fish presence might be limiting oviposition.

Water Quality Data

The five water quality variables (temperature, pH, dissolved oxygen, depth, and turbidity) measured ranged widely both among and within ponds over time (Appendix N). A repeated measures ANOVA showed that all water quality variables differed significantly among the five ponds over time (Table 17). Figure 20 depicts the variation in the five water quality variables. None of the water

quality variables indicated conditions that might limit or deter amphibian oviposition in the study ponds.

Table 17 – Comparison of water quality variables among pond, February-April 2000
All tests in the following comparison were significant at $P < 0.001$.

Water Quality Variable	F Values of Effects		
	Time	Pond	Time * Pond Interaction
	(df 2)	(df 4)	(df 8)
pH	52	59	5
Temperature	3380	257	102
Turbidity	9	24	6
Dissolved Oxygen	37	86	13
Depth	30	15	11

Figure 20 – Water quality

V. Discussion

Vegetation

I was unable to consistently reject the directional null hypothesis that amphibians used native vegetation for oviposition at frequencies equal or greater than that of the exotic species present. I was able to reject this hypothesis in three ponds (Oak, Sedge, and Muskrat), but I failed to reject it in the remaining four (Table 6). Lack of consistent rejection indicates that vegetation status (i.e., exotic versus native) is likely not important in selecting an oviposition brace.

As exotic vegetation was often used more frequently than expected, and the dominant exotic species was reed canarygrass, I chose to examine the possibility that reed canarygrass and alternative oviposition substrates might differ in strength as a basis for amphibian selection of an oviposition brace.

Substrate Strength

The different vegetation braces used for oviposition differed significantly in overall strength. However, egg masses were not found with significantly greater frequency on stronger braces (Tables 10-13). I found no evidence to conclude that substrate strength, at least as I measured it, might be the basis for selecting of an oviposition substrate.

Cover

The most frequently used substrate, reed canarygrass, also differed in structure from the other brace species in having a more complex growth form (i.e., complex of radiating leaves on closely spaced stems). Stronger woody-stemmed species, such as hardhack and willow, were much less abundant or less complex. Sedge, like reed canarygrass, grows as a relatively dense vertical structure, but has a more limited lateral complexity. Water pepper has some lateral complexity, but typically spreads out more horizontally over the water surface, and has less of an emergent component than reed canarygrass.

Analysis taking structural complexity of each plant species into account did reveal significant patterns of use among plant species with differences in complexity (Tables 10-13). The rankings were based on visual observations and not actual measurements, but did provide a comparison between species. Structural complexity relates to local cover density. Egg masses and ovipositing adults may be more concealed, and thus, protected from predators in more complex vegetation. More refined examination of this idea, especially with experimental manipulation, is needed to determine whether this pattern is consistent.

Distribution of the egg masses of both northern red-legged frogs and northwestern salamanders within the ponds seemed somewhat clumped, but the nearest edge analysis did not reveal any significant associations between egg masses and the structural types of edge vegetation (Table 14). Nonetheless, the

mapping analysis revealed that the ponds with the most red-legged egg masses (i.e., > 10) all had $\geq 50\%$ marginal shrub and tree cover (Figure 18).

Reed canarygrass was the most abundant species providing dense cover when all ponds were considered. Northern red-legged frog egg masses were found more frequently in vegetation providing denser cover than northwestern salamander egg masses, therefore, they were frequently found in reed canarygrass. This difference may reflect differences in vulnerability resulting from differences in mating and oviposition behavior between the two species.

I found no significant correlation between the number of red-legged frog egg masses and the amount of cover within the pond. However, one might not expect such a relationship if certain threshold-cover conditions are required. A refined sampling approach may yet reveal such a pattern, and my finding that northern red-legged frogs showed greater oviposition in ponds with greater cover would seem to agree with this idea. Northern red-legged frog need for cover may be linked to their seasonal movements from densely vegetated upland habitats (active season, non-breeding) to aquatic habitats (breeding). Several studies have suggested that northern red-legged frogs may be cover sensitive, and a shrub or similar low structural matrix may be important (Aubry 1997, Ritson and Hayes 2000). More northwestern salamanders were found in the larger ponds, which also had a greater percentage of tree/shrub cover available, but the sample size was small. Other studies on amphibian reproductive sites have correlated reproduction

mainly with emergent vegetation (Monella and Wright 1999,)Thompson *et al.* 1980). However, the significance of emergent vegetation as an oviposition brace versus its ability to provide cover to egg-laying amphibians has typically not been distinguished. Therefore, significant association between egg mass distribution and emergent vegetation may yet be revealed through examination of vegetation density with careful attention to scale.

Other Habitat factors

Lack of relationship between egg mass numbers and different water quality variables suggests that the water quality variables may not influence oviposition over the range of conditions measured. Likewise, lack of relationship to fish suggests that fish were not important to oviposition in the systems examined. However, selection of those systems may be occurring because they are fish poor, a condition that has been repeatedly demonstrated elsewhere (e.g., Kats and Sih 1992, Holomuzki 1995). However, the pond sample sizes being small, water quality conditions being relatively benign, and fish presence being limited likely all restricted my ability to recognize water quality or fish effects, even if these existed. I would anticipate that conditions with more abundant fishes or more extreme water quality parameters might significantly influence oviposition.

The only other habitat factor I measured implying that a constraint on pond use might exist was water column invertebrates, the abundance of which was

correlated with abundance of northwestern salamander egg masses. Further investigation would be needed to determine just how water column invertebrates and oviposition conditions might be confounded for the northwestern salamander.

Implications and Conclusions

The original focus of this study was to examine whether exotic vegetation might be less favorable for oviposition among stillwater-breeding amphibians. My data suggest that vegetation status (exotic or native), at least in the manner I measured it, does not seem to influence selection of oviposition sites. Similarly, strength of the oviposition brace seems unrelated to oviposition site selection. The one factor that may play a role in oviposition site selection is the cover characteristics of the oviposition substrate. My seemingly inconsistent results related to vegetation cover, either at the level of oviposition substrate or where the oviposition site is located within the pond, may arise from several factors. Species response to cover may differ. My data showed that northern red-legged frogs selected oviposition sites that with significant more near-space cover than northwestern salamanders. This pattern needs further verification. If true, it is unclear whether denser cover close to the oviposition site is an advantage to ovipositing adults, to developing embryos in the egg mass, or both. Moreover, other studies have shown that northern red-legged frogs are highly cryptic dependent (Ritson and Hayes 2000), and in terrestrial habitats appear to be dependent on a complex, low structural matrix for cover (e.g., ferns: Aubry 1997).

Failure to demonstrate that marginal cover characteristic of the study ponds were spatially related to oviposition patterns may mean the cover characteristics of the aquatic habitat are unimportant for northern red-legged frogs. Alternatively, it may mean that either I did not measure the appropriate cover metric or measure cover at the appropriate scale. In either case, further investigation of cover is warranted.

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VII. Appendices

Appendix A - Burlington Bottoms Oviposition Study 2000

400 0 400 800 Meters

- Permanent Fish Slough
- Sedge Pond
- Pancreas Pond
- Oak Pond
- Muskrat Pond
- Spirea Pond
- Butterfly Pond
- Kidney Pond

Appendix B -- Vegetation Survey Effort

Pond	Dates	<u>Effort</u> (hr)	<u>Number of</u> #People	<u>Effort</u> (person * hours)
Oak	9/10/99	3.0	2	6.0
	9/16/99	2.0	2	4.0
Spirea	9/17/99	1.0	2	2.0
Butterfly	9/17/99	1.0	2	2.0
	9/24/99	1.0	2	2.0
Muskrat	9/17/00	1.0	2	2.0
	9/24/00	1.5	2	3.0
Kidney	9/24/00	2.0	2	4.0
Pancreas	10/1/99	2.5	3	7.5
Sedge	10/1/99	2.0	3	6.0
Totals	<u>5 days</u>	17.0	22	38.5

Appendix C: Survey Transects

Butterfly Pond Survey Transects

Appendix C- Survey Transects

Kidney Pond Survey Transects

Appendix C - Survey Transects

Muskrat Pond Survey Transects

Appendix C: Survey Transects

Oak Pond Survey Transects

Appendix C: Survey Transects

Pancreas Pond Survey Transects

Appendix C: Survey Transects

Sedge Pond Survey Transects

Appendix C; Survey Transects

Spirea Pond Survey Transects

Appendix D: Egg Mass/Water Quality Survey Effort

Pond	Dates	Effort (hrs)	Number of (#people)	Effort (person*hours)
Butterfly	2-Feb	2.3	6	13.5
	11-Feb	2.5	4	10.0
	21-Feb	1.0	1	1.0
	13-Mar	1.3	2	2.6
	27-Mar	1.0	3	3.0
	30-Apr	1.0	2	2.0
	Subtotal	6 days	9.1	32.1
Kidney	2-Feb	0.5	6	3.0
	11-Feb	3.0	4	12.0
	23-Feb	1.6	2	3.2
	10-Mar	0.8	2	1.5
	13-Mar	0.5	1	0.5
	14-Mar	0.2	2	0.3
	24-Mar	0.8	3	2.4
	4-Apr	1.0	2	2.0
	30-Apr	0.8	2	1.6
	Subtotal	9 days	9.1	26.5
Muskrat	3-Feb	0.6	5	3.0
	9-Feb	2.0	6	12.0
	3-Mar	2.6	4	10.4
	6-Mar	2.8	3	8.4
	30-Mar	0.3	1	0.3
	31-Mar	1.5	1	1.5
	1-Apr	0.2	1	0.2
	28-Apr	1.0	1	1.0
	Subtotal	8 days	11.0	36.8
Oak	19-Jan	2.0	2	4.0
	3-Feb	2.0	3	6.0
	14-Feb	6.5	2	16.0
	16-Feb	5.0	2	16.0
	25-Feb	1.3	2	2.6
	8-Mar	1.3	6	7.8
	10-Mar	3.0	3	9.0
	16-Mar	0.5	1	0.5
	17-Mar	0.3	1	0.3
	21-Mar	0.3	1	0.3
	22-Mar	0.3	1	0.3
	23-Mar	0.3	2	0.5
	4-Apr	1.0	2	2.0
	27-Apr	1.3	3	3.9
	28-Apr	0.3	1	0.3
	Subtotal	15 days	23.9	69.4
Pancreas	2-Feb	0.5	6	3.0
	11-Feb	2.5	4	10.0
	23-Feb	1.5	5	7.3
	1-Mar	1.0	2	2.0
	10-Mar	0.5	2	1.0
	14-Mar	0.8	2	1.6
	4-Apr	1.3	2	2.5
	30-Apr	2.0	2	4.0
	Subtotal	8 days	10.0	31.4
Sedge	27-Jan	1.6	4	6.4
	4-Feb	2.5	5	12.5
	16-Feb	1.8	6	10.8
	18-Feb	1.5	2	3.0
	16-Mar	0.6	1	0.6
	17-Mar	1.5	3	4.5
	21-Mar	0.3	1	0.3
	22-Mar	0.2	1	0.2
	23-Mar	0.3	2	0.6
	4-Apr	0.8	2	1.6
	27-Apr	0.8	3	2.4
	28-Apr	0.3	1	0.3
	Subtotal	12 days	12.1	43.1
Spirea	2-Feb	0.4	6	2.4
	11-Feb	1.5	3	4.5
	23-Feb	0.2	1	0.2
	1-Mar	0.1	1	0.1
	14-Mar	0.3	3	0.9
	27-Mar	0.2	3	0.5
	29-Mar	0.1	1	0.1
	30-Apr	0.8	2	1.6
	Subtotal	8 days	3.5	10.2
TOTALS	33 days	78.7		249.4

Appendix E: Mapping Vegetation and Pond Margins Effort

Pond	Dates	<u>Effort</u> (hr)	<u>Number of</u> #People	<u>Effort</u> (person * hours)
Muskrat	3/06/00	1.5	2	3.0
Sedge	3/17/00	2.0	3	6.0
Oak	3/10/00	3.0	3	9.0
Pancreas	3/24/00	1.0	3	3.0
Kidney	3/24/00	1.0	3	3.0
Spirea	3/30/00	0.5	2	1.0
Butterfly	3/30/00	0.5	2	1.0
Totals	<u>5 days</u>	9.5	-	26.0

Appendix F: Data Sheet Used for Egg Mass Surveys

Burlington Bottoms Amphibian Oviposition Survey Data Sheet															Page ____ of ____	
Date:	Begin Time:	Begin AT (C):	End AT (C):	Ref Map:												
Pond:	End Time:															
Observers			Wind:													
Weather:			Water Level:													
	Polar coord:	Water	Distance	Plant	Dia/L Ax	S Axis	Density	Veg								
Time	Pole	Dist	off N	#	Stage	L Axis	S Axis	Height	Depth	to Mass	Sbstr	Sbstr	mm	mm	WT	Notes
0:00	M	deg				mm	mm	mm	cm	cm	mm	mm	mm	mm	%	C
1																
2																
3																
4																
5																
6																
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Species Codes: RAJU=Kana aurea, AMGR=Ambystoma gracile, HYRE=Hyla regilla, AMMA=Ambystoma macrodactylum

PI substrate Codes: PHAR=Red Canary Grass, SALA=Pacific Willow, SPDO=Spiron douglasii, CAQB=Carex obnupta, CAAP=Carex speta, POHY=Polygonum hydropiperoides

POHYR=Polygonum hydropiper, POPE=Polygonum perfoliatum, ELAC=Eleocharis acicularis, ELPA=Eleocharis palustris, FEAR=Festuca arundinacea, PACA=Panicum capillare, GASP=Gallium spp., SAGLA= Sagittaria latifolia, SPDM=Sparganium angustatum, LUPA=Ludwigia palustris, BICE=Bidens cernua

Appendix G: Protocol for Egg Mass Surveys

Protocol for Egg Mass Data Collection	
1. When first arriving at pond, assign jobs to observers,	
a. Data recorder - in charge of data sheets	
b. Data collectors - 1 person for mapping, spp id, staging, and egg mass measurements 1 person for Plant substrate, Dia substrate, Density sample, Vegetation Density and WT	
c. Mapper - in charge of compass, tape measure, giving angles and distances to data recorder	
2. Review protocols before beginning- Review maps and place and measure poles for polar coordinates	
3. Data recorder fill out top of sheet while others getting equipment ready	
4. Begin systematic survey of pond:	
5. When egg mass has been located:	
1. Notify data recorder, record time.	
2. Take measurements and observations in order they occur on data sheet	
3. Mark R.L. and N.W. egg masses with flagging on vegetation within 10 cm at water surface.	
Write the number of the egg mass from data sheet on flagging before tying on	
5. After entire pond has been surveyed: record end time and air temp on data sheet.	
Data and Measurement Protocol	
1. Time: Record time in hours and min (24 hrs) Ex. 13:30	
2. Pole: Give the compass direction label from the pole from which egg mass is being measured from.	
3. Distance: Use measuring tape to record distance from pole to center of egg mass in nearest 1/10 of M	
4. Degrees off North: Set the plastic compass to North on center of pole using magnetic compass	
Then use the plastic compass to read the angle of the measuring tape.	
5. #: number each egg mass consecutively for each pole. Record number on flagging marking egg mass: ex. NE1, NE2, SW5, SE1	
6. Species: Record species of egg mass using codes at bottom of data sheet. If not sure on HYRE and AMMA then take sample.	
7. Stage: Record stage of egg mass using Gosner chart. Always record modal condition (range if possible).	
8. Long Axis mm: Measure the long axis of the top of the egg mass in mm	
9. Short Axis mm: Measure the short axis of the top of the egg mass in mm	
10. Height mm: Measure the height of the egg mass in mm at tallest point	
11. Water Depth cm: Using measuring pole with white circular base, place pole in water until base is at bottom of water column (at point where you begin to hit muck)	
12. Distance to mass in cm: Measure the distance from the surface of the water to the top of the attachment part of egg mass.	
13. Plant sbstr: Record the plant spp the egg mass is attached to. If more than one species record both. Make note. Use four letter abbreviations provided.	
14. Dia/Laxis Sbst mm: the diameter of the substrate at bottom of the egg mass; If Leaf/group of leaves then record length of long axis	
15. Dia/Saxis Sbst mm: Measure the length of short axis of leaf/group of leaves if sbstr is not a stem/twig	
16. Density Sample: 10 per sbstr type per pond. Cut at least 10 cm long sample of same veg sbstr as attachment sbstr. Place in labeled plastic bag: pond, date, spp.	
17. Veg Density %: Estimate the cover of veg in water around the egg mass within a 60 cm diameter circle.	
18. WT in C: Water temp in Celsius using digital thermometer to nearest 0.1 C	
19. Notes: Record special measurements, conditions, observations	
ex. Predation on egg mass, loose egg mass, unfertilized eggs or deceased embryos, more than one plant substrate, egg mass on top of veg clump.	

[illegible]

Appendix I: Plant Species Addressed in This Study

Common Name	Scientific Names	Code	Status ¹	Structural Group ²	Macrohabitat ³
Beggar's ticks	<i>Bidens cernua</i>	BICE	N	H	T/E
Columbia sedge	<i>Carex aperta</i>	CAAP	N	H	E
Slough sedge	<i>Carex obnupta</i>	CAOB	N	H	E
Needle spikerush	<i>Eleocharis acicularis</i>	ELAC	N	H	E/T
Ovate spikerush	<i>Eleocharis ovata</i>	ELOV	N	H	E/T
Creeping spikerush	<i>Eleocharis palustris</i>	ELPA	N	H	E/T
Common horsetail	<i>Equisetum arvense</i>	EQAR	N	H	T
Tall fescue	<i>Festuca arundinacea</i>	FEAR	I	H	T
Bedstraw	<i>Galium spp.</i>	GAsp	N	H	T
Pointed rush	<i>Juncus acuminatus</i>	JUAC	N	H	T/E
Water purslane	<i>Ludwigia palustris</i>	LUPA	I	H	F/S
Witch grass	<i>Panicum capillaris</i>	PACA	N	H	T/E
Reed canarygrass	<i>Phalaris arundinaceae</i>	PHAR	I	H	E/T
Waterpepper, smartweed	<i>Polygonum spp.</i>	POsp	N	H	E/T
Wapato	<i>Sagittaria latifolia</i>	SGLA	N	H	E
Pacific willow	<i>Salix lasiandra</i>	SALA	N	T	E/T
Nightshade	<i>Solanum dulcamara</i>	SODU	I	H	T
Simple-stem burreed	<i>Sparganium emersum</i>	SPEM	N	H	E
Hardhack or Douglas' spirea	<i>Spirea douglasii</i>	SPDO	N	Sh	E/T

¹ N = native, I = introduced, nonnative² H = Herbaceous, T= Tree, Sh= shrub³ E = Emergent, F= Floating, S = Submerged, T = Terrestrial

Note: Data for this table are based on a combination of (Hitchcock and Cronquist 1973, Guard 1995, Cooke 1997)

Appendix J –Attachment Brace Variation

Direct counts of egg masses found on the different vegetation brace types regardless of plant species. Combinations of stems and leaves were abbreviated with “S” for stem and “L” for leaf. The number of letters represents the number of stems or leaves found within the egg mass.

Attachment Brace Variation for RAAU Egg Masses

Pond	# Attached																# Not Attached
	Single		Multiple														
	Leaf	Stem	Leaves			Stems			Leaves and Stems								
			2	3	4	2	3	4	6	SL	LLS	SSL	LLLS	SSSL	SLLLL	SSSSL	
Butterfly		1	1														
Kidney		2															
Muskrat		18				1	1										
Oak	5	44	4	1		27	12	7	1	3	1	1	2	1	1		5
Pancreas	2	11															
Sedge	1	11	3	1	1	8	3	2		2	2					1	1
Spirea		8	1			1											

Attachment Brace Variation for AMGR Egg Masses

Pond	# Attached															# Not Attached	
	Single		Multiple														Attached
	Leaf	Stem	Leaves			Stems			Leaves and Stems								
			2	3	4	2	3	4	6	SL	LLS	SSL	LLLS	SSSL	SLLLL	SSSSL	
Butterfly																	
Kidney		14		1													
Muskrat		1															
Oak		3															
Pancreas		21	1														
Sedge		2	1			1											
Spirea																	

Attachment Brace Variation for AMMA Egg Masses

Pond	# Attached															# Not Attached	
	Single		Multiple														
	Leaf	Stem	Leaves			Stems				Leaves and Stems							
			2	3	4	2	3	4	6	SL	LLS	SSL	LLLS	SSSL	SLLLL		SSSSL
Butterfly	5	15	2			1				1							floating
Muskrat	4	24															

Attachment Brace Variation for HYRE Egg Masses

Pond	# Attached																# Not Attached
	Single		Multiple														
	Leaf	Stem	Leaves			Stems			Leaves and Stems								
			2	3	4	2	3	4	6	SL	LLS	SSL	LLLS	SSSL	SLLLL	SSSSL	
Butterfly	7	12	2	1	1	1	1										
Muskrat	5	15		3						1							

Appendix K: Pond Oviposition Sites

Butterfly Pond Oviposition Sites

Appendix K - Pond Oviposition Sites

Kidney Pond Oviposition Sites

Appendix K - Pond Oviposition Sites

Muskrat Pond Oviposition Sites

Appendix K: Pond Oviposition Sites

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Oak Pond Oviposition Sites

Appendix K: Pond Oviposition Sites

Pancreas Pond Oviposition Sites

Sedge Pond Oviposition Sites

Appendix K - Pond Oviposition Sites

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Spirea Pond Oviposition Sites

Appendix L: Macroinvertebrate Sampling Results

These surveys were done the week of 27 March to the 4 April 2000. The ponds are listed alphabetically. Macroinvertebrates collected from the sweep net surveys were identified to the family level. The number of different morphospecies was totaled for each pond. Muskrat had the greatest number of species (12) while Sedge pond had the fewest (5). The other ponds had between 8 and 10 species. Grouping the macroinvertebrates by feeding type (herbaceous versus predator species) showed that all of the ponds had greater than 69% macroinvertebrate herbivores present. Fairy shrimp (Order Crustacea: Eubranchipodia) and snails (Gastropoda) were the most frequent macroinvertebrates encountered in all the ponds. The presence of copepods and cladocera were noted but a relative count was not obtained. A comparison of the numbers of macroinvertebrates to the number of northwestern salamander egg masses revealed only significant correlations for water column species like fairy shrimp and mayflies.

Sweep Net Survey Results								
Pond	Predators	Herbivores	# Morphospecies	Dominant Species (> 10%)	Count	Percent	Total Count	Other MorphoSpecies Groups
Butterfly	2%	98%	8	Eubranchipoda (fairy shrimp)	570	85%	671	Gastropoda, Ephemeroptera, Isopoda, Limnephillidae, Arachnida, Elmidae, Hirudinidae
Kidney	32%	69%	9	Eubranchipoda (fairy shrimp)	9	32%	28	Corixidae, Tipulidae, Hemiptera, Chaoboridae, Dytiscidae
				Gastropoda (snail)	7	25%		
				Elmidae (beetle)	4	14%		
				Simuliidae (Diptera)	3	11%		
Muskrat	11%	89%	12	Eubranchipoda (fairy shrimp)	181	44%	416	Limnephillidae, Chaoboridae, Chironomidae, Dytiscidae, Elmidae, Ephemeroptera, Hydra, Sialidae, Simuliidae
				Gastropoda (snail)	107	26%		
				sow bugs	63	15%		
Oak	9%	91%	8	Eubranchipoda (fairy shrimp)	101	68%	149	Isopoda, Limnephillidae, Simuliidae, Chaoboridae, Elmidae, Hirudinidae
				Gastropoda (snail)	28	19%		
Pancreas	19%	81%	10	Gastropoda (snail)	32	38%	84	Halipidae, Limnephillidae, Tipulidae, Chaoboridae, Dytiscidae, Elmidae, Hemiptera, Bactidae
				Eubranchipoda (fairy shrimp)	27	32%		
Sedge	2%	98%	5	Eubranchipoda (fairy shrimp)	80	46%	175	Dytiscidae, Limnephillidae
				Gastropoda (snail)	45	26%		
				sow bugs	39	23%		

Appendix M: Minnow Trap Results

All the traps were place for 24 hour cycles on the dates listed. Three traps were placed in randomly spaced locations throughout the pond each day. All ponds where fish were caught are connected to a permanent slough. Minnow traps captured only two peamouth (*Mylocheilus caurinus*), one each in Kidney and Pancreas ponds respectively, and several bullfrog (*Rana catesbeiana*) tadpoles in Oak and Sedge ponds (Table 20). A dead three-spine stickleback (*Gasterosteus aculeatus*) was observed and collected during sweep netting in Kidney pond. I observed another dead three-spine stickleback along the shore of Kidney Pond during egg mass sampling. I also observed one mosquito fish (*Gambusia affinis*) in Pancreas pond while surveying for egg masses. These results agree with the fact that the ponds where nothing was trapped were isolated and temporary in comparison to Oak, Kidney, and Pancreas, which had beaver channels connecting them to a small, permanent slough.

Minnow Trap Results										
Water Depth of Traps and Contents										
Date	Pond	Time		cm	Species	cm	Species	cm	Species	Notes
		In	Out	#1	Present	#2	Present	#3	Present	
10-Mar	Kidney	915	1530	42	0	45	0	35	0	5 prs of Mallards on pond when I arrived
13-Mar	Kidney	915	1530	42	0	45	0	35	0	5 prs of Mallards on pond when I arrived
24-Mar	Kidney	1332	1400	30	1 Minnow (peamouth)	30	0	35	0	
11-Feb	Muskrat	1300	1300				AMGR juvenile, AMMA adult	NA		
31-Mar	Muskrat	1300	1330	37	0	20	0	30	2 HYRE Tadpoles	Tadpoles immediately swam out when trap lifted
1-Apr	Muskrat	1330	1330	37	3 Dytiscidae	20	0	30	0	
3-Feb	Oak	1330	1320	36	0	19	0	28	0	
17-Mar	Oak	1100	1100	55	0	65	0	57	0	
21-Mar	Oak	1414	1148	55	0	65	0	57	1 RACA Tadpole -	no measurements taken on tadpole
23-Mar	Oak	1415	1415	47	0	50	1 adult beetle (Dytiscid family)	44	2 RACA tadpoles	#1HT 7.5 cmTL 5.0 cm; #2HT8.0cm, TL5.2cm
28-Apr	Oak	1040	1408	55	2 RACA tadpoles	58	1 snail	NA	0	Two baited traps tried
11-Feb	Pancreas	1415	1420	47	0	50	1 RACA tadpole	44	1 RACA Tadpole -	#2HT8.0cmTL5.0cm#3HT9.0cmTL5.5cm
10-Mar	Pancreas	949	1600	40	0	50	0	40	0	Some caddis fly larva in traps
14-Mar	Pancreas	1351	1409	28	1 Minnow (peamouth)	50	0	22	0	Length of fish - 56 mm , Width - 14 mm
17-Mar	Sedge	1012	1207	46	0	32	1 snail, 3 Caddis fly larva (Trichopteras)	40	1 adult beetle (Dytiscid family)	
22-Mar	Sedge	1012	1335	37	0	49	0	55	0	
23-Mar	Sedge	1335	1448	37	0	49	0	55	0	
28-Apr	Sedge	1150	1350	68	10 RACA tadpoles	50	2 RACA tadpoles	NA	0	Two baited traps tried
11-Feb	Spirea	1448	1350	37	0	49	0	55	0	Water levels dropped too low to do anymore

Note: Time Out is 24 hours later than date listed

Appendix N: Hydrolab Water Quality Measurements

Hydrolab Water Quality Measurements												
Date	Pond	Weather	Time	Depth	Water Temp C		pH		D.O.		Turbidity NTU	
			2400hrs	m	Average	Range	Average	Range	Average	Range	Average	Range
2/11	Spirea	100% clouds, wind 1	951-1043	1-3	5.98	5.5-6.2	6.34	6.25-6.43	22.91	15.6-29.3	96.10	28-120
2/21	Butterfly	100% Cloud cover, Wind 0, light drizzle	1237-1315	2-4	6.81	6.6-7.0	6.44	6.1-6.56	21.74	11.6-33.4	34.20	25-50
2/21	Muskat	100% cloud cover, wind 0-1	1115-1202	1-4	6.007	5.44-6.44	6.77	6.52-6.97	17.12	10.6-28.1	23.56	8-100
2/23	Kidney	50% cloud, wind 1	1451-1630	2-4	10.866	10.1-11.6	6.67	6.55-6.72	70.70	57.4-74.9	NA	86-500+
2/23	Pancreat	50% cloud, wind 1	1438-1604	3-8	9.65	8.8-10.4	6.63	6.44-6.79	57.25	45-69	NA	NA
2/25	Oak	100% clouds, light rain, Wind 1	1411-1528	3.5-8	5.83	8.8-10.4	6.29	6.15-6.55	44.49	18-69	23.20	19-32
2/25	Sedge	100% cloud, light precip, wind 0-1	1556-1644	3-5	5.56	4.8-6.8	6.31	6.19-6.47	24.02	17-30	13.70	10-16
3/31	Muskat	Clear, Wind 1	1330-1500	16-37	12.96	8.8-10.4	6.62	6.37-6.73	21.26	6-32.6	30.90	0-81
4/4	Kidney	100% cloud, wind 1	1342-1437	18-3	13.81	16.3-18.7	6.44	6.21-6.63	56.33	59-80.5	208.10	17-28
4/4	Oak	100% cloud cover, wind 1, slight rain	1000-1100	3-71	13.29	10.6-14	6.12	5.81-6.44	38.67	19.1-77.5	24.40	11-71
4/4	Pancreat	90% cloud, wind 0.5	1449-1527	22-48	13.69	12.7-14.2	6.40	6.33-6.48	43.62	31.7-55.3	55.30	9-224
4/4	Sedge	100% Cloud, sprinkles, wind 0	1135-1228	18-3	13.55	10.3-12.2	6.13	5.92-6.37	15.12	7.7-29.2	23.50	10-78
4/27	Oak	100% clouds, wind 0	920-1040	35-75	14.21	5.1-6.4	6.03	5.79-6.32	25.53	10.9-42	24.70	11-57
4/27	Sedge	95% cloud, wind 1-2	1100-1153	49-64	6.41	13.6-14.2	6.16	5.94-6.29	36.22	26-50.2	12.10	5-36
4/28	Muskat	100% cloud, wind 1, light precip	1548-1645	21-36	14.03	12.7-14.2	6.73	6.43-6.84	33.65	15.7-52.2	33.30	12-48
4/30	Butterfly	10% cloud, wind 1-3 gusts	1432-1528	34-6	17.67	16.3-18.7	6.36	6.21-6.63	68.34	59-80.5	20.20	17-28
4/30	Kidney	10% cloud, wind 1-3 gusts	1552-1646		23.19	22.2-23.7	6.53	6.36-6.85	99.56	90-131	125.20	59-214
4/30	Pancreat	10% cloud, wind 1-3 gusts	1556-1747	26-47	18.57	17.5-19	6.20	6.05-6.29	65.13	50.2-80.6	43.30	11-148
4/30	Spirea	10% cloud, wind 1-3 gusts	1340-1428	3-5	14.22	13.7-14.7	6.30	6.18-6.41	59.24	55.6-64.1	18.10	15-33