DETERMINING HABITAT CHARACTERISTICS THAT PREDICT OVIPOSITION

SITE SELECTION FOR POND-BREEDING NORTHERN RED-LEGGED FROGS

(*RANA AURORA*) IN HUMBOLDT COUNTY, CALIFORNIA

By

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We certify that we have read this study and that it conforms to acceptable standards of
scholarly presentation and is fully acceptable, in scope and quality, as a thesis for the
degree of Master of Arts.

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ABSTRACT

In species where offspring are restricted to neonatal habitats, fitness is dependent upon oviposition site selection by females, a concept often referred to as the preference-performance hypothesis. Before determining if this hypothesis can be applied to a particular species, it is necessary to know what habitat characteristics influence site selection. My study assessed which habitat characteristics are preferred for oviposition in *Rana aurora* (Northern Red-legged Frog) in northern Humboldt County, California. I had two objectives: (1) to determine what habitat characteristics predict oviposition site use by *R. aurora*, and (2) to identify what characteristics predict the density of egg masses at a pond. This study took place at 30 ponds across northern Humboldt County, California, which were visited weekly from December-April during the 2007-2008 breeding season. Pond characteristics assessed were number of egg masses, water temperature, pond area, canopy cover, emergent vegetation, floating vegetation, woody vegetation, and presence/absence of other amphibians and fish.

There was a higher probability of egg masses being found in ponds with relatively low canopy cover, and greater densities of egg masses occurred in ponds with high levels of emergent and floating vegetation. These findings are supported by previous studies that show that *R. aurora* attach their egg masses to emergent vegetation and tadpoles use this vegetation as a nursery. Both of these results suggest that female *R. aurora* are actively selecting sites to increase offspring performance, but more research will be needed to determine if offspring survival is affected.

ACKNOWLEDGEMENTS

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INTRODUCTION

The ability of an individual to survive from one life stage to the next is dependent, in part, on the quality of the surrounding habitat. The decision parents make for their offspring's first habitat influences the survival of their offspring (Kaplan 1998). For example, temperature and predators impact survival of offspring in oviparous species (Chivers et al. 2001; Kolbe and Janzen 2002; Johnson et al. 2003; Lloyd and Martin 2004). The consequences of parental decisions affect offspring survival with ultimate consequences for parental fitness.

The preference-performance hypothesis, also known as the "mother knows best" principle, states that parents select oviposition sites to optimize fitness of their offspring (Jaenike 1978; Thompson 1988). This hypothesis was originally proposed for phytophagous insects with juvenile stages that often lack the capability to relocate (Jaenike 1978; Johnson et al. 2006), but it has also been applied to soil-dwelling insects, birds, and reptiles (Kolbe and Janzen 2002; Lloyd and Martin 2004; Johnson et al. 2006). Selective pressure to choose high quality sites should be greatest on species that exhibit oviparity and lack parental care (Crump 1991; Garcia-Gonzales and Gomendio 2003). When organisms leave their eggs unattended they are relying on the habitat to provide protection and food for their offspring. By contrast, parental care gives individuals a chance to increase their offspring's survival even if parents are forced into poor habitats.

The preference-performance hypothesis has only recently been applied to amphibians. Amphibians, like phytophagous insects, have several life stages as they transition from

embryos to adults. Depending on the species, these stages, such as salamander larvae or anuran tadpoles, are often incapable of changing habitats (Duellman and Trueb 1994; Kaplan 1998). Many amphibians also lack parental care (Kaplan 1998) and thus a strong selective pressure should exist to maximize their fitness by selecting optimal oviposition sites. To date, only one study has explicitly tested the preference-performance hypothesis on amphibians (Reigler 2004). In that study, survival of *Hyla femoralis* (Pinewood Treefrog) tadpoles was higher when adults oviposited in predator-free environments. Other studies have explored oviposition site selection in amphibians (e.g., Resetarits 1996), but none of these studies related their results to the preference-performance hypothesis nor determined offspring survival or estimated parental fitness.

A critical assumption of the preference-performance hypothesis is that an individual has the ability to choose oviposition sites. One potential contraindication of applying the preference-performance hypothesis to amphibians is that many amphibians exhibit high levels of breeding site fidelity (philopatry), which implies that individuals of such species may not be capable of selecting oviposition sites (Duellman and Trueb 1994; Hayes et al. 2001; Pope and Matthews 2001; Matthews and Preisler 2010). However, philopatry is not static. Studies have shown that the intensity of philopatry can vary among species and even individuals in a population (Smith and Green 2005; Semlitsch 2008). There may be a continuum of philopatric behavior from extremely philopatric to highly vagile (Semlitsch 2008). Site fidelity varies, not only by individual, but also by habitat type. High site fidelity has been linked with stable ponds in late successional stages, while low site fidelity is associated with new ponds in early successional stages (e.g., open canopy) (Semlitsch 2008).

Several studies have measured philopatry in *Rana sierrae* (Sierra Nevada Yellow-legged Frog, formerly called *Rana muscosa*). Populations of *R. sierrae* have been rapidly disappearing from lakes in protected forests of the high Sierra Nevada. The local extirpation of this species from several basins has been correlated with the introduction of non-native trout for recreational purposes (Knapp and Matthews 2000; Knapp et al. 2007). This species exhibits very high site fidelity, which is thought to be due to the tadpoles' reliance on water for two to four years before metamorphosing, and the short period of movement due to winter snow packs (Matthews and Preisler 2010).

Although herpetologists have long thought that oviposition site choice in frogs is restricted by high levels of site fidelity, new research is showing that amphibians are capable of moving greater distances and have larger home ranges than previously realized, and are able to recolonize areas that have been restored to high quality sites. Despite the small size of frogs and their need for moisture, radio telemetry studies have shown that frogs can travel several kilometers, even over land (Matthews and Pope 1999; Pope and Matthews 2001; Hayes et al. 2001; Bulger et al. 2003; Fellers and Kleeman 2007). Long term monitoring of these movements provides strong evidence for large home ranges that include multiple over-wintering and breeding sites (Matthews and Pope 1999; Pope and Matthews 2001). Such movements suggest that amphibians are able to survey their habitats and take note of changes in their environment. Anecdotal evidence for this comes from an experiment in northwestern California in which canopy cover surrounding a pond was removed, and within a few years there was increase in numbers of *Ambystoma gracile* and *Rana aurora* that used the pond for breeding (Ryan Bourque, pers. comm.). Direct evidence for recolonization following

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improvement in habitat quality comes from a nine year study on *Rana sierrae* (Knapp et al. 2007). Multiple studies have looked at various effects of the experimental removal of trout from roughly a dozen alpine lakes in two basins located in Kings Canyon National Park. In these studies there was a quick recolonization by *R. sierrae* in lakes previously occupied by fish (Knapp et al. 2001; Vredenburg 2004; Knapp et al. 2007). Such research supports the notion that though amphibians exhibit site fidelity, they are capable of long distance movements and may be selecting ponds based on habitat quality.

Before the preference-performance hypothesis can be tested on any particular species of amphibians, one must determine what constitutes a high quality oviposition site for that species. For my study, I investigated the oviposition habitat preferences of *Rana aurora* (Northern Red-legged Frog) in northern Humboldt County, California. I chose *R. aurora* for my subject species because it is a fairly common local species, although there are many areas throughout its range (northern California north to Vancouver, BC) where populations have been declining (Pearl 2005). *Rana aurora* is listed as a California Species of Special Concern because of habitat loss due to urban development (DFG 2006). Thus, determining what types of habitats that it prefers for oviposition could help in future conservation efforts for the species. Information about this species is limited, taken from individuals in the northern portion of the range, and/or extrapolated from its sister species, *Rana draytonii*, and thus more data are needed to better understand this species, especially in the southern portion of its range. *Rana aurora* are aquatic breeders that lay their eggs in a wide range of aquatic habitats including marshes, ponds, lakes and reservoirs (Stebbins 2003). Females exhibit no parental care and lay one egg mass per season with clutch sizes averaging 530-830 eggs/mass

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(Pearl 2005). Egg masses are located on emergent vegetation, in shallow waters with an average water depth between 50 and 100 cm (Storm 1960; Pearl 2005). The tadpoles hatch after 10-30 days of development, after which they rely on dense aquatic vegetation for a protective foraging area (Pearl 2005). Known predators of tadpoles and juveniles are various fish and amphibians (e.g., *Gambusia affinis*, Mosquitofish, and *Lithobates catesbeianus*, American Bullfrog) (Calef 1973; Gamradt and Kats 1996; Kiesecker and Blaustein 1997 1998; Goodsell and Kats 1999; Kiesecker et al. 1999, 2001; Lawler et al. 1999).

The objectives of my study were two-fold: (1) to determine what habitat characteristics predict oviposition site use by *R. aurora*, and (2) to elucidate what characteristics predict the density of egg masses at a pond. The second objective was included because density of egg masses can increase competition amongst offspring and thus impact survival of young. Water temperature was monitored since it can influence developmental rates. Colder temperatures decrease metabolic rates, which consequently impact growth rates of egg masses and tadpoles (Bachmann 1969). Slower growth rates lead to smaller individuals which increases the chance for predation (Bachmann 1969; Voss 1993). Another potentially influential characteristic is water depth because it can impact the survival of egg masses and tadpoles, as both these life stages require a minimum amount of water for survival (Duellman and Trueb 1994; Pearl 2005). Other habitat characteristics that could affect embryo survival include canopy cover, amount of emergent vegetation, presence or absence of other amphibians, and the presence or absence of fish. Canopy cover can impact water temperature by decreasing the amount of sunlight reaching the pond, and thereby influencing developmental rate and survival of embryos. Decreased canopy cover

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leads to increased sunlight, which allows for more algal growth, a primary food source for tadpoles (Mallory and Richardson 2005). The amount of emergent vegetation also impacts survival because *R. aurora* females attach their egg masses to emergent vegetation in shallow waters (average water depth: 50-100 cm) (Storm 1960; Pearl 2005). Tadpoles also rely on vegetation as a protective foraging area, providing them cover from predators such as other amphibians and fish (Werschkul and Christensen 1977; Lawler et al. 1999; Pearl 2005).

By monitoring multiple ponds over the course of one breeding season, I assessed multiple habitat characteristics and conducted egg mass surveys to gain a better understanding of the correlation between pond habitat composition and oviposition for *R. aurora*. This study provides information that will allow for future research to determine if the breeding behavior in *R. aurora* operates under the preference-performance hypothesis. Results of this study will determine what pond characteristics will be indicators of a high quality site and, therefore, allow for increased offspring survival and parental fitness for a species in need of conservation.

MATERIALS AND METHODS

Study sites

My study was conducted during the winter of 2007-2008 (December-April) using 30

permanent (N=17) and temporary (N=13) ponds located in northern Humboldt County,

California (Figure 1; Appendix A). Eleven ponds contained fish. Twenty of the ponds were

natural; the rest were manmade. Pond area was measured by tracking the perimeter of the

pond using a Garmin GPS Map 60 (Garmin Ltd. 2010). Ponds ranged in size from 7.5 to

6105 m^2 (mean = 926.8 m²) and elevation ranged from 5 m to around 500 m, with

surrounding habitat varying from residential yards to dense redwood forests. Ponds were

located on lands owned or protected by Humboldt Bay National Wildlife Refuge, Green

Diamond Resource Company, City of Arcata, and private landowners. I selected ponds

based on permission of landowners and accessibility.

Monitoring water temperature

Throughout the breeding season, water temperature was measured using HOBO® data

loggers (Onset Computer Corporation, Bourne, MA), each deployed in a watertight

container. I situated data loggers just below the water surface about 1 m from the northern

shoreline to maintain consistency across ponds in regards to the amount of sunlight received

throughout the day (Pearl 2005).
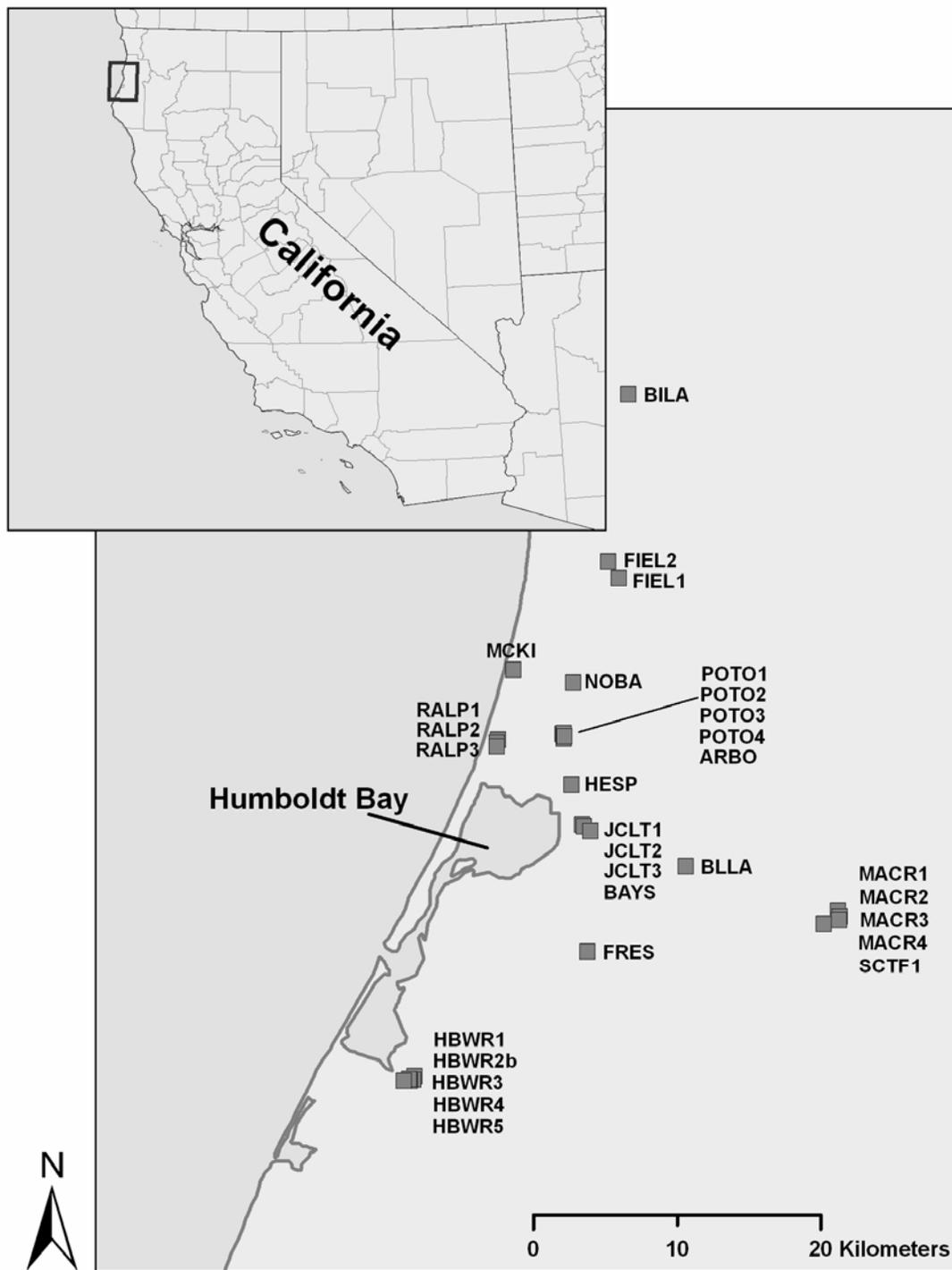
Figure 1. Map of study sites along the northern California coast in Humboldt County. Squares represent study ponds that are labeled with pond abbreviation names. (Courtesy of Judd Patterson.)

During the 2007-2008 breeding season I was only able to record water temperature at 22 of the 30 ponds due to an insufficient number of data loggers. Of those, data from nine loggers were unusable because all or a significant portion of the data were inaccurate, either because water levels dropped (thereby loggers measured air rather than water temperature) or batteries died between visits. To obtain water temperature data for all ponds, I installed loggers in all 30 ponds for 2-3 weeks in February 2009, which is the peak oviposition period for *R. aurora*. Egg masses were present during the same 2-3 week period in both 2008 and 2009. All ponds monitored in 2007-2008 showed similar daily mean temperatures throughout the season, with only a small increase during the warmer months (Appendix A). There was no significant difference between the two years (p=0.239) when daily mean water temperatures were compared between 2008 and 2009. Therefore, only 2009 daily mean water temperature data were used in further analyses.

Fish sampling

Sampling for fish occurred at the beginning (December 2007/January 2008) and end (April) of the *R. aurora* breeding season. Before egg masses were laid, I used seine nets for sampling shallower ponds (maximum depth < 3 m). I divided each pond into quadrants based on cardinal directions and randomly selected two of the four quadrants for seining. Minnow traps were used for sampling deeper or heavily vegetated ponds. Two to six baited (canned cat food) minnow traps were positioned in random areas of the pond and left out for three hours. The number of minnow traps used per pond was proportional to the size of the

pond, and the locations of traps were chosen by flipping a coin and using a grid system. After egg masses hatched, I sampled for fish using minnow traps baited with hot dogs (because cat food fell through holes in traps). Since minnow traps proved to be more effective than seine nets at capturing fish and were easier to deploy, they were the sole method used for sampling at the end of the breeding season.

Captured fish were temporarily stored (maximum 1 hour) in 5-gallon buckets that were filled with local pond water, and supplied with oxygen using battery-powered aerators. Fish were anesthetized prior to handling using CO_2 (one-quarter tablet Alka Seltzer® Gold per five gallons of water) and total length (TL) of each fish was measured (Page and Burr 1991). When over 200 fish were caught in a trap, I sub-sampled by measuring every fifth fish (of the same species) in order to minimize the time the fish remained outside of the pond. Handling of each fish took less than one minute. After handling, fish were placed in a recovery bucket filled with aerated local pond water and were released after a recovery period of at least five minutes. The minnow traps also captured various species of amphibians (including *R. aurora* adults and tadpoles). I recorded the number of each species caught per trap.

All handling protocols were approved by Humboldt State University's Institutional Care and Use Committee (Protocol # 07/08-B.33-A) and the California Department of Fish and Game (Scientific Collection Permit # SC-9794).

Egg mass surveys

All ponds, except those at Humboldt Bay National Wildlife Refuge, were visited weekly from 1 December 2007 to 10 April 2008, weather permitting. Ponds at the refuge were visited every two weeks in order to minimize the disturbance to waterfowl populations during the hunting season. If it was raining or the ponds were frozen, visits were postponed because rain reduces visibility, and breaking the ice would have drastically decreased the water temperature below the surface. There were only two surveys postponed due to ice and this only affected eight ponds. Visits postponed due to rain were frequent in January and February 2008, often occurring once or twice a week. But in other months, rain delays were sporadic, happening only four or five times a month, affecting only a few surveys instead of most.

During each survey I searched ponds for egg masses by carefully walking line transects through the pond. I flagged and numbered all egg masses, while also taking note of the presence of fish and other amphibians (of any life stage).

Determining pond vegetation and canopy cover

I determined the percent vegetation for each pond by first visually dividing the pond into quadrants, using cardinal directions, in order to obtain four values to average. For each quadrant, I estimated the percentage of the pond surface area that contained woody vegetation (stumps, logs, trees, etc.; WOOD), percent floating vegetation (FVEG), and percent emergent vegetation (EMVEG). I subtracted the sum of these three estimates from 100 to obtain percent of open water. Average percent emergent vegetation was chosen as a

variable because *R. aurora* attaches its egg masses to emergent vegetation, although there are

also some instances when extremely dense floating vegetation is used for oviposition (pers.

obs.). Woody debris and open water were included as categories that represented areas

where oviposition does not occur.

Average percent canopy cover was estimated using a spherical densiometer. Percent

canopy cover is the proportion of an area (in this case, pond surface) shaded by the vertical

projection of the tree canopy (Jennings et al. 1999). The number of canopy cover

measurements made was proportional to the size of the pond. I took only one measurement

for ponds smaller than 100 m², two measurements for ponds between 100 and 1000 m², and

three measurements for ponds greater than 1000 m². Measurements were taken where the

greatest density of egg masses appeared. If a pond did not contain any egg masses, then

either the middle of the pond (if accessible) or an area that was most representative of the

surrounding habitat was selected.

Statistical analysis

All statistical tests were conducted using the statistical package R (R Development

Core Team 2010). To assess if any habitat characteristics differed between occupied and

unoccupied ponds, I used binary multiple regression to test for significant relationships

between the presence/absence of egg masses and the following explanatory variables:

average percent emergent vegetation, sum of percent emergent vegetation and floating

vegetation, average percent woody vegetation, average daily water temperature, average

percent canopy cover, presence/absence of other amphibians, and presence/absence of fish

(Table 1). Pearson's correlations were used to determine relationships between variables.

To determine habitat characteristics that impacted the density of egg masses per pond,

I first transformed the total number of egg masses using the natural log in order to meet

assumptions of normality. For this analysis, only ponds with at least one egg mass were

included (N=24). I used *a priori* model selection and inference. *A priori* model selection is a

contemporary statistical tool that is similar, in nature, to ANOVA and multiple linear

regression (MLR) (Burnham and Anderson 2002). It allowed me to compare models based

on weights calculated from corrected Akaike Information Criterion (AIC$_c$) values. The best

fitting model is the one with the lowest AIC$_c$ and fewest variables. The area of a pond

impacted the total number of egg masses, so it was included in all models. Having area in

Table 1. List of predictor variables used in analysis.

Objective 1: Predicting presence or absence of egg masses at a pond (N = 30).

Average water depth	WD
Average percent emergent vegetation	EMVEG
Sum of percent emergent vegetation and floating vegetation	EMFVEG
Average percent woody vegetation (e.g., logs)	WOOD
Average daily water temperature (2-3 wks 2009) (°C)	TEMP
Average percent canopy cover	CC
Presence/absence of other amphibians (any life stage)	AMPHI
Presence/absence of fish	FISH

Objective 2: Habitat characteristics potentially impacting the density of egg masses (N = 24).

Pond Area (m^2)	AREA
Sum of EMVEG and FVEG	EMFVEG
Average water depth	WD
Average daily water temperature (2-3 wks 2009)	TEMP
Average percent canopy cover	CC

each model and the response variable as the natural log of the total number of egg masses

allows the habitat variables to be assessed in terms of density (Table 2). Density could not be

used as a response variable because normalizing density (taking the natural log) produces

negative values, because density is expressed as a decimal. Models 1, 2, 3, and 4 include the

individual variables plus pond area. Models 5, 6, 7, and 8 include interaction terms that

could impact numbers of egg masses. I also chose models to minimize the effect of

potentially confounding factors, such as canopy cover and water temperature.

Table 2. *A priori* models with the response variable as the natural log of the total number of
egg masses. (see Table 1 for explanation of variables)

Model	Covariates
1	EMFVEG + AREA
2	CC + AREA
3	TEMP + AREA
4	WD + AREA
5	EMFVEG + CC + AREA
6	TEMP + EMFVEG + AREA
7	EMFVEG + CC + WD + AREA
8	EMFVEG + CC + TEMP + WD + AREA

RESULTS

Egg mass surveys

Egg masses were found in 24 of the 30 ponds, with individual ponds containing from 0 to 526 masses (mean=85) (Appendix B). The mean density was 0.1 egg masses/m² (± 0.2) for all ponds, and 0.2 egg masses/m² (± 0.2) for those ponds that contained at least one egg mass. Eight of the 30 ponds had densities greater than 0.2 egg masses/m², with the highest at ponds MACR2 (0.661/m²), NOBA (0.551/m²), and BAYS (0.532/m²) (Appendix B).

Previous studies state that *R. aurora* attach their egg masses to emergent vegetation (Pearl 2005), however, during my study I observed *R. aurora* regularly attaching egg masses to floating vegetation. Though I did not record the specific types of vegetation used, the common emergent vegetation included cattails (*Typha spp.*), Tule (*Scirpus acutus*) and horticulture plants. The two most common floating plants (excluding Duckweed, which is not dense enough to hold an egg mass) were Parrot Feather (*Myriophyllum aquaticum*) and Hydrocotyle (*Centella asiatica*). Both of these create dense floating mats, thereby increasing the number of oviposition sites and shelter for tadpoles. However, there are trade offs as some of these plants are invasive and can take over a pond (Ruiz-Avila and Klemm 1996).

I observed the presence of a water-mold (*Saprolegnia* sp.) on several egg masses in pond POTO 1. *Saprolegnia* is transferred from fish and may lethally attack amphibian egg masses (Kiesecker *et al.* 2001, pers. obs.); it has infected *Pseudacris regilla* and *R. aurora* egg masses at other locations in Humboldt County (Jamie Bettaso, pers. comm.). Photographs of infected egg masses were sent to a local biologist (Jamie Bettaso) who

conjectured that the water mold was *Saprolegnia* by the general effects this genus has on egg

masses. Infected egg masses had embryos that were clumped together in the center of the

mass with multiple embryos turned gray or white. In several cases, only the center of the egg

mass was infected and I observed that the uninfected regions were still able to develop and

hatch as tadpoles. I did not follow the tadpoles to determine their survival, but embryonic

survival for infected egg masses varied from 50-80% (pers. obs.).

Predicting the presence/absence of egg masses

The only variable that showed a significant relationship with the presence or absence

of egg masses was average percent canopy cover (Table 3). Egg mass presence was more

probable in ponds that had low average percent canopy cover (p<0.000; adj R^2=84.4) (Figure

2).

Table 3. Results from binary multiple regressions using presence/absence of an egg mass
as the response variable. Adjusted R^2: 84.29

Variable	Estimate of Coefficient	Standard Error	T-value	P value
WD	0.0008	0.0022	0.351	0.729
EMVEG	-0.0012	0.0024	-0.513	0.613
EMFVEG	-0.0005	0.0021	-0.238	0.814
WOOD	0.0019	0.0045	0.436	0.667
TEMP	-0.0495	0.0447	-1.108	0.281
CC	-0.0126	0.0016	-8.119	0.000
AMPHI	-0.0931	0.0697	-1.336	0.196
FISH	0.1135	0.0720	1.577	0.130
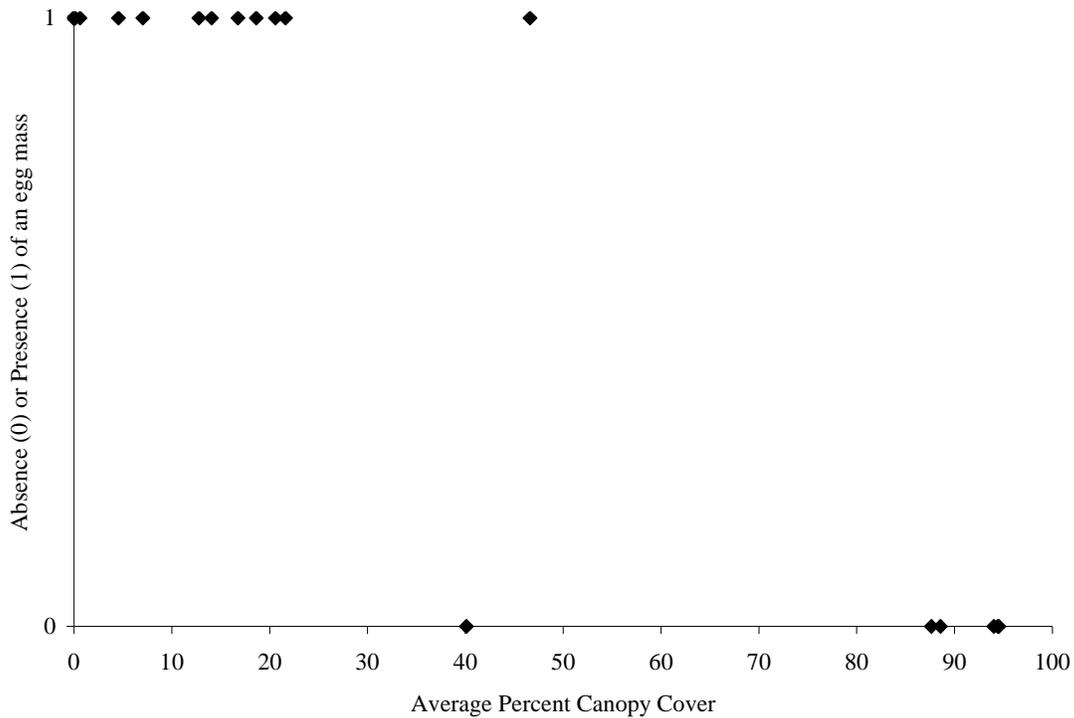
Figure 2. The relationship between egg mass presence/absence and canopy cover (N=29 ponds).

Water temperature did not vary significantly (p=0.281) between ponds with egg masses (mean = 8.37 °C, std = 0.68) and those without them (mean = 8.98 °C, std = 0.82) during the 2-3 week peak period of oviposition measured in 2009 (Figure 3). There was no relationship (p=0.196) between presence of egg masses and the presence or absence of other amphibians (AMPHI) (Table 3), nor was presence of *R. aurora* affected by the presence/absence of fish (p=1.000).

Impacts on egg mass density per pond

The best-fitting model for explaining the number of egg masses per pond was EMFVEG + AREA (AIC$_c$ = 86.501, w$_i$ = 54.7%, Table 4). The ln-transformed number of egg masses increased with both EMFVEG and pond area (adj R^2 =48.2, P=0.0187, Figure 4). The weight of the best-fitting model indicates a 54.7% probability that the model represents the true information from the data, assuming the best-fitting model can be found among the candidate models (Burnham and Anderson 2002). Due to this high probability, no model averaging was conducted.
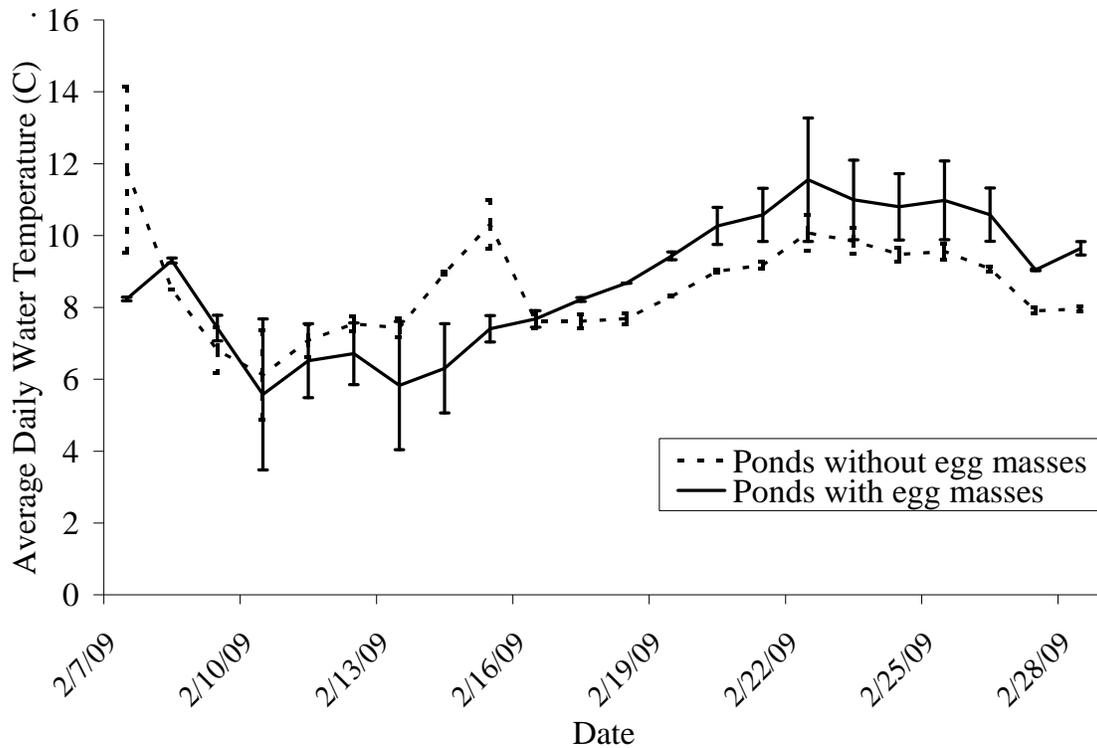
Figure 3. Temperature (°C) variation (measured in 2009) in ponds with and without egg masses. Egg mass presence was assessed in 2008.

Table 4. *A priori* models with the response variable as the natural log of the total number of egg masses.

Covariates	Residual Standard Error	adj R^2	P-value	DF	AIC_C[a]	Likelihoods	w_i[b]
EMFVEG+AREA	1.270	0.417	0.001	21	86.501	1.000	0.547
EMFVEG+CC+AREA	1.278	0.410	0.004	20	88.858	0.308	0.168
EMFVEG+TEMP+AREA	1.294	0.395	0.007	20	89.455	0.228	0.125
EMFVEG+CC+WD+AREA	1.282	0.407	0.007	19	91.359	0.088	0.048
WD+AREA	1.425	0.267	0.015	21	92.002	0.064	0.035
CC+AREA	1.426	0.266	0.015	21	92.042	0.063	0.034
TEMP+AREA	1.426	0.266	0.015	21	92.057	0.062	0.034
EMFVEG+WD+CC+TEMP+AREA	1.301	0.389	0.014	18	94.834	0.016	0.008

[a]Corrected Akaike Information Criterion

[b]Akaike Weights
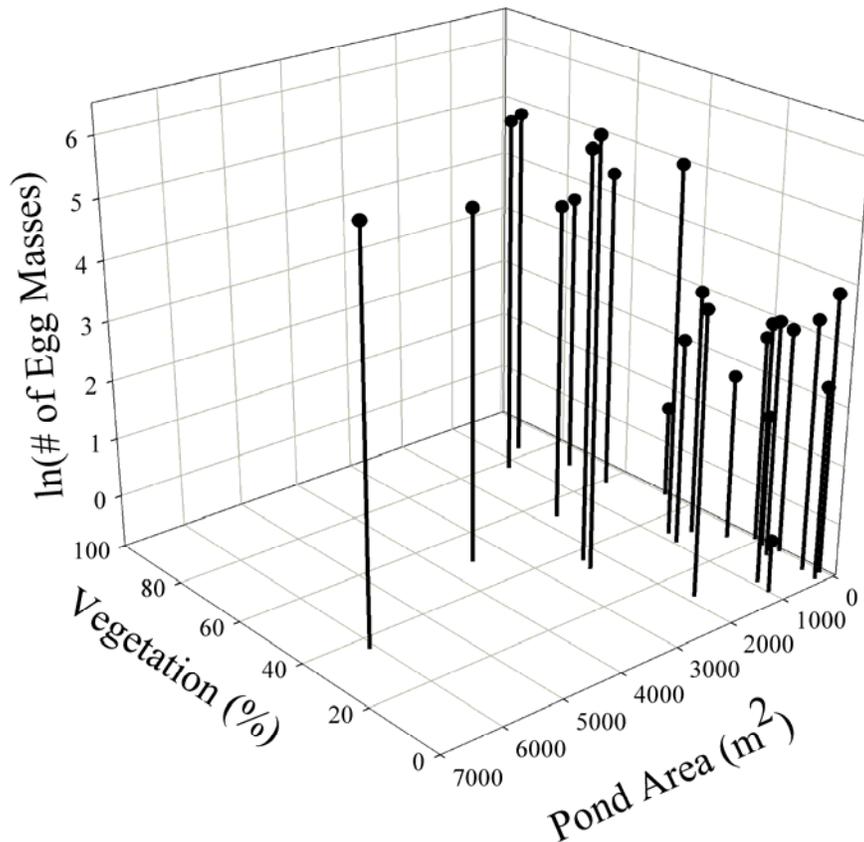
Figure 4. Three dimensional graph of the natural log of the total number of egg masses and the percent of emergent/floating vegetation and pond area (m²).

Other observations

       Fish were found in 11 of the 30 ponds, with a total of seven species observed or caught in minnow traps (Appendix C). Goldfish (*Carassius auratus*), Koi (*Cyprinus carpio*), and Mosquitofish (*Gambusia affinis*) were more common in residential ponds than elsewhere. Three-spine Stickleback (*Gasteroulus aculeatus*) was the most abundant and widespread overall, being found in seven of the 11 ponds containing fish. The abundance of fish captured per pond varied immensely. In JCLT2, only one Mosquitofish was caught out of two minnow traps, but in RALP3 pond, three minnow traps caught a total of 892 Stickleback.

       Most study ponds contained at least one other species of amphibian (Appendix C). These were Coastal Giant Salamander (paedomorph) (*Dicamptodon tenebrosus*), Pacific Chorus Frog (*Pseudacris regilla*), Rough-skinned Newt (adults only) (*Taricha granulosa*), and Northwestern Salamander (*Ambystoma gracile*). Though I did not record exact numbers, I did note the general abundance of *A. gracile* egg masses at each pond and categorized them accordingly: 0=0 egg masses, 1=1-5 egg masses, 2=5-10 egg masses, 3=greater than 10 egg masses. *Ambystoma gracile* oviposited more often in ponds with deeper water ($p < 0.001$) and higher canopy cover ($p = 0.007$). There was no relationship between the relative number of *A. gracile* egg masses and the total number of *R. aurora* egg masses ($p=0.850$) but there was a marginally significant negative relationship between the relative number of *A. gracile* egg masses and the presence/absence of *R. aurora* egg masses ($p=0.057$).

DISCUSSION

Oviposition site selection has been shown to affect offspring survival in numerous species of insects, birds, reptiles, and amphibians (Kolbe and Janzen 2001; Mezquida 2004; Garcia-Gonzalez and Gomendio 2003; Reiger et al. 2004; MacCracken 2007). Before the impact of oviposition preference on offspring performance can be measured in *Rana aurora,* one must first determine what habitat characteristics females prefer for oviposition. Various studies have shown the influence of one or more of the following factors on pond choice for oviposition: presence of predators, amount of vegetation cover, water temperature, amount of UV-B radiation, substrate type, hydrology (i.e., desiccation risk), pH, amount of food available for offspring, and density or presence of conspecifics (Crump 1991; Sadinski and Dunson 1992; Duellman and Trueb 1994; Moore and Townsend 1998; Dillon and Fiaño 2000; Halloy and Fiaño 2000; Orizaola and Brana 2003; Palen et al. 2005; Waltson and Mullin 2007). My study examined what habitat characteristics might influence a female *Rana aurora* in her choice of pond for oviposition.

Predicting presence/absence of egg masses

Percent canopy cover was negatively related to the presence of egg masses. Increased canopy cover reduces light levels, which can potentially decrease water temperature and vegetation growth. There was no evidence that water temperature was correlated with presence or absence of egg masses, though I did not measure water temperature for all ponds for the entire period of oviposition. A recent study showed a link between increased canopy

cover and decreased survival of *Ascaphus truei* (Pacific Tailed Frog) tadpoles due to a

reduction in the density of algae, the tadpoles' food source (Mallory and Richardson 2005).

The study demonstrated that high artificial canopy cover decreased the amount of sunlight

hitting ponds, causing less algae to grow. The higher number of egg masses oviposited in

ponds with decreased canopy cover may represent an assessment by females of increased

food availability for tadpoles. It is possible that percent canopy cover may influence water

temperatures beyond the period of my temperature measurements. Cold temperatures slow

embryonic development, leading to smaller individuals more susceptible to predation

(reviewed by Bachmann 1969; Voss 1993; Duellman and Trueb 1994) or perhaps pond

drying.   Both of these possible effects of canopy cover (algal productivity, temperature)

suggest that when females select a pond for oviposition, they not only select habitat

characteristics to increase embryonic survival, but also tadpole survival.

Many species of amphibians, including *Rana aurora*, have shown a preference for

oviposition sites that lack fish (Kats and Sih 1992; Hopey and Petranka 1994; Pope and

Matthews 2001; Blaustein et al. 2004; Egan and Paton 2004; Hazell et al. 2004; Reiger et al.

2004; MacCracken 2007; Waltson and Mullin 2007). Fish are predators of adults and

tadpoles, and some fish species eat egg masses as well (Kiesecker and Blaustein 1998;

Lawler et al. 1999; Monello and Wright 2001). In one study, *Lithobates sylvaticus* (formerly

Rana sylvatica; Wood Frog) and *Ambystoma maculatum* (Spotted Salamander) both

oviposited more frequently in ponds with lower numbers of fish (Egan and Paton 2004). My

finding that presence of fish correlated with the presence egg masses was unexpected.

However, this result was most likely based on the locally-specific low risk of predation. The

fish caught in the study ponds were mainly Three-spine Stickleback and Mosquitofish, both of which have body lengths less than the body size of adult *R. aurora* (Moyle 2002). Small body length is associated with a small gape size, which places a limitation on prey size (Moyle 2002). FIEL 2, MCKI, and BAYS were the only ponds that had fish large enough to eat tadpoles (Appendix C). Of these three ponds, *R. aurora* egg masses were only sighted at BAYS pond. The absence of egg masses at the other two ponds cannot be definitively attributed to the presence of fish, however, because these ponds also had high levels of canopy cover, which has shown to be negatively correlated with presence of egg masses.

Many studies have shown that the presence of other amphibians, or even conspecifics, can influence oviposition site preference in frogs (Crump 1991; Dillon and Fiaño 2000; Halloy and Fiaño 2000; Matsushima and Kawata 2005). My results showed that the presence/absence of *Rana aurora* egg masses was not correlated with the presence/absence of *Ambystoma gracile* adults or egg masses, or presence/absence of other amphibians. However, my data suggest that *A. gracile* egg masses are found more often in ponds with deeper waters (p < 0.001) and higher percent canopy cover (p = 0.007) than those used by *Rana aurora*. These two species also frequently share the same ponds for oviposition, and both attach their egg masses to emergent vegetation, but *A. gracile* egg masses are found below the surface and *R. aurora* egg masses float near the surface (Pearl 2005; Shaffer 2005; pers. obs.).

This study did not reveal a relationship between the presence/absence of *Rana aurora* egg masses and pond temperature during the peak of oviposition (Figure 3). This result contrasts with studies on other frog species showing temperature influences oviposition site

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selection. The lack of a temperature effect in my study appears attributable to modest

variation in temperature among ponds, and the fact that all ponds achieved the minimum

temperature for *R. aurora* oviposition of 6 °C (Pearl 2005) (Appendix A). However, more

complete temperature records covering the entire developmental period might yield a

different conclusion about the influence of temperature on site selection.

Impacts on egg mass density per pond

The density of egg masses was influenced by average percent emergent and floating

vegetation and pond area. The amount of aquatic vegetation has been shown to impact

oviposition site preference in other amphibian species. For example, in a study monitoring

four species of pond-breeding amphibians, all species laid more egg masses in ponds with

higher levels of vegetation (Hazell et al. 2004). Since *R. aurora* attach their egg masses to

emergent vegetation, ponds with greater amounts of vegetation can hold more egg masses.

The preference for high amounts of emergent and floating vegetation suggests that female *R.*

aurora might select oviposition sites that increase survival probability for embryos and

tadpoles. A secure, permanent position for embryonic development increases chances for

survival (Licht 1974; Kiesecker and Blaustein 1998; Pearl 2005). Once the embryos hatch,

tadpoles rely on vegetation for shelter from predators.

Conclusion

My study demonstrates that *Rana aurora* prefer to oviposit in ponds with low levels

of canopy cover and that a greater density of egg masses is associated with ponds with higher

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percentages of emergent and floating vegetation. Lower canopy cover levels have been

shown (in other studies) to increase food for tadpoles and increased levels of vegetation

provide more oviposition sites and shelter for tadpoles. These results show that females are

choosing oviposition sites with specific habitat characteristics, those which provide the

necessary elements for high embryonic survival, which, in turn, affects tadpole survival.

The next step in this research is to determine if these characteristics affect offspring

performance. If they do influence offspring performance, then the preference-performance

hypothesis can be applied to *Rana aurora*.

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Appendix A: Graph of water temperature from 24 of the 30 ponds during the 2007-2008 breeding season. The bolded lines represent those ponds with greater than 40% canopy cover. Vertical, dashed lines represent the 2-3 weeks in February that correspond to water temperature measurements taken in 2009.
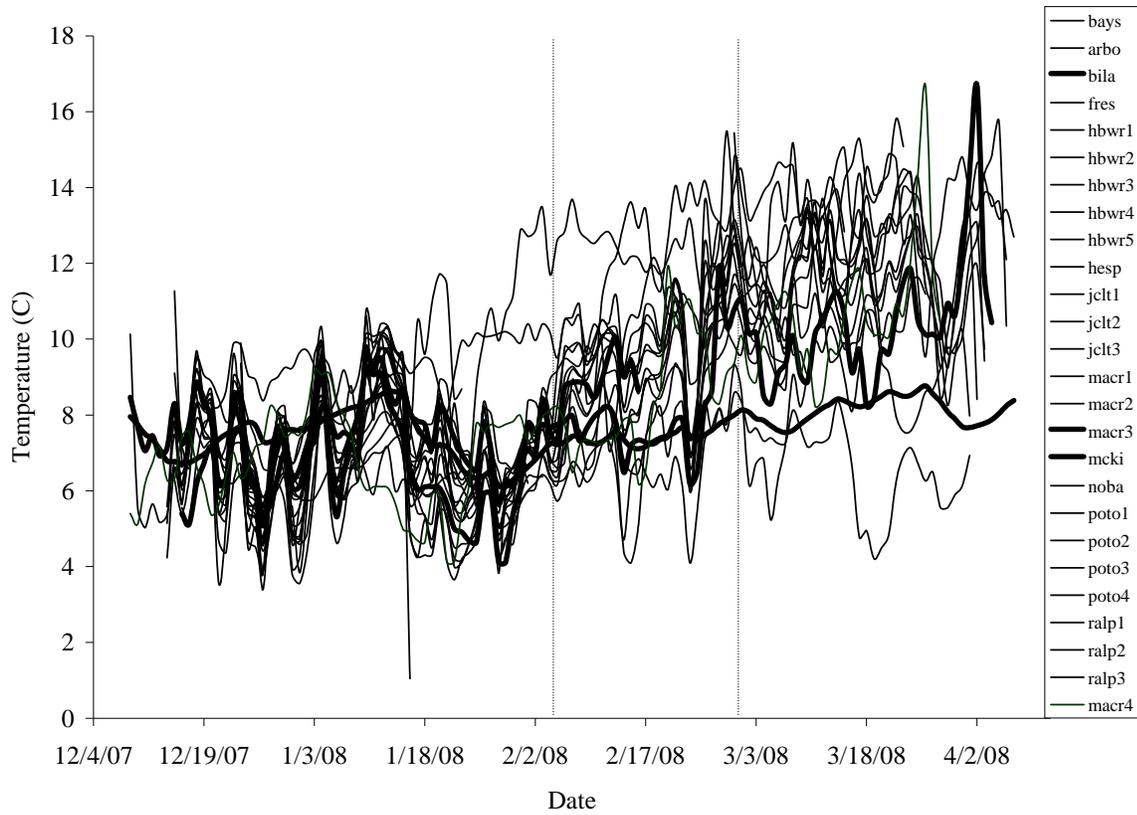
Appendix B: Background information and raw data for statistical analysis for the 30 ponds.

Pond	Location	UTM E	UTM N	Natural	Hydrology	Habitat	Elevation (m)	Area (m^2)	Avg Water Depth (cm)
ARBO	Arcata, CA	408558	4527442	no	permanent	residential	9	28.3	64.17
BAYS	Bayside, CA	409843	4521466	no	permanent	residential	2	26.3	32.63
BILA	Big Lagoon	413033	4551501	yes	permanent	cleared forest	240	563.4	66.03
BLLA	Blue Lake, CA	417077	4518589	yes	permanent	forest	350	308.7	39.73
FIEL1	Fieldbrook, CA	412499	4538534	yes	permanent	forest	75	400.0	60.20
FIEL2	Fieldbrook, CA	411655	4539841	yes	permanent	forest	67	631.0	43.57
FRES	Freshwater, CA	410220	4512611	no	permanent	cleared forest	30	913.0	64.00
HBWR1	Loleta, CA	398162	4503868	yes	seasonal	open field	-3	1008.9	35.17
HBWR2	Loleta, CA	398071	4503623	yes	seasonal	open field	-1	6105.4	45.40
HBWR3	Loleta, CA	397809	4503558	no	seasonal	open field	-2	849.8	38.60
HBWR4	Loleta, CA	397792	4503685	no	seasonal	open field	-3	2336.0	28.37
HBWR5	Loleta, CA	397424	4503525	yes	seasonal	open field	-4	256.0	23.30
HESP	Arcata, CA	409096	4524227	no	seasonal	residential	6	3537.0	29.27
JCLT1	Bayside, CA	409946	4521344	yes	seasonal	residential	5	92.9	18.70
JCLT2	Bayside, CA	409970	4521333	yes	seasonal	residential	6	293.0	23.17
JCLT3	Bayside, CA	410392	4521005	no	permanent	open field	8	2426.6	58.46
MACR1	Maple Creek, CA	427661	4515097	no	permanent	cleared forest	440	1732.1	54.03
MACR2	Maple Creek, CA	427681	4515469	no	seasonal	cleared forest	445	69.6	17.47
MACR3	Maple Creek, CA	427797	4515079	yes	seasonal	forest	442	94.5	18.73
MACR4	Maple Creek, CA	427759	4514827	no	permanent	cleared forest	425	258.6	31.73
MCKI	McKinnleyville, CA	405061	4532232	no	permanent	residential	9	7.5	41.33
NOBA	McKinnleyville, CA	409219	4531336	no	permanent	cleared forest	45	34.5	33.77
POTO1	Arcata, CA	408484	4527786	no	permanent	open field	7	1203.8	35.80
POTO2	Arcata, CA	408568	4527744	no	seasonal	open field	5	451.0	24.50
POTO3	Arcata, CA	408565	4527818	no	permanent	open field	9	188.4	19.77
POTO4	Arcata, CA	408593	4527598	no	seasonal	open field	3	434.8	50.52
RALP1	North Spit (Arcata, CA)	403956	4527352	no	permanent	open field	-3	1867.3	10.27
RALP2	North Spit (Arcata, CA)	403871	4527171	no	permanent	open field	-3	660.9	43.83
RALP3	North Spit (Arcata, CA)	403878	4526914	no	seasonal	open field	-2	950.3	29.23
SCTF1	Maple Creek, CA	426676	4514554	yes	seasonal	forest	220	74.6	20.87

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Appendix B: Background information and raw data for statistical analysis for the 30 ponds.

Pond	Avg % EMVEG	Avg % FVEG	Avg % EMFVEG	Avg % WOOD	Avg % H$_2$0	Daily Avg Water Temp (°C)	% Canopy Cover	Presence/absence other amphibians	Presence/absence fish	Total # R. aurora egg masses	Density of egg masses/pond
ARBO	11.5	35.3	46.8	0.0	53.3	8.06	0.00	yes	yes	2	0.071
BAYS	16.8	4.0	20.8	0.0	79.3	9.11	14.06	yes	yes	14	0.532
BILA	31.3	0.0	31.3	12.5	56.3	7.71	46.61	yes	no	27	0.048
BLLA	11.3	0.0	11.3	42.5	46.3	9.06	87.63	yes	no	0	0.000
FIEL1	1.3	0.0	1.3	2.8	96.0	7.74	94.41	yes	no	0	0.000
FIEL2	21.3	0.0	21.3	21.3	57.5	8.13	88.56	yes	yes	0	0.000
FRES	7.5	0.5	8.0	0.0	92.0	7.89	20.58	yes	no	32	0.035
HBWR1	3.8	0.0	3.8	0.0	96.3	9.15	0.00	no	yes	1	0.001
HBWR2	36.3	0.0	36.3	0.0	61.3	9.82	0.00	yes	no	351	0.057
HBWR3	33.8	0.0	33.8	0.0	66.3	8.93	0.13	yes	no	223	0.262
HBWR4	36.3	0.0	36.3	0.0	63.8	8.48	0.00	yes	no	389	0.167
HBWR5	14.5	0.0	14.5	0.0	85.5	9.81	0.00	no	no	23	0.090
HESP	50.0	0.0	50.0	1.8	48.3	9.68	0.00	yes	yes	157	0.044
JCLT1	18.0	0.0	18.0	0.0	78.0	9.04	0.00	yes	no	4	0.043
JCLT2	25.0	0.0	25.0	0.0	73.8	9.41	0.00	yes	yes	7	0.024
JCLT3	32.8	0.0	32.8	6.3	61.0	9.98	0.00	yes	no	526	0.217
MACR1	20.0	33.8	53.8	0.0	50.0	9.69	16.75	yes	no	91	0.053
MACR2	2.8	0.0	2.8	0.0	97.3	9.83	21.61	yes	no	46	0.661
MACR3	0.0	0.0	0.0	2.0	98.0	8.49	94.53	yes	no	0	0.000
MACR4	0.8	0.8	1.5	0.3	98.3	8.86	18.62	yes	no	11	0.043
MCKI	12.5	0.5	13.0	0.0	87.0	9.24	40.10	no	yes	0	0.000
NOBA	14.0	0.0	14.0	0.0	86.0	7.59	12.76	yes	no	19	0.551
POTO1	77.5	0.0	77.5	0.0	22.5	7.60	0.00	no	no	199	0.165
POTO2	70.0	0.0	70.0	0.0	30.0	7.75	0.61	yes	no	51	0.113
POTO3	4.8	1.0	5.8	0.0	94.3	8.74	7.04	no	yes	29	0.154
POTO4	58.8	0.0	58.8	0.0	41.3	9.57	4.56	no	no	101	0.232
RALP1	11.8	0.0	11.8	0.0	88.3	9.50	0.00	yes	no	49	0.026
RALP2	4.5	78.8	83.3	0.0	16.8	10.10	0.00	yes	yes	176	0.266
RALP3	30.0	0.0	30.0	0.0	70.0	9.24	0.00	yes	yes	14	0.015
SCTF1	12.8	0.0	12.8	26.3	61.0	7.59	94.01	no	no	0	0.000

Appendix C: Amphibian and fish sighting.

Pond	Common Name, life stage	Scientific Name	TOTAL
ARBO	Northern Red-legged Frog, adult	*Rana aurora*	3
	Pacific Chorus Frog, adult	*Pseudacris regilla*	5
BAYS	Goldfish	*Carassius auratus*	6
	Northern Red-legged Frog, adult	*Rana aurora*	1
	Rough-skinned Newt, adult	*Taricha granulosa*	1
	Mosquitofish	*Gambusia afinis*	36
BILA	Northern Red-legged Frog, adult	*Rana aurora*	1
	Northwestern Salamander, egg mass	*Ambystoma gracile*	17
	Northestern Salamander, larvae	*Ambystoma gracile*	5
	Pacific Chorus Frog, egg mass	*Pseudacris regilla*	6
BLLA	Northern Red-legged Frog, juvenile	*Rana aurora*	2
	Northwestern Salamander, egg mass	*Ambystoma gracile*	6
	Rough-skinned newt, adult	*Taricha granulosa*	16
	Pacific Chorus Frog, adult	*Pseudacris regilla*	2
	Coastal Giant Salamander, paedomorphic	*Dicamptodon tenebrosus*	2
FIEL1	Northwestern Salamander, egg mass	*Ambystoma gracile*	27
	Cutthroat Trout, juvenile	*Oncorhynchus clarki*	1
FIEL 2	Northwestern Salamander, egg mass	*Ambystoma gracile*	12
FRES	Northern Red-legged Frog, egg mass	*Rana aurora*	30
	Northwestern Salamander, larvae	*Ambystoma gracile*	25
	Northwestern Salamander, egg mass	*Ambystoma gracile*	1
HBWR1	Three-spine Stickleback	*Gasterosteus aculeatus*	6
HBWR2	Northern Red-legged Frog, adult	*Rana aurora*	1
	Rough-skinned newt, adult	*Taricha granulosa*	1
	Pacific Chorus Frog, adult	*Pseudacris regilla*	1
HBWR3	Pacific Chorus Frog, egg mass	*Pseudacris regilla*	6
	Pacific Chorus Frog, adult	*Pseudacris regilla*	4
	Rough-skinned newt, adult	*Taricha granulosa*	3
HBWR4	Northern Red-legged Frog, adult	*Rana aurora*	2
	Pacific Chorus Frog, adult	*Pseudacris regilla*	1
HESP	Pacific Chorus Frog, egg mass	*Pseudacris regilla*	1
	Northwestern Salamander, egg mass	*Ambystoma gracile*	2
	Three-spine Stickleback	*Gasterosteus aculeatus*	14
JCLT1	Pacific Treefrog, adult	*Pseudacris regilla*	4
JCLT2	Three-spine Stickleback	*Gasterosteus aculeatus*	1
JCLT3	Northern Red-legged Frog, adult	*Rana aurora*	2
	Northwestern Salamander, egg mass	*Ambystoma gracile*	1
	Pacific Chorus Frog, egg mass	*Pseudacris regilla*	100
	Rough-skinned newt, adult	*Taricha granulosa*	9

Appendix C: Amphibian and fish sightings.

Pond	Common Name, life stage	Scientific Name	TOTAL
	Northern Red-legged Frog, adult	*Rana aurora*	1
	Northwestern Salamander, egg mass	*Ambystoma gracile*	6
MACR1	Northwestern Salamander, larvae	*Ambystoma gracile*	11
	Pacific Chorus Frog, adult	*Pseudacris regilla*	1
	Rough-skinned Newt, adult	*Taricha granulosa*	26
MACR2	Mosquitofish	*Rana aurora*	2
	Pacific Chorus Frog, adult	*Pseudacris regilla*	8
MACR3	Rough-skinned Newt, adult	*Taricha granulosa*	2
MACR4	Northern Red-legged Frog, adult	*Rana aurora*	2
	Pacific Chorus Frog, adult	*Pseudacris regilla*	1
	Goldfish	*Carassius auratus*	3
MCKI	Koi	*Cyprinus carpio*	1
	Northern Red-legged Frog, adult	*Rana aurora*	4
NOBA	Rough-skinned Newt, adult	*Taricha granulosa*	3
	Northwestern Salamander, egg mass	*Ambystoma gracile*	1
POTO1	Northern Red-legged Frog, adults	*Rana aurora*	6
POTO2	Northern Red-legged Frog, adult	*Rana aurora*	2
	Pacific Chorus Frog, adult	*Pseudacris regilla*	2
POTO3	Three-spine Stickleback	*Gasterosteus aculeatus*	13
	Northern Red-legged Frog, adult	*Rana aurora*	3
	Pacific Chorus Frog, adult	*Pseudacris regilla*	1
RALP1	Prickly Sculpin	*Cottus asper*	1
	Tidewater Goby	*Eucyclogobius newberryi*	2
	Three-spine Stickleback	*Gasterosteus aculeatus*	21
	Northern Red-legged Frog, adult	*Rana aurora*	4
	Pacific Chorus Frog, egg mass	*Pseudacris regilla*	9
RALP2	Pacific Chorus Frog, adult	*Pseudacris regilla*	5
	Rough-skinned Newt, adult	*Taricha granulosa*	7
	Three-spine Stickleback	*Gasterosteus aculeatus*	155
	Northern Red-legged Frog, adult	*Rana aurora*	5
	Pacific Chorus Frog, egg mass	*Pseudacris regilla*	1
RALP3	Pacific Chorus Frog, adult	*Pseudacris regilla*	5
	Three-spine Stickleback	*Gasterosteus aculeatus*	900

Appendix C continues: Amphibian and fish sightings totals

Totals

Northern Red-legged Frog, adult*^	*Rana aurora*	60
Northwestern Salamander, egg mass*	*Ambystoma gracile*	46
Northwestern Salamander, larvae^	*Ambystoma gracile*	41
Pacific Chorus Frog, egg mass*	*Pseudacris regilla*	117
Pacific Chorus Frog, adult*^	*Pseudacris regilla*	39
Rough-skinned Newt, adult*^	*Taricha granulosa*	78
Coastal Giant Salamander, paedomorphic^	*Dicamptodon tenebrosus*	2
Goldfish	*Carassius auratus*	9
Koi	*Cyprinus carpio*	1
Prickly Sculpin^	*Cottus asper*	1
Tidewater Goby^	*Eucyclogobius newberryi*	2
Mosquitofish^	*Rana aurora*	38
Cutthroat Trout, juvenile^	*Oncorhynchus clarki*	2
Three-spine Stickleback^	*Gasterosteus aculeatus*	1110

*casual observation (not full survey)

^caught in minnow trap