HABITAT CHARACTERISTICS OF CALIFORNIA RED-LEGGED FROGS (*Rana aurora draytonii*): ECOLOGICAL DIFFERENCES BETWEEN EGGS, TADPOLES, AND ADULTS IN A COASTAL BRACKISH AND FRESHWATER SYSTEM

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ABSTRACT

HABITAT CHARACTERISTICS OF CALIFORNIA RED-LEGGED FROGS (*Rana aurora draytonii*): ECOLOGICAL DIFFERENCES BETWEEN EGGS, TADPOLES, AND ADULTS IN A COASTAL BRACKISH AND FRESHWATER SYSTEM

By Dawn Kathleen Reis

I designed a multivariate field study to investigate differences in the habitat characteristics of California red-legged frog (*Rana aurora draytonii*) eggs, tadpoles, and adults. The MANOVA indicated that adult frogs selected shallow and warm water locations over either cold or deep-water locations for laying eggs (Wilks' Lambda = 0.547, $p<0.001$, df = 3, 34). Egg masses were attached to emergent vegetation and dead, free-floating vegetation, but plant species type was unimportant for egg attachment (goodness of fit G adj = 14.69, $p<0.025$). Tadpole habitat was characterized by pondweed (especially at high abundance), cattails, water with salinity less than 6.5 ppt, water temperatures between 15.0°C and 24.9°C, and water depths no greater than 0.75 m [Logistic Regression X2 (8, N=223) = 85.41, $p<0.001$]. After laying eggs, adults were more likely to be found in deeper water. Plant type was unimportant to adult frogs.

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INTRODUCTION

Understanding the ecology of a species is crucial for effective conservation management. This is especially important for species such as the California red-legged frog (*Rana aurora draytonii*), for which range and population reductions warranted protection as a "threatened" species under the federal Endangered Species Act (Miller *et al.* 1996).

The historic range of California red-legged frogs (CRLF) extended from the Sierra foothills to the Pacific coast and from Shasta County to Mexico, excluding the Coast Range north of Marian County. It is estimated that CRLF have disappeared from over 99 % of their inland and southern California localities and from at least 75 % of all localities within their entire historic range (Jennings *et al.* 1993). Populations of CRLF in the Coast Range between Marin and Santa Barbara counties are more intact than populations in the rest of the state. The estimated disappearances of historical populations in the Coast Range are 50 %.

California red-legged frogs are known to occur in both fresh and brackish-water habitats of Pescadero Marsh (Jennings and Hayes 1990, Smith and Reis 1997). When CRLF were federally listed, only three localities were thought to support over 350 adult CRLF: Pescadero Marsh Natural Preserve in San Mateo County; Point Reyes National Seashore in Marin County; and Rancho San Carlos in Monterey County (Miller *et al.* 1996). However, more recent surveys have indicated additional areas that support such population levels, including Elkhorn Slough National Estuarine Research Reserve in Monterey County (Reis 1999).

Habitat descriptions and a limited number of more intensive studies of habitat use have been conducted for CRLF adults and eggs, but information is sparse for tadpoles and juveniles. Existing CRLF habitat descriptions are based principally on observations of sites where adult frogs and eggs were present (Jennings and Hayes 1990, Rathbum *et al.* 1993, Jennings and Hayes 1993, Cook 1997).

Cook (1997) made quantitative comparisons between the microhabitat uses of a freshwater marsh by adult frogs and egg masses where both were present. Studies at Pescadero Marsh (Jennings and Hayes 1990) and at San Simeon and Pico creeks (Rathbum *et al.* 1993) quantitatively described areas where CRLF adults and egg masses were present in a variety of coastal habitats, including freshwater creeks, freshwater ponds, and brackish-water marshes. These three studies mainly characterized water depth and plant species to which egg masses were attached. Jennings and Hayes (1993) also described habitat characteristics where adult CRLF were found and collected from within the Sacramento River system in the Central Valley. However, each of these four studies were based on the presence of CRLF; they did not quantitatively compare areas were frogs were absent. Other general habitat descriptions for CRLF adults (Stebbins 1985, Miller *et al.* 1996) are qualitative in nature. Thus, important questions remain for adult CRLF habitat. Furthermore, habitat descriptions for CRLF at life-history stages other than adults and eggs are even less defined. Published field investigations of habitat utilization by CRLF tadpoles (larvae hatched from eggs) habitat utilization and limiting factors are limited a studies where field investigations showed differences in the distributions of adults, tadpoles, and egg masses relative to salinity levels in Pescadero Marsh (Jennings and Hayes 1990). These field investigations were followed by controlled experiments of survival rates of CRLF tadpoles at different salinities (Jennings and Hayes 1990) and eucalyptus-oil concentrations (Jennings 1996 pers. comm.). However, there have been no previous studies investigating a broad spectrum of CRLF tadpole habitat variables.

An understanding of CRLF egg mass (embryos and pre-hatchling larvae) and tadpole habitats is crucial. The ecological needs and microhabitat uses of CRLF eggs and tadpoles may differ greatly from those of adults. This is true for many other species. For example, among Western toads (*Bufo boreas*), water temperature tolerances of tadpoles differ from those of adult (Lillywhite *et al.* 1973). Other behavioral and physiological adaptations evolved by western toad tadpoles but not retained by adults include: darker pigmentation, clustering with other individuals to increase heat absorption, and the use of warmer water in shallower areas (Brattstrom 1962 and Lillywhite *et al.* 1973). There are numerous instances where conservation efforts did not succeed until researchers attained a detailed understanding of ecological differences between eggs, larvae, and adults and their respective needs. Significant examples include Lange's metalmark butterfly

(*Apodemia mormo langei*), the monarch butterfly (*Danaus plexippus*), and salmon (*Oncorhynchus spp.*) (Thelander 1994).

Water temperatures and the dry-down date of the water body can affect growth and development conditions for frog eggs and tadpoles. However, there is no published information on CRLF egg and tadpole temperature tolerances. Studies on Northern red-legged frogs (*R. a. aurora*) have shown that there are critical ranges in temperatures and time needed to for the completion of development (Storm 1960, Licht 1969, Calef 1972). Storer (1925) stated that preferred breeding habitat for *R. a. aurora* includes water temperatures of less than 20° C (68° F) while Calef (1972) stated that eggs of *R. a. aurora* may be killed if they freeze. Cook (1997) found CRLF egg masses in water with temperatures ranging between 7.5°C and 13.7°C. However, in Cook's study, sites with higher or lower water temperatures were not available. Cunningham (1955) speculated that CRLF tadpoles have a low critical thermal maximum and that high temperatures may cause developmental defects; however, specific temperatures are unknown. Jennings and Hayes (1990) noted that adult CRLF die of heat exposure at 29 °C. It is assumed that a smaller-bodied tadpole is less tolerant of higher and lower water temperatures and more susceptible to internal temperature fluctuations than an adult frog with more mass and relatively less surface area. It is speculated that CRLF tadpoles require a minimum of three months to complete development to metamorphose (Jennings and Hayes 1993). Although water temperatures influence the growth rate of tadpoles (Hayes *et al.* 1993), it is likely that the dry-down date is also important, as it limits the length of time available for tadpoles to grow.

Differences in water depth can change water temperature, the dry-down date, and protection from predators. Museum specimens of adult CRLF collected from the Central Valley were found in areas with water depths of "at least 0.75 meters deep" (Jennings and Hayes 1988). Wildlife consultants and managers have often interpreted this statement to mean that water depths less than 0.75 meters were inadequate for CRLF, and certainly not for CLRF reproduction and larval development (personal observation). This is an example where a habitat description was not supported by further investigations of the limiting factors and/or comparisons of the habitat with and without the species. In this case, minimum water levels had not been investigated. Furthermore, differences in the ecology of CRLF adults, tadpoles, and eggs may result in differences in the habitat utilization of different water depths.

Water salinity can also affect development and growing conditions for frog eggs and tadpoles. Surface-area-to-volume differences between small and large bodies (such as pre-hatched larvae, tadpoles, and adults) should also effect salinity tolerances. In controlled experiments Jennings and Hayes (1990) found that CRLF embryos and pre-hatchling larvae died with prolonged exposure to salinity concentrations of 4.5 ppt. They also found that tadpoles did not survive in salinity concentrations greater than 7.5 ppt; however, these tolerance levels should change with tadpole size.

Specific plants species that may be important for food and cover for CRLF tadpoles, as weil as an attachment substrate for eggs, are unknown. It is known that various plant species affect dissolved oxygen levels and temperature of the aquatic environments differently (Prescott 1980). Cook (1997) found that CRLF at his study site predominantly used spike rush (*Eleocharis macrostachya*) and cattail (*Typha latifolia*) for egg mass attachment. However, whether the plant species used for egg mass attachment reflected plant species abundance, or whether the frogs were selectively using specific plant species, has not been investigated.

Competition and predation relationships of CRLF tadpoles with other species are largely unknown. Controlled studies showed that mosquitofish (*Gambusia affinis*) competed for food and harassed CRLF tadpoles, resulting in smaller size among CRLF tadpoles without affecting tadpole mortality rate (Lawer *et al.* 1997). Known predators of both CRLF adults and tadpoles include San Francisco garter snakes (*Thamnophis sertalis tetrataenia*) (Wharton *et al.* 1989) and non-native bullfrogs (*Rana catesbeiana*) (Moyle 1973, Cook 1997). However, prey-predator relationships are likely to be different for CRLF tadpoles compared to adults. There are potentially more predators capable of capturing the smaller tadpoles; and, unlike adults, tadpoles can only use aquatic escape cover. Known predators of *R. a. aurora* tadpoles include rainbow trout (*Oncorhynchus mykiss*), rough-skined newt (*Taricha granulosa*), northwestern salamander (*Ambystoma gracile*), giant diving bug (*Lethocerus americanus*), damselfly and dragonfly naiads, and garter snakes (*Thamnophis sertalis*) (Calef 1972). Predators of CRLF tadpoles are likely to be similar species.

The objective of this study was to conduct a field investigation that went beyond visual descriptions of the environment and identified environmental variables that may be important to either the presence or absence of CRLF eggs and tadpoles. To assess differences in the physical environment, I compared water temperatures, salinities, and water depths between sites where eggs were present and sites where eggs were absent. The purpose of this was two-fold. I wanted to determine if eggs required specific water temperatures, water depths, and salinity ranges. Secondly, I wanted to determine if these physical variables could be used to predict where egg masses were found. I also examined the relative importance of different vegetation types to which egg masses were attached to assess whether CRLF were selectively using particular plant types.

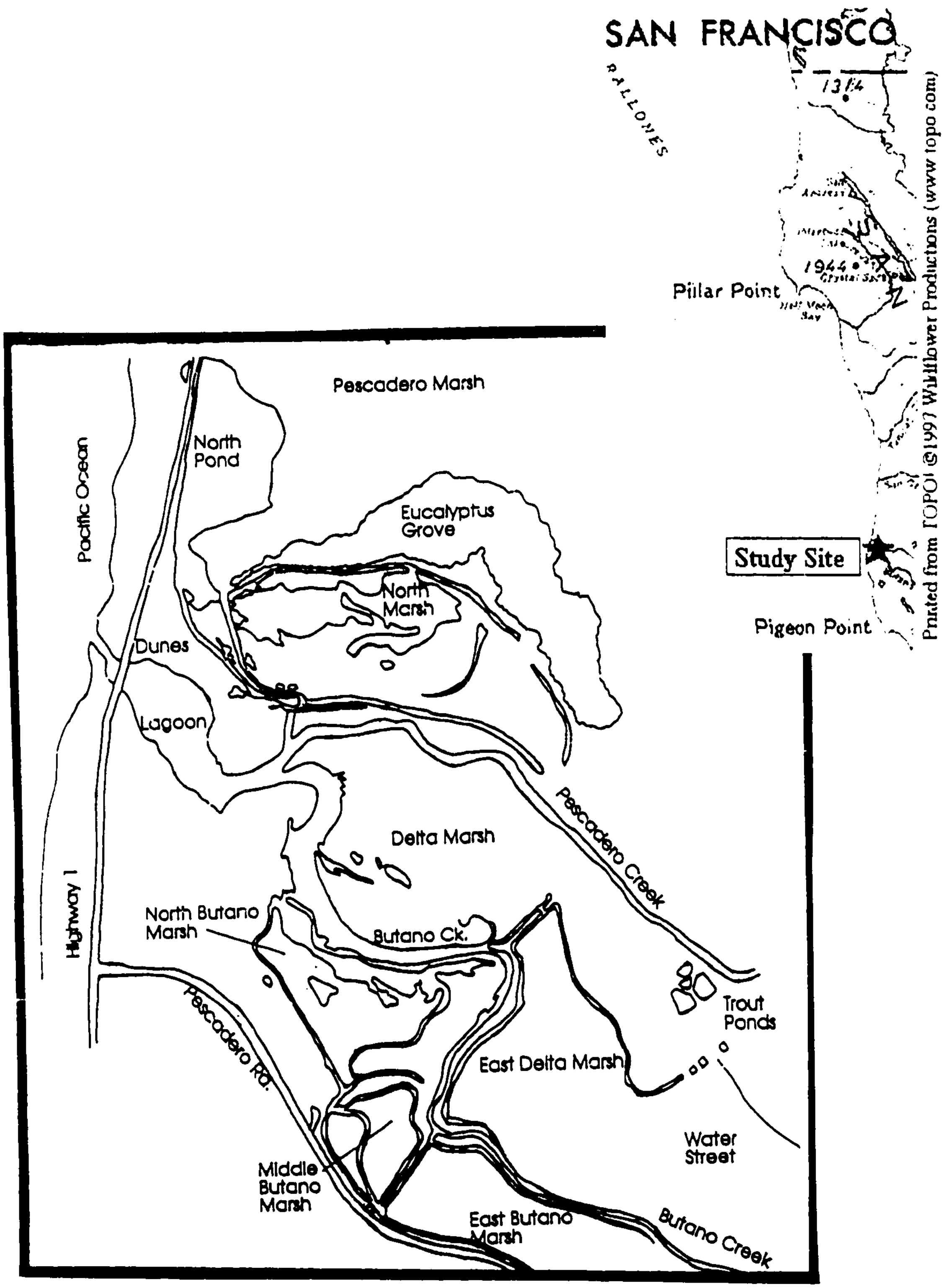
For CRLF tadpoles, my goal was to determine which abiotic, plant, and animal variables accounted for their presence or absence in various locations. I investigated water temperatures, salinities, water and mud depths, and dry-down date of the water body in association with CRLF tadpole growing conditions and development. I examined individual plant species and plant community structure (height and cover) as potential foraging and cover habitat for tadpoles. Finally, I also investigated potential tadpole prey and predators.

METHODS

Study Area.- Pescadero Marsh Preserve is a 320-acre, state-owned coastal wetland, located in the central Coast Range of California between San Francisco and Monterey bays (Figure 1). The preserve includes a seasonal estuary at the confluence of Pescadero and Butano creeks, seasonal brackish and freshwater marshes, freshwater ponds, and riparian habitats along the creeks. In most years a sandbar forms at the estuary mouth during late spring or summer and blocks tidal flow. Freshwater inflow impounds behind the sandbar resulting in decreased salinity levels in the estuary.

During the summer of 1993, the California Department of Parks and Recreation began its first phase of a Pescadero Marsh restoration project to restore tidal fluctuations to North Pond and portions of North Marsh and enlarge estuary habitat (Figure 1). This restoration involved removing the levee between North Pond and North Marsh and installing tide gates through the levee between North Marsh and the estuary. In addition, a levee was constructed in North Marsh in an attempt to keep most of North Marsh as a freshwater habitat separated from the estuary. Other levees were breached in the northwestern, middle, and eastern sections of Butano Marsh to increase flood and tidal flow through the marsh. With all these environmental alterations underway, the study site provided a large sampling area with a diverse mix of habitat variables, along with a large population of CRLF (Jennings and Hayes 1990).

Figure 1. Study site at Pescadero Marsh Natural Preserve, San Mateo County, California.



Dominant plant species in the aquatic habitats of Pescadero Natural Preserve include: cattail (*Typha* spp.); California bulrush (*Scirpus californicus*); fennel leaf or sego pondweed (*Potamogeton pectinatus*); pickleweed (*Salicornia virginica*); cinquefoil (*Potentilla ededei* var. grandis) (Munz and Keck 1973), commonly known as silverweed (*Potentilla pacifica*) (Mason 1969); *Sparganium* spp; and several species in the sedge (Cyperacea) family, including: (*Scirpus robustus*), spike rush (*Eleocharis palustris*), and beak rush (*Rynchospora macrostachya*). Other plant species present in low numbers included willows (*Salix* spp.), duckweed (*Lemna minor*), brass buttons (*Cotula coronopifolia*) and *Juncus* spp.

Egg mass methods.-To compare the physical environments in which egg masses were present and absent, I recorded habitat type, water temperature, salinity concentration and water depth associated with each egg mass and measured the same variables at sites without egg masses. I conducted visual surveys for egg masses on sixteen days in 1996, from 22 February through 30 April by walking transect lines across all macro-habitat types where possible. These included freshwater marsh, freshwater pond, brackishwater marsh with open, or no, tidal gates, and brackish water marsh areas with closed tidal gates. Transect-line length varied depending on available habitat; however, in general, transect lines were spaced approximately two meters apart in densely vegetated areas and four meters apart in less densely vegetated areas. I flagged egg masses so as not to recount them on later surveys dates. Eighteen sites without egg masses were randomly selected. A numbered grid was over-laid on the map at each macro-habitat type so that random numbers that were drawn corresponded to a mapped grid number. These eighteen sites without CRLF egg masses were sampled during the sampling period in which egg masses were located. Salinity (ppt) and water temperature (C) were measured adjacent to the egg mass, or at the surface in plots without egg masses, with a Yellow Springs Instruments Model 30 Salinity-Temperature meter. A PVC pole marked at 0.25-meter increments was used to measure water depth.

A multivariate analysis of variance (MANOVA: Tabachnick and Fidell 1996) was used to test whether the physical environment (water temperature, water depth, and salinity) where CRLF egg masses were present differed from the physical environment where egg masses were absent. Preliminary tests indicated that salinity was highly correlated with water temperature (Pearson's Product Moment Correlation coefficient: $r=0.673$). Based on these results, I performed a Roy-Bargmann stepdown analysis (Tabachnick and Fidell 1996) to determine if the MANOVA results were influenced by the dependent variable correlations. Preliminary tests also showed that the assumptions for normality, homogeneity of variance-covariances, linearity and multicollinearity were met.

In order to examine whether CLRF were selecting a specific plant type [e.g., cattail, pickleweed (*Salicornia virginica*), California bulrush (*Scripus californicus*) or other members of the Cyperaceae family] for egg mass attachment, I compared frequencies of egg mass attachment vegetation types to the availability of those plant types in the marsh. Percent cover of plant species type was recorded from 223 randomly selected, four-meter by four-meter plots, within areas of the marsh that were known to contain some standing water during the time of the egg mass survey. To insure a proportional representation of all macro-habitat types, a numbered grid was overlain on the map at each macro-habitat type and a proportional number of random numbers were drawn that corresponded with a mapped grid number.

A goodness of fit test (Zar 1997) was used to determine if the observed frequency distribution of plant species to which egg masses were attached differed from the relative frequency of the dominant vegetation types in the marsh. Relative percent of dominant plant species types (Rp) in the marsh was calculated as: $Rp_i = \frac{Cover_i}{Cover_{all}} * 100$

where $Cover_i$ is the total percent cover of an individual plant species and $Cover_{all}$ is the total percent cover of all plant species types. Expected frequency ($\hat{F}_i$) of an attachment plant species for egg mass was calculated by multiplying the relative percent cover for the marsh by the number of locations were CRLF egg masses were located ($\hat{F}_i = Rp_i *$ 40). The expected frequencies of attachment vegetation types was then compared with the observed frequencies (F_{ob}) of egg mass attachment vegetation types with a Goodness of Fit Test, as described by Zar (1997).

To further aid microhabitat descriptions of egg mass sites, I noted whether the egg mass was unattached to vegetation or whether the attachment vegetation was alive, dead, rooted, or free-floating. The condition of each egg mass was recorded as the percent of the mass with dead embryos at specific Gosner Development Stages (Gosner 1960). Other microhabitat information collected included depths of egg mass submergence and water clarity (Secchi depth).

Tadpoles methods.-To examine the effect of habitat characteristics on the presence or the absence of CRLF tadpoles in a field setting required multivariate habitat measurements in a large number of sample plots that varied in habitat characteristics. The same 223 random, four-meter by four-meter study plots used to provide plant species availability for egg mass micro-habitat preference analysis were sampled for tadpoles on twenty days from 16 April through 21 June 1996. A seine net with a mesh diameter of three mm was hauled toward the shore. In open water plots, the seine was hauled for four meters and then lifted from the water. Captured tadpoles were identified to species, numbers were tallied, and all tadpoles were released back to their original location unharmed. If no CRLF tadpoles were found in the first seine haul, up to three additional samples were made and recorded. Sampling was stopped when CRLF tadpoles were captured. A dipnet was used in areas where vegetation was too dense to seine. Dipnetting continued until a CRLF tadpole was captured or the plot had been thoroughly sampled. The number of dipnet samples was recorded along with tadpole capture counts.

Abiotic variable measurements were taken at each study plot immediately before the plot was sampled for tadpoles. A Yellow Springs Instruments Model 30 meter was used to take water salinity and temperature profiles. Salinity and temperature readings were taken at the surface, the bottom, and at each 0.25 meter depth increment in each plot . A two meter PVC pole was used to extend the salinity and temperature probe cord out into the channel (like a fishing pole). Means were calculated for each salinity and temperature profile. Preliminary analysis indicated little differences between surface, bottom and profile mean values for both temperature and salinity, therefore, only the profile means were used in the multivariate analysis. Water temperature and salinity category levels tested in the Hierarchical Log-linear Analysis are presented in Table 1.

The PVC pole, marked in 0.25 meter increments, was also used to measure water depth and mud depth. Water depth was measured in the deepest location within a plot. The month in which the plot dried down was recorded as the dry-down date. Water pH was measured at the surface using a standard litmus paper test kit. Water depth, pH and dry-down date category levels tested in the Hierarchical Log-linear and Logistic Regression Analysis are presented in Table 1.

Study plots with tide gates that remained closed to tidal flow from early spring to September were recorded as closed. Brackishwater study plots without tide gates or with tide gates that remained open to tidal flow any time between early spring and September were recorded as open. Freshwater plots (ponds and non-brackish regions of the creeks) were recorded as non-tidal (Table 1). A sandbar that formed in late summer and remained until late fall stopped tidal flow into areas with open tidal gates and backed-up fresh water. The tidal categories were not indicative of daily tidal flows but of sharp

Table 1. Category levels for abiotic variables tested in the California red-legged frog tadpoles data set.

Variable	Category Levels
Water Temperature Profile Mean	As (1) 10.0-14.9 C (4) 25.0-29.9 C (2) 15.0-19.9 C (5) 30.0-35.0 C (3) 20.0-24.9 C
Water Depth	As (1) 0.05 m - 0.25 m (3) 0.51 m - 0.75 m (2) 0.26 m - 0.5 m (4) 0.76 m - 1.5 m
Dry-down Date	As (1) February- April (2) May- July (3) August- Year round sites
Tidal Flow	As: (1) Open Tidal Gates (brackish water) (2) Closed Tidal Gates (brackish water) (3) Non-tidal (freshwater ponds or creek)
Surface water pH	As (1) 0.0-3.9 (7) 7.0-7.4 (2) 4.0-4.9 (8) 7.5.-7.9 (3) 5.0-5.4 (9) 8.0-8.4 (4) 5.5-5.9 (10) 8.5-8.9 (5) 6.0-6.4 (11) 9.0-9.4 (6) 6.5-6.9 (12) 9.5-10
Salinity Profile Mean	As (1) 0.0-3.9 PPT (6) 6.6-6.9 PPT (2) 4.0-4.9 PPT (7) 7.0-7.5 PPT (3) 5.0-5.5 PPT (8) 7.6.-7.9 PPT (4) 5.6-5.9 PPT (9) $\geq$ 8.0 PPT (5) 6.0-6.5 PPT

seasonal variations in salinity levels during tadpole development.

I visually estimated percent cover of each plant species at different structural heights (sub-surface to surface, above surface to 0.33 m, 0.33 m to 1.0 m, 1.0 m to 2 m, and over 2 m). Totals were then calculated for plant types and structural heights in each plot. These visual estimates were later lumped in to the following categories: low (1-15%), medium (16-75%) or high (76-100%). In addition, presence or absence of algae and its growth pattern (surface, stem, or bottom) was recorded. The condition of the algae was noted by color, with green indicating healthy or orange indicating decomposing or dead algae.

To investigate potential prey or predator relationships with CRLF tadpoles, I recorded the presence or absence of predaceous fish, amphibian and invertebrates in each plot. Sampling for predators occurred when seining and dipnetting for CRLF tadpoles. Captured fish and amphibians were identified to the species. Predaceous invertebrates included diving beetles (family Dytiscidae), dragonflies (order Odonata) and back-swimmers (family Notonectidae).

Before the study plot was sampled for tadpoles, the plot was visually surveyed for adult and juvenile amphibians. Total counts were recorded for adult and juvenile CRLF and for predatory bullfrog adult and juveniles. Pacific tree frog (*Psuedacaris regilla*) adults, juveniles, tadpoles and eggs were recorded as present or absent only.

Night surveys for adult frogs were conducted by spot lighting for eye-shine and listening for calls on six nights in March. In order to test for differences between the habitat utilization of CRLF adults, tadpoles, and egg masses, the distributions and habitat use of CRLF at these different life-history stages were compared.

Statistical Analysis.-In order to test if the abiotic and botanical variables accounted for either the presence or absence of tadpoles, a SPSS 8.0 sequential Logistic Regression Analysis (Tabachnick and Fidell 1996) was used to determine which habitat variables were most important for predicting the presence or absence of CRLF tadpoles and to develop a predictive model. Habitat data from all 223 plots were used; 71 of the plots contained CRLF tadpoles and 152 plots did not. The Logistic Regression Analysis required that the predictor variables were independent. Therefore, I used a Hierarchical Log-linear Analysis and Pearsons' product moment correlation to identify and eliminate dependent predictors.

Table 2 provides a list of correlated habitat variables, variable choices made and variables included in the Logistic Regression. Continuous habitat variables that showed a correlation with other variables (Pearsons' $r \geq 0.60$) were eliminated so that all variables used in the final Logistic Regression where independent of each other (Table 2). Variables with the largest number of multiple correlations were eliminated first. Final variable choices between two correlated habitat variables were based on biological reasoning. Two choice types occurred. Plant species type was used instead of vegetation structure (Table 2), as differences in vegetation structure above the water surface did not affect or describe the aquatic tadpole environments as much as plant species type. Further, knowledge of plant species type provided more detailed information regarding potential food and cover type, as well as effects on dissolved oxygen. Dry-down date was chosen over tidal flow (Table 2) because it limits the amount of time in which

Table 2. Habitat variables used and eliminated from the Logistic Regression Analysis due to significant Pearsons' correlations or logistic linear associations.

Variables Used	Variables Eliminated
Abiotic Factors	
Water Temperature Profile Mean	
Water Depth	
Dry-down date	Tidal Flow (Pearsons' r = -0.756)
Salinity Profile Mean	
Plant Species	
Fennel Leaf Pondweed	Vegetation structure at subsurface to surface level (Pearsons' r = 0.501)
Pickleweed	Vegetation structure above Surface to 0.33 m (Pearsons' r = -0.793)
Cattails	Vegetation structure at 1.0 m to 2.0 m (Pearsons' r = -0.768) Total Vegetation (Pearsons' r = -0.4.03)
"Sedges"other than California Bulrush.	Vegetation structure at 1.0 m to 2.0 m (Pearsons' r = -0.897)
California Bulrush	Total vegetation over 2.0 m (Pearsons' r = 0.637)
Filamentous Surface Algae	

CRLF tadpole development could occur. In addition, tidal flow was eliminated because it did not reflect daily tides (some plots were behind levees or tide gates) or salinity levels.

Table 2 also summarizes the same type of information for discrete variables with significant Hierarchical Log-linear associations. Variables were included in the Logistic Regression if they (1) had an association with either the presence of tadpoles or the absence of tadpoles and (2) had no significant associations with other habitat variables. Table 1 provides the category levels for the abiotic variables tested in the Hierarchical

Log-linear and Logistic Regression Analysis. Due to the numerous variables investigated, the Hierarchical Log-linear variable sets with plant species and vegetation structures were first tested at the presence/absence level. If an association occurred at the presence or absence level, the variable was then further tested at different percent cover levels (low = 1-15%, medium = 16-75%, or high = 76-100%).

The full model used in the sequential Logistic Regression included the following habitat variables as predictors: pondweed (absent, low, medium, high), cattail (present or absent), bulrush (present or absent), Cyperaceae other than bulrush (present or absent), pickleweed (present or absent), surface algae (healthy or decaying), salinity (low = 0.0-6.5 ppt or high 6.6 or greater), water depth (shallow = 0.05-0.75 m, deep = 0.76-1.75 m), dry-down date (February - April, May – July, August –year round) and water temperature (low 10.0 - 14.9 C, medium 15.0 - 24.9 C, high 25.0 C or greater). The cut value used in the model equaled 0.50.

Tadpole and Adult Requirements Of Specific Habitat Variables.- Specific requirements of CRLF tadpoles were analyzed by reviewing the descriptive statistics (means, modes, minimums and maximum) of habitat variables with significant Logistic Regression or Hierarchical Log-linear results. The same study method was used for a separate analysis for CRLF adults.

Other Animals and CRLF Tadpoles. – A Hierarchical Log-linear Analysis was used to test for significant associations between the presence or absence of prey or predator species with either the presence or absence of CRLF tadpoles.

Differences Between The Distributions And Habitat Requirements Of CRLF Adults, Tadpoles, And Egg masses.- Study plots with the CRLF at different life-history stages (adults, tadpoles and eggs) were compared in order to compare distribution within the marsh and habitat utilization at a macro-habitat level. Habitat variables that showed associations (with the Hierarchical Log-linear Analysis) or correlations (with the Logistic Regression) from the above analyses were compared against the descriptive measures of the same habitat variable type in areas where adult, tadpole and egg masses occurred. In addition, the final Logistic Regression model that best described tadpole habitat was compared against the final Logistic Regression model that best described adult habitat characteristics.

RESULTS

Differences Between The Physical Environment Where CRLF Egg Masses Were Present Verses Where Egg Masses Were Absent.-The MANOVA results indicated that there was a significant multivariate difference (Wilks' Lambda = 0.547, $p<0.001$, df = 3, 34) in the physical environment between sites with egg masses and those without. A greater number of plots with egg masses were found in areas with warmer water temperatures and with shallower water depths (Figure 2). Water temperature was scored inversely so low scores for water temperature showed higher mean temperatures. Water temperatures in sites with egg masses were greater than in sites without egg masses, with a difference

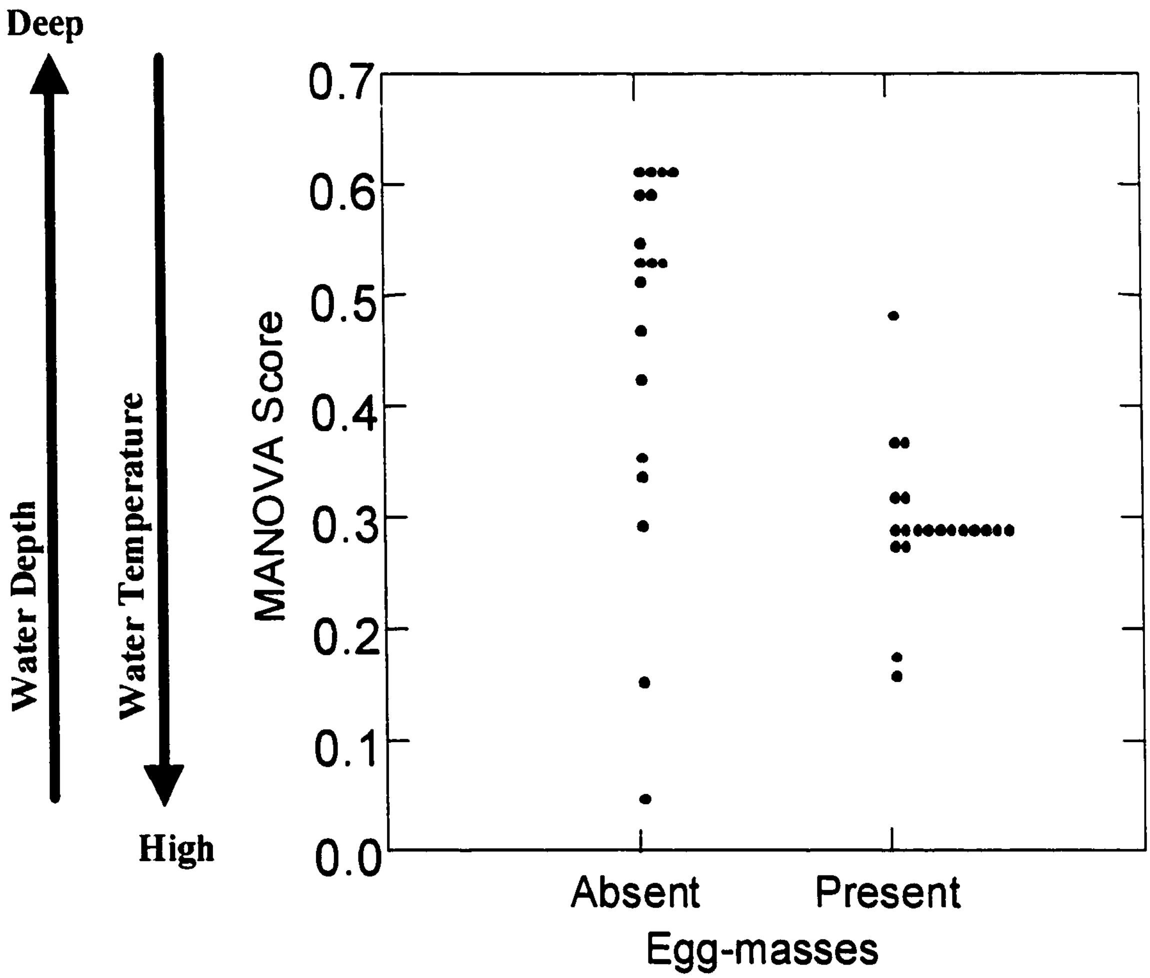


Figure 2. MANOVA scores for Pescadero Marsh sites with and without CRLF egg masses. MANOVA scores show that a greater number of plots with egg mass are found in areas with warmer water temperatures and at shallower water depths (Wilks' Lambda = 0.547, p<0.001, df =3, 34).

of 3.2 C between means (Table 3). Water depth was scored such that low scores represented shallower water depths (Figure 2). Mean water depth adjacent to egg masses was lower than the mean depth for sites without egg masses, with a relatively small differences of 0.10 m between sites with and without egg masses (Table 3). Table 4 provides descriptive statistics of water temperature, water depth and salinity concentrations between sites without egg masses and sites with egg masses.

Table 3. Means, modes, and ranges for water temperature, depth and salinity in plots with CRLF egg masses present and in plots without egg masses at Pescadero Marsh.

Abiotic Variable(s)	**Egg mass Present:** N = 18 mean ± standard deviation mode (range)	**Egg masses Absent:** N = 18 mean ± standard deviation mode (range)
Temperature (C)	μ 20.0 ± 2.79 mode 20.4 (13.3-27.2)	μ = 16.8 ± 3.0 mode 13.6 and 14.0 (12.1-22.)
Depth (m)	μ = 0.35 ± 0.29 mode 0.30 (0.10-1.00)	μ = 0.45 ± 0.36 mode 0.30 (0.10-1.50)
Salinity (ppt)	μ = 2.2 ± 0.8 mode 2.3 (0.1-3.8)	μ = 1.8 ± 1.5 mode 0.1 (0.1-5.4)

Table 4. MANOVA and Roy-Bargmann canonical loadings: percent of the differences between sites with CRLF egg masses verses sites without egg masses explained by water temperature, water depth, and salinity.

Dependent Variable	**MANOVA Canonical Loadings (significant > 0.30)**	**Roy-Bargmann Stepdown Analysis Canonical Loadings**
Water Temperature (C)	-0.78	+0.999
Water Depth (m)	+0.31	-0.279
Salinity Concentration (ppt)	-0.15	Not tested

The canonical loadings from the MANOVA (Table 4) showed that water temperature was the most important of the three physical variables in differentiating between sites with egg masses and sites without. Water depth also proved to be an important characteristic between sites with egg masses verses sites without egg masses (Table 3). The small loading for salinity (Table 4) indicated that this variable was not as critical in differentiating between sites with egg masses and sites without. The results from the Roy-Bargmann stepdown Analysis were virtually identical to the MANOVA results, which showed that the results of the MANOVA were robust to potential correlations between the dependent variables (Table 4).

Adult CRLF Selection Of Specific Plant Type For Egg Mass Attachment.- Egg mass attachment plants included all dominant plant types in the marsh: pickleweed, cattails, bulrush, brass buttons and sedges. However, the Goodness of Fit test showed that the observed relative abundance of plant species types with egg masses was different from what was expected by the relative abundance of available plant species in the marsh (G adj = 14.69, $p<0.025$). The observed relative frequency of pickleweed attachment sites was three times greater than expected, while the observed relative frequencies of cattail, sedges and spike rush were substantially less than expected (Table 5). California bulrush and gum plant were used as attachment sites in proportional to their availability (Table 5). All the pickleweed used for egg mass attachment was both rooted (Table 6) and found exclusively in shallow water locations. All bulrush, sedge, and cattails used for egg mass attachment were dead and free-floating (not rooted).

Table 5. Observed and expected frequencies of plant species used for CRLF egg mass attachment (overall G adj = 14.69, p<0.025 between observed and expected).

Plant Species	Observed	Expected
Pickleweed	16	5.3
Gum plant	1	0.5
Cattail	8	14.4
"Sedge" and spiked rush	5	15
Bulrush	4	4.7

Table 6. CRLF egg masses vegetation attachment types.

CRLF Egg mass Attachment Types	Frequency	Percent
Egg mass attached to rooted vegetation	**22**	**56.4**
Pickleweed	16	41.0
Gum plant	1	2.6
Cattail	0	0
"Sedge" and spiked rush	4	10.3
Bulrush	1	2.56
Egg mass attached free-floating (dead) vegetation	**15**	**33.3**
Pickleweed	0	0
Gum plant	0	0
Cattail	8	20.5
California bulrush	4	10.3
Sedges other than California bulrush	1	2.6
Egg mass unattached to vegetation	**4**	**10.3**

Micro-habitat Descriptions and Condition of Egg Masses.- All 41 egg masses appeared to contain healthy embryos or pre-hatched larva, except for approximately 5 to 20 % of the eggs nearest the point of attachment, which often became desiccated, killing exposed eggs. The conditions of the egg masses are summarized in Table 7. All egg masses observed were either at the surface or within 0.1 m of the surface. Water was generally turbid with Secchi depths ranging form 0.1 to 0.25 m, with a mean of 0.13 m, so eggs in deeper water would not have been visible.

Table 7. Condition of CRLF egg masses observed at Pescadero Marsh.

Condition of CRLF Egg masses	Frequency	Percent
Some dead embryos or pre-hatched larva (percent of dead eggs in the mass ranged from 0 to 1 %)	20	48.78
Pre-hatched larva dead at Gosner stage 13 (percent of dead eggs in the mass ranged from 0 to 1 %)	10	24.39
Some observed fungus on embryos (percent of dead eggs in the mass ranged from 0 to 1%)	3	7.3
Egg masses with no mortality observed	8	19.5

Overview of Habitat Characteristics Associated with the Presence or Absence of CRLF Tadpoles and Their Value as Predictors- The Logistic Regression model which best predicted the presence of CLRF tadpoles included five habitat characteristics (Table 8). The order of importance as habitat predictors was based on the significance of Logistic Regression, as the Wald statistic is not as reliable (Tabachnick and Fidell 1996). The predictability for the presence CRLF tadpoles in the brackishwater and freshwater macro-habitats at Pescadero Marsh was 80.00 % when pondweed was present (especially at medium and high percent cover levels), cattails were present, salinity levels were below 6.6 ppt, water temperatures were between 15.0 and 24.9 C and not 14.9 C or less, water depths were between 0.05 and 0.75 m and not 0.76 m or greater. The model predicted even better for CRLF tadpole absence (84.2%) and the over all model prediction rate was 82.9% (Figure 3). The test of this model with all five predictors against the constant-only model was statistically reliable, X2 (8, N = 223) = 85.41, $p < 0.001$, indicating that as a set, these predictors reliably distinguished between plots with and plots without CRLF tadpoles. In addition, each of the five habitat characteristics used significantly

Table 8. Logistic regression model with an 80.0 % prediction rate for the presence of CRLF tadpole when using eight habitat characteristics.

Variable (in order of importance)	B	Wald	Sig. of Wald	R	Exp(B)	Sig. Log LR
Pondweed			31.4703	0.000	0.3034	> 0.001
Pondweed Absent	-2.7541	24.9516	0.0000	-0.2880	0.0637	
Pondweed Low Cover (1-15 % cover)	-1.2672	2.6142	0.1059	-0.0471	0.2816	
Pondweed Medium Cover (16-75 % cover)	-0.2251	0.0833	0.7729	0.0000	0.7984	
Cattail Absent	-1.5177	13.7395	0.0002	-0.2060	0.2192	> 0.001
Water Salinity						> 0.001
Low Salinity Profile mean between 0.0 and 6.5 ppt	2.2426	12.1100	0.0005	0.1911	11.318	
Water Temperature			12.909	0.0016	0.1794	> 0.001
Water Temperature Profile mean between 10.0 C and 14.9 C	-1.2496	0.6094	0.4350	0.0000	0.2866	
Water Temperature Profile mean between 15.0 C and 24.9 C	2.2670	4.3331	0.0374	0.0918	9.6501	
Water Depth						0.7920
Depth between0.05-0.75 m	0.1646	0.0691	0.7927	0.0000	1.1790	
Constant	-2.0727	1.9189	0.1660			
Variables Removed						
California Bulrush						
Sedges other than California Bulrush						
Pickleweed						
Surface Algae						
Dry-down date						

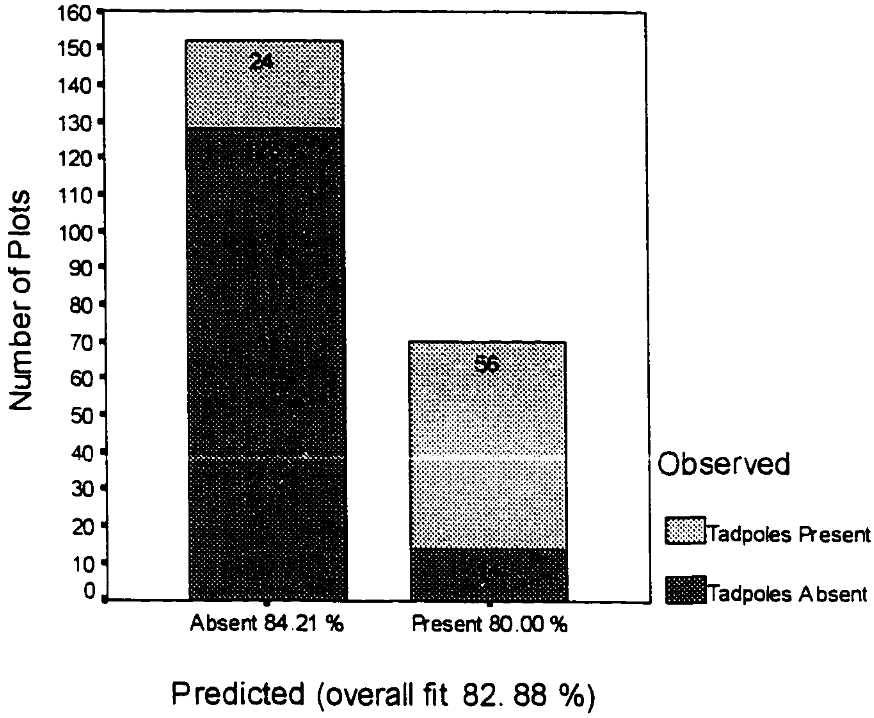


Figure 3. SPSS Logistic Regression model that used eight habitat characteristics to predict the presence of California red-legged frog tadpoles (*Rana aurora draytonii*) in sample plots at Pescadero Marsh Natural Preserve 80.00% of the time.

contributed as a predictor. The most important predictor of tadpole presence was the presence of pondweed (Table 8 and Figure 4). The most important abiotic variable for predicting the presence of CRLF tadpoles was salinity levels of 6.5 ppt or less.

The Hierarchical Log-linear Analysis results that indicated interdependent habitat variables (abiotic and botanical) are reported in Table 2. Significant Hierarchical Log-linear associations with either the presence or absence CRLF tadpoles and habitat variables are reported in Tables 9, 10 and 11. Additional Hierarchical Log-linear Analysis indicated that there were specific levels (such as low, medium or high) at which the association of some of the variables was the strongest (Table 9, 10 and 11). Hierarchical Log-linear and descriptive statistic results for each variable type are reported in the following three sections.

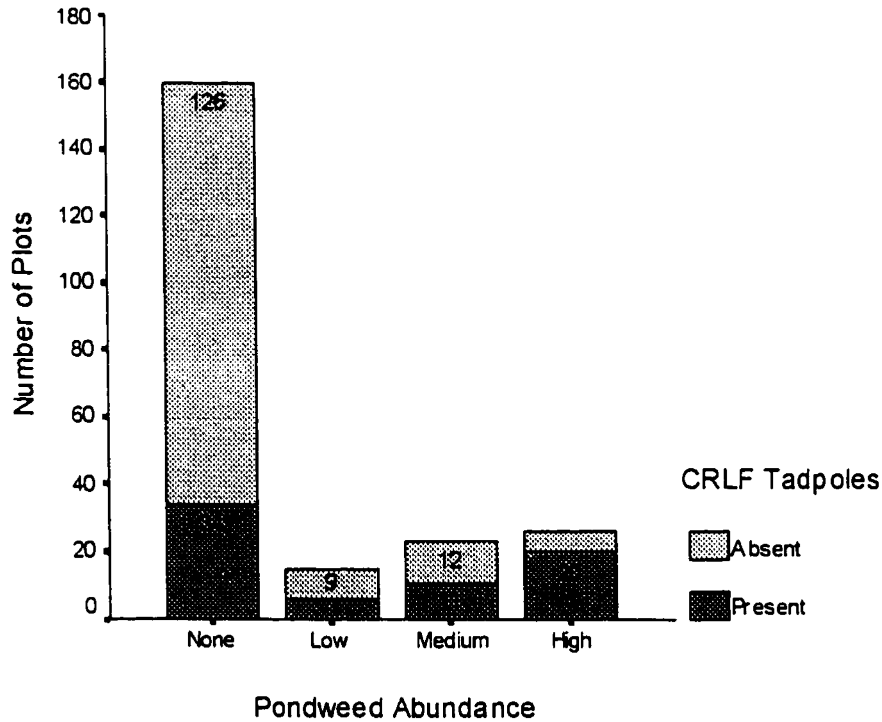


Figure 4. Occurrences of CRLF tadpoles at different abundance levels of pondweed.

Abiotic Factors and CRLF Tadpoles.-The Hierarchical Log-linear Analysis indicated that the were no significant differences between the proportion of plots with tadpoles present verses absent at all salinity levels tested below 6.6 ppt (Figure 5 and Table 9). However, salinity profile means of 6.6 ppt or greater were associated with the absence of CRLF tadpoles (Hierarchical Log-linear $p < 0.001$). Plots with CRLF tadpoles had a mean salinity of 3.6 ppt, with a range of 0.1 ppt to 9.9 ppt (Table 12). Plots that did not have CRLF tadpoles had a mean salinity profile of 4.4 ppt, with a range of 0.1 ppt to 23.3 ppt.

Table 9. Abiotic variables that showed a significant relationship with presence or absence of California red-legged frog tadpoles in the Hierarchical Log-linear Analysis.

Abiotic variable(s)	p	Type of relationship
Water Temperature Levels Tested: 10.0-14.9 C 15.0-19.9 C 20.0-24.9 C 25.0-29.9 C 30.0-35.0 C	< 0.001	The proportion of plots with tadpoles was greatest when mean water temperature between 15.0-24.9 C. Tadpoles were more likely to be absent in plots that had mean water temperatures between 10.0-14.9 C and 25.0 C or higher. See Figure 3.
Water Depth Levels Tested: 0.05 m - 0.25 m 0.26 m - 0.55 m 0.60 m - 0.75 m 0.76 m - 1.75 m	< 0.001	The proportion of plots with tadpoles was greatest when water depths were between 0.05 m and 0.75 m, with the greatest number of tadpoles occurring at depths of 0.26-0.5 m. Tadpoles were more likely to be absent in plots with water depths between 0.76 m and 1.75 m
Salinity Levels Tested: 0.0-3.9 ppt 4.0-4.9 ppt 5.0-5.5 ppt 5.6-5.9 ppt 6.0-6.5 ppt 6.6-6.9 ppt 7.0-7.5 ppt 7.6-7.9 ppt 8.0-4.0 ppt	< 0.001	There was no significant difference between the proportion of plots with tadpoles present vs. absent at salinity levels between 0.0 and 6.5 ppt. However, proportion of plots without tadpoles were greatest in plots with salinity levels between 6.6 and 40.0 ppt.
Tidal Flow Levels Tested: Open Tidal Gates Closed Tidal Gates Non-tidal	< 0.001	The proportion of plots with tadpoles was significant in all three plot types (non-tidal fresh water ponds, brackish water locations within areas both open to tidal flow and closed to tidal flow).
Dry-down Date Dates Tested: May, June, July, Aug., Sept., Oct., Nov., or Perennial		No relationship for the levels test.
Mud		no relationship
pH		no relationship

Table 10. Plant species that showed a significant relationship with presence or absence of California red-legged frog tadpoles in the Hierarchical Log-linear Analysis.

Variables Tested	p	Type of relationship
Pondweed	< 0.001	The number of plots with tadpoles present was greatest when pondweed was also present. In addition, the greatest number of plots with tadpoles present occurred when percent cover of pondweed was high.
Pickleweed	< 0.021	Tadpoles were absent more often when percent cover of pickleweed was at a medium level.
Cattails	< 0.001	A significant proportion of plots with tadpoles occurred when cattails were present. However, there is no relationship at low, medium or high levels of cattail cover.
Sedge	< 0.001	No relationship, main effect: the proportion of plots with tadpoles present and sedge present were similar to proportion of plots with tadpoles absent and sedge absent.
Bulrush	< 0.001	A significant proportion of plots with tadpoles occurred when bulrush was absent. However, there is no relationship at low, medium or high levels of bulrush cover.
Algae		No relationship
Algae Type	< 0.001	The number of plots with tadpoles present was greatest when green surface algae were also present.

While tadpoles were found in salinity levels up to 9.9 ppt, they may not have survived prolong exposures at this level. Seventy-seven percent of the sample plots with tadpoles present had salinity levels below 5.0 ppt (Figure 5).

Water temperature between the ranges of 15.0 C and 24.9 C was significantly correlated with the presence of CRLF tadpoles (Logistic Regression $p < 0.001$) while water temperature between 10.0-14.9 C was correlated with the absence of tadpoles (Table 8

Table 11. Aquatic animals that showed a significant relationship with presence or absence of California red-legged frog tadpoles in the Hierarchical Log-linear Analysis (N= number of sites with CRLF tadpoles present or absent).

Variable	P	Type of relationship
Bullfrog Adult N present =10, N absent =5	< 0.001	A significant proportion of plots with CRLF tadpoles occurred when bullfrog adults were present.
Predaceous Invertebrates N present =41, N absent =118	< 0.001	The proportion of plots without CRLF tadpoles was a greatest when predaceous invertebrate where present.
Fish N present = 129, N absent = 23	< 0.001	No relationship, main effect
Red-legged Frog Adult N present =20, N absent =16	< 0.001	A significant proportion of plots with CRLF tadpoles occurred when CRLF frog adults were present.
Pacific Treefrog Adult N present =11, N absent =8	< 0.001	A significant proportion of plots with CRLF tadpoles occurred when treefrog adults were present.
Pacific Treefrog Tadpoles N present =16, N absent =24	< 0.001	A significant proportion of plots with CRLF tadpoles occurred when treefrog tadpoles were present.
Pacific Treefrog Eggs N present =9, N absent =9	< 0.001	A significant proportion of plots with CRLF tadpoles occurred when treefrog eggs were present.

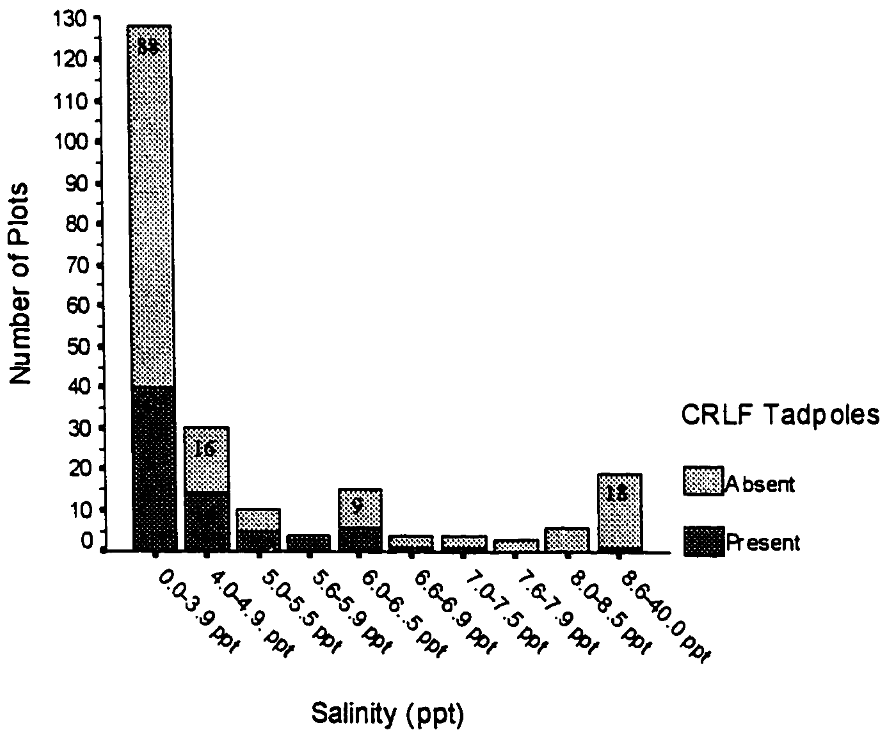


Figure 5. Occurrences of CRLF tadpoles at different mean water column salinities.

Table 12. Summary of means, modes, and ranges for abiotic habitat variables with and without CRLF tadpoles and adults present.

Abiotic Variable(s)	Mean ± Standard Deviation Mode (Range)	Mean ± Standard Deviation Mode (Range)
Tadpoles	Present: N plots = 71	Absent: N plots = 152
Water Salinity (ppt)	μ 3.6 ± 2.0 mode 0.1 (0.1-9.86)	μ 4.4 ± 3.4 mode 0.2 (0.1-23.3)
Water Temperature (C)	19.4 ± 2.8 μ mode 17.6 (13.4 -25.9)	μ 20.53 ± 3.92 mode 25.5 (11.9-31.4)
Water Depth (m)	μ 0.5 ± 0.22 mode 0.5 (0.05-1.1)	μ 0.42 ± 0.30 mode 0.25 (0.05-1.70)
Adults	Present: plots = 37	Absent: N plots = 186
Water Salinity (ppt)	μ 2.7 ± 2.14 mode 0.1 (0.1-9.86)	μ 4.4 ± 3.1 mode 0.1 (0.1-23.3)
Water Temperature (C)	18.32 ± 2.7 μ mode 16.4 (12.4 -24.8)	μ 20.53 ± 3.7 mode 25.5 (11.9-31.4)
Water Depth (m)	μ 0.64 ± 0.27 mode 0.5 (0.25-1.5)	μ 0.41 ± 0.37 mode 0.25 (0.05-1.70)

and Figure 6). The difference between mean water temperatures in plots with tadpoles and without tadpoles was small (1.1 C) but the mode for plots without tadpoles was 8.1 C higher (Table 12).

The difference between mean water depths in plots with and without tadpoles was small (0.12 m) but the modal depth was substantially (0.25 m) lower for sites without tadpoles (Table 12). Sixty three percent of the sites with tadpoles had water depths less than 0.5 m and 23.9 % of the sites with tadpoles had depths less than 0.26 m (Figure 7).

The mean dry-down date for plots with red-legged frog tadpoles present was mid-September. Although this variable was included in the Logistic Regression it did not prove to be a good predictor variable. The first dry-down date for a plot known to have CRLF tadpoles occurred in May, while 39.4 % of plots with tadpoles present were at sites that stayed wet year round (Figure 8).

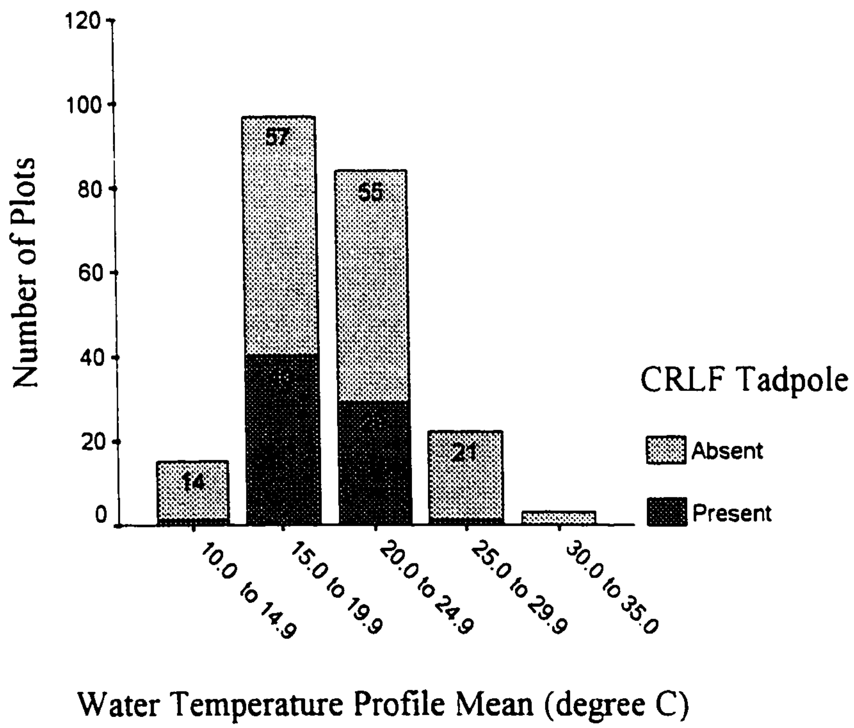


Figure 6. Occurrences of CRLF tadpoles at different mean water column temperatures.

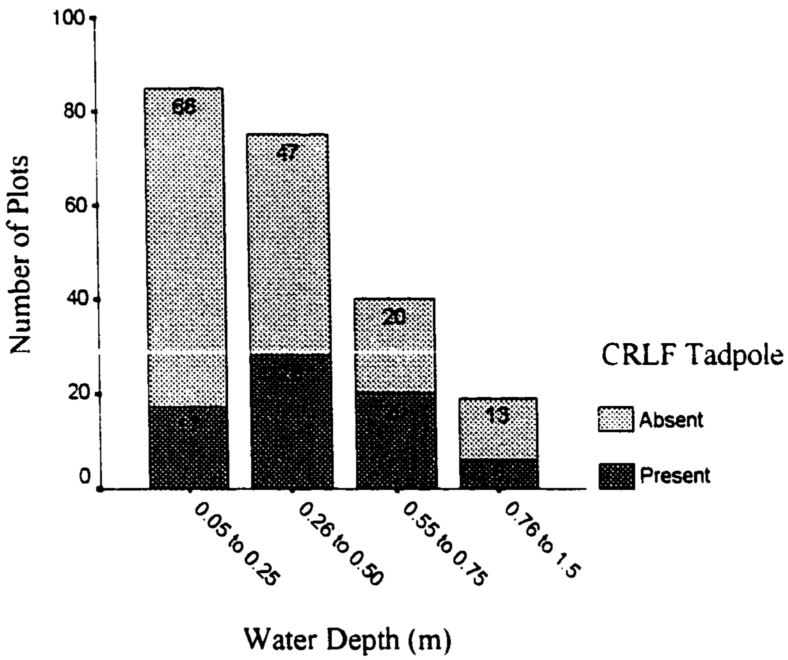


Figure 7. Occurrences of CRLF tadpoles at different water depths (m).

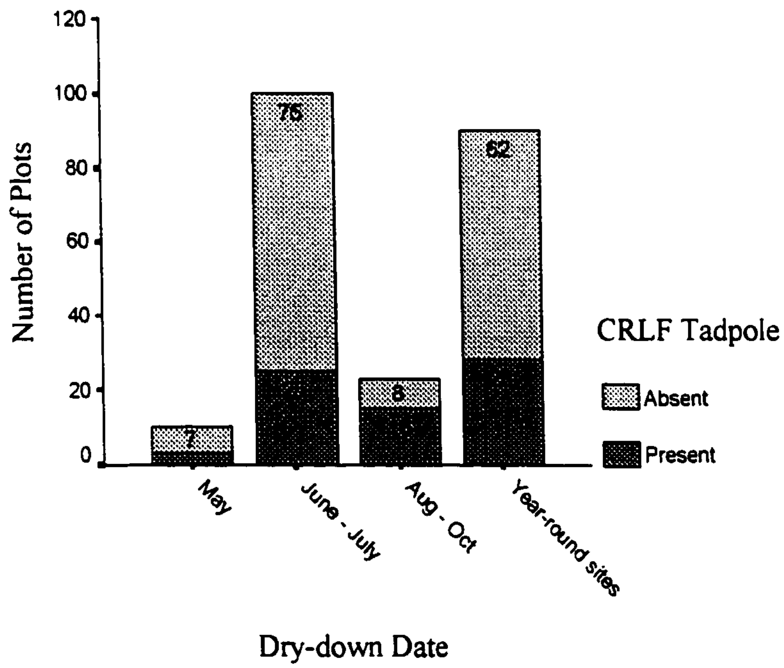


Figure 8. Occurrences of CRLF tadpoles in plots with different dry-down dates.

Site locations that were non-tidal freshwater, tidal brackishwater (no tidal gates or open tide gates), and non-tidal brackishwater (closed tidal gates) were all significantly associated with the presence of red-legged frog tadpoles (Hierarchical Log-linear $p<0.001$). Sample plots with tadpoles present occurred most often in brackishwater plots with open tidal gates (Figure 9), but non-tidal brackishwater sites had the highest proportion of tadpole occurrence. During the time of the study, tidal regimes were interrupted during the late summer when a sandbar formed at the lagoon mouth and remained in place until late fall. This stopped tidal flow into areas with open tidal gates,

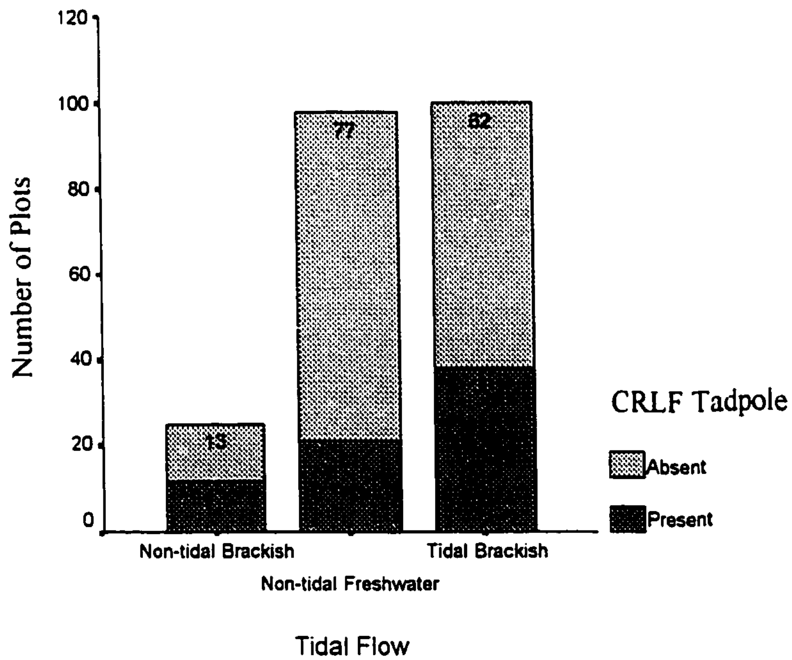


Figure 9. Occurrences of CRLF tadpoles in plots with different tidal flow conditions. Non-tidal brackish water sites occurred in sites with tidal gates that were closed until late July. Non-tidal freshwater plots included freshwater ponds and upstream sites. Tidal brackishwater plots did not have tide gates and were open to tidal flow.

and backed-up fresh water coming down from Pescadero Creek and Butano Creek. The tidal brackish sites were subject to occasional high salinity levels at very high tides before the sandbar closed.

The Hierarchical Log-linear showed that there was no significant association between mud depth or pH levels and the presence or absence of CRLF tadpoles. Therefore these variables were eliminated from the Logistic Regression Analysis.

Plant Species and CRLF tadpoles.-The most significant predictor of CRLF tadpole presence was pondweed, especially pondweed at high percent cover levels (Table 8 and Figure 4). CRLF tadpoles occurred more often when some pondweed was present (Table 10). Further, the greatest proportion of plots with both pondweed and CRLF tadpoles present was when the percent cover of pondweed was high (Figure 4). The next most significant predictor of CRLF presence was the presence of cattails (Table 8 and Figure 10). However, there was no difference in interaction at low, medium or high levels of cattail cover.

The proportion of plots with both bulrush and tadpoles present was greater than the proportion of plots with both tadpoles and bulrush absent. However, the number of plots with both bulrush and tadpoles absent was far greater than when both were present (Figure 11). Therefore, bulrush was not a significant predictor for the presence of CRLF tadpoles (Table 8). However, when bulrush was included in the Logistic Regression, the model predicted the absence of CRLF tadpoles better, but at the cost of a lower prediction fit for CRLF presence.

There was no association between the presence of algae and the presence of CRLF tadpoles (Hierarchical Log-linear main effect), and there was no relationship with tadpoles and algae at low, medium or high levels. However, there was a significant positive association between the type of algae present (green surface algae) and CRLF

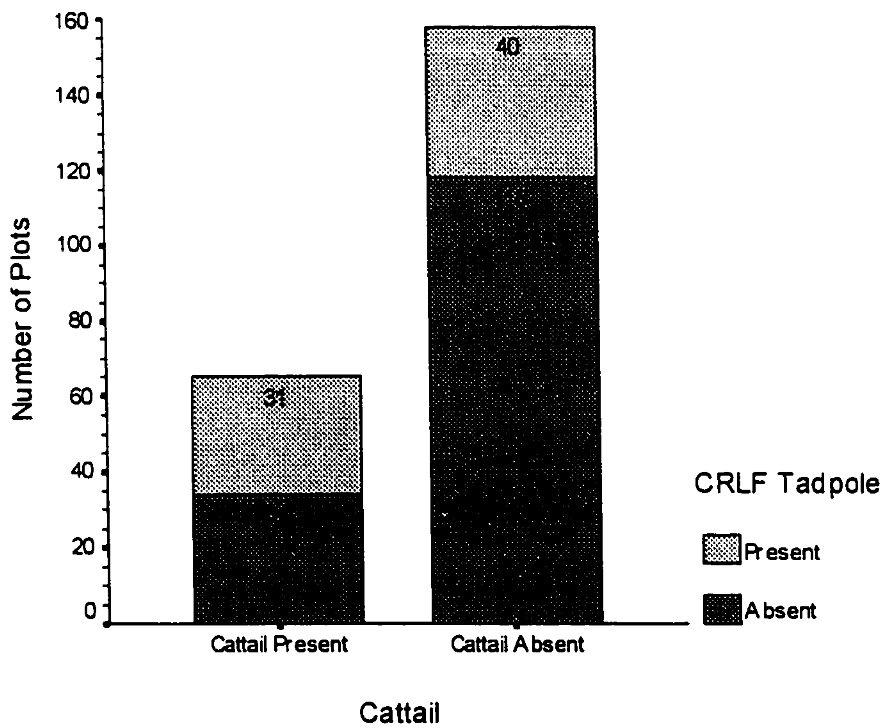


Figure 10. Occurrences of CRLF tadpoles with the presence or absence of cattails.

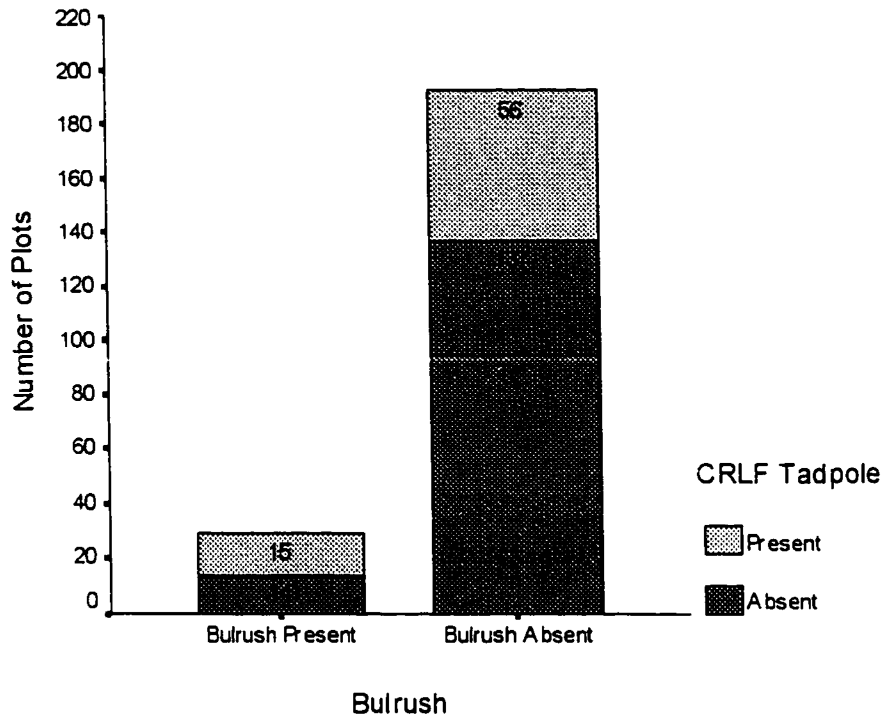


Figure 11. Occurrences of CRLF tadpoles with the presence or absence of bulrush.

tadpoles (Hierarchical Log-linear $p<0.001$) (Figure 12). Sites with dying discolored algae were much less likely to have tadpoles.

CRLF tadpoles were found in plots that contained abundant pickleweed (Figure 13). However, the Hierarchical Log-linear Analysis showed that the absence of CRLF tadpoles was significantly associated with the presence of pickleweed. Further, tadpoles were absent more often when percent cover of pickleweed was at a medium level (Hierarchical Log-linear $p= 0.021$). Although pickleweed was associated with egg masses, there was no positive association with tadpoles. There were no plots with both

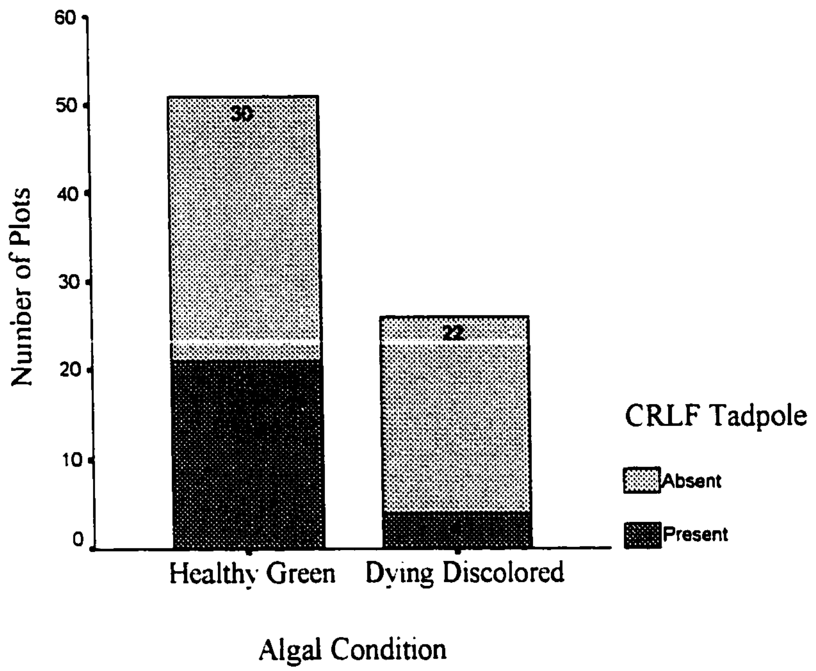


Figure 12. Occurrences of CRLF tadpoles with condition of surface algae.

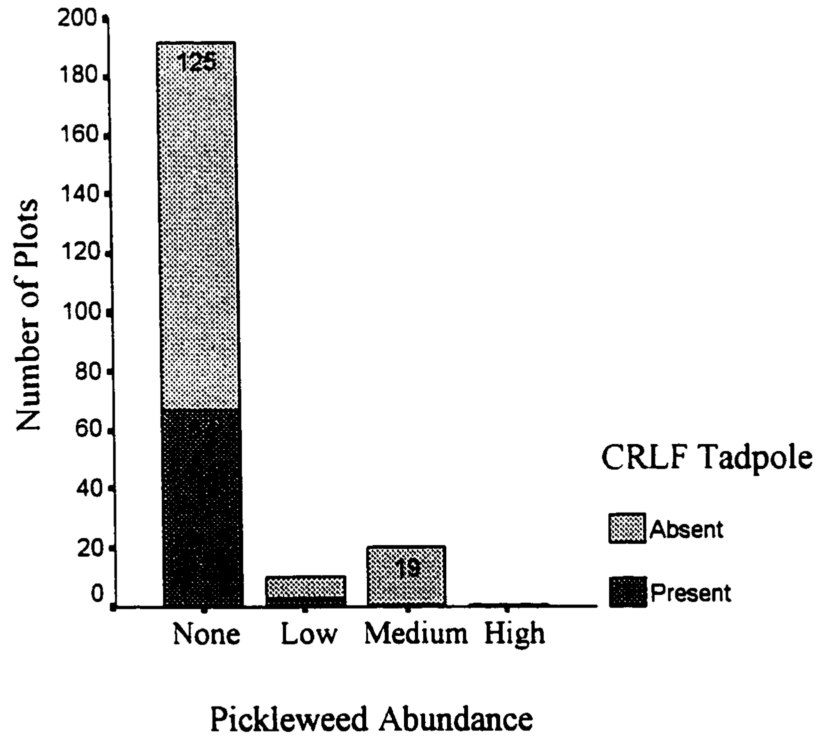


Figure 13. Occurrences of CRLF tadpoles at different abundance levels of pickleweed.

CRLF tadpoles and willows, and only nine plots without CRLF tadpoles and willows. In addition, there were only a total of seven plots that contained duckweed (two with tadpoles present). It is therefore concluded that pickleweed, duckweed and willows were not important to the presence of CRLF tadpoles at Pescadero Marsh.

Plants in the sedge family had no interaction with either the presence or absence of CRLF tadpoles (Hierarchical Log-linear main effect $p < 0.001$). There were no significant associations with CRLF tadpoles when percent cover bulrush, Cyperacea and cattails were categorized into low, medium or high percent cover.

Other Aquatic Animals and CRLF Tadpoles.-Bullfrog adults (*Rana catesbeiana*) were significantly associated with the presence of CRLF tadpoles (Hierarchical Log-linear $p < 0.001$, Table 11). Bullfrog tadpoles and juveniles were observed only in the freshwater portion of Butano Creek. Except for the freshwater portion of Butano Creek, bullfrogs did not appear to be reproducing in this system. No calling was heard during the night surveys for frogs and no egg masses were found. Sampling for bullfrog tadpoles in the late summer could not be conducted due to the federal listing of CRLF. However, freshwater habitats suitable for bullfrog tadpole development were scarce; most of the study plots either dried down by late fall or, if they were year round sites, they had high salinity concentrations during the late summer or winter months. CRLF frog adults, as well as all life history stages of Pacific treefrogs were significantly associated with the presence of CRLF tadpoles (Hierarchical Log-linear $p < 0.001$, Table 11). However there were differences in the distribution of CRLF tadpoles and Pacific treefrogs within the marsh.

The proportion of plots without CRLF tadpoles was greatest when predaceous invertebrates where present (Figure 14). One-hundred and twenty-nine sampled plots had fish. Fish species included three-spinned stickleback (*Gasterosteus aculeatus*), mosquito fish (*Gambusia affinis*), and tidewater Goby (*Eucyclogobius newberryi*). The Hierarchical Log-linear showed that there was no association between the presence of fish and the presence of CRLF tadpoles (Hierarchical Log-linear main effect).

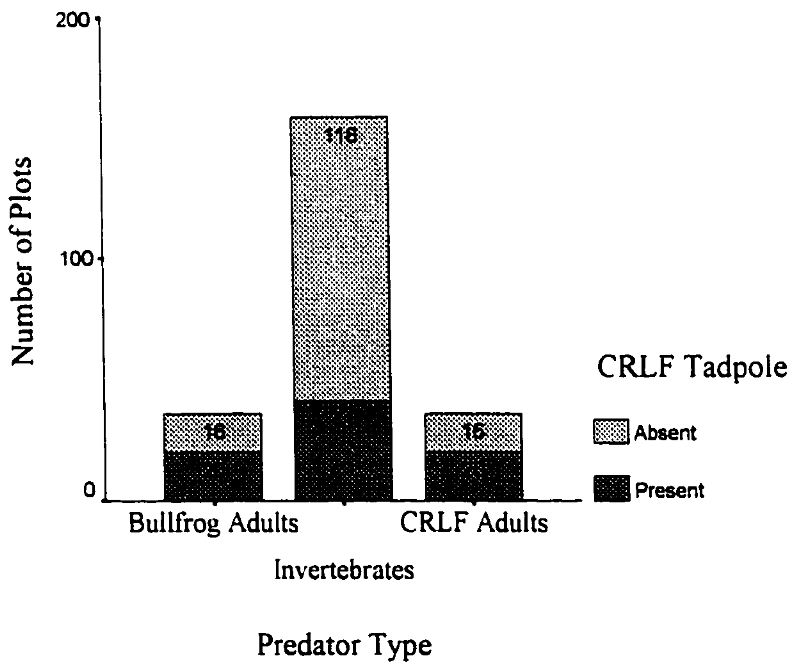


Figure 14. Occurrences of CRLF tadpoles with different potential predator types.

Habitat Characteristics Associated with the Presence or Absence of Adult CRLF and Their Value as Predictors.- The Logistic Regression indicated that none of the habitat variables, or combinations of habitat variables, that was tested against the constant-only model was not statistically reliable, X2 (8, N = 223) = 8.45, p> 0.05. Therefore, as a set, these predictors did not reliably distinguish between plots with and plots without CRLF adults. Further, the Hierarchical Log-linear Analysis results indicated that there were no significant associations between CRLF adults and either the presence or absence of individual plant species. Also, there were no significant associations when plant species type was lumped, as adults occurred in nineteen plots without emergent vegetation and in twenty plots with emergent vegetation. Hierarchical Log-linear analysis results did not show any positive associations between adult frogs and water depth, water salinity or water temperature. Table 12 provides a summery of the means, modes and ranges for plots with and without adult CRLF.

Differences in the Distribution and Habitat Utilization Between CRLF Adults, Tadpoles, and Egg masses.-Tadpoles were found over a greater area of the marsh than where egg masses. The CRLF tadpoles were located in all macro-habitat areas where egg masses were also found. However, tadpoles were also found in the following additional areas: East Delta Marsh, East Butano Marsh, Trout Pond One and in the east-end of the North marsh irrigation ditch which parallels Pescadero Creek (Figure 1). The distribution of adults and tadpoles were also different. Adult frogs were not found in the shallow, brackishwater regions in the middle of North Marsh and in the middle of East Delta

Marsh, but these sites contained both CRLF eggs and tadpoles. Adult frogs were observed in four freshwater locations were tadpoles or egg masses apparently did not occur, including along Pescadero Creek, Butano Creek, in a sag pond in North Butano Marsh, and in Trout Pond Three (Figure 1). The locations along Pescadero Creek and Butano Creek are perennial freshwater habitats, but the sag pond in North Butano Marsh and Trout Pond 3 are ephemeral ponds.

DISCUSSION

One of the challenges in this study was to identify and remove potentially non-independent habitat variables from the large number of measured variables to meet the assumption of independence required by the analytical techniques. The sampling design and analytical techniques I used yielded results not generated by previous studies, because they allowed me to distinguish between meaningful habitat descriptors and merely coincidental factors in relation to CRLF occurrences. The two techniques used to identify non-independent habitat characteristics were correlation analysis (for continuous data) and log linear analysis (for discrete data). The simplification of the list of habitat variables made it easier to recognize specific habitat characteristics (or combinations of characteristics) which were then associated with CRLF. If non-independent habitat variables were to be included, problems can occur where an association is falsely made between CRLF and a habitat characteristic. For example, while adult CRLF are known to occur in habitats that contain willows (Jennings and Hayes 1988), describing the presence of willows as an important habitat characteristic for frogs depends upon a testing procedure that evaluates if willows are independent of total vegetation cover; adult frogs may require some form of vegetation as escape cover, but not specifically willows. In addition, using a random plot design in this study allowed for the comparative analysis of plots with and without adults, tadpoles, and eggs, so that basic assumptions regarding what comprises frog habitat could be tested. Further, for each variable I tested a wide range of values, which allowed for analysis of the different habitat preferences at each life-history stage.

Egg mass habitat was characterized by warm, shallow water and the presence of either free-floating or emergent vegetation. Temperature and depth were independent; combining data on the two variables increased the ability to accurately predict egg masses sites, with water temperature ranking as the most significant predictor. Adult frogs selected warm, shallow water for egg deposition over cooler or deep-water locations. It is often assumed that shallower habitats have warmer water temperatures (Cole 1994). However, freshwater inflow, fetch, and shade can decrease water temperature in shallow water areas (Cole 1994). The only plant species observed to be selected for egg mass attachment was pickleweed, but further analysis showed that the pickleweed was a subset of water depths of ≤ 0.30 m. Pescadero Marsh is the only locality where CRLF eggs have been found in pickleweed. However, the MANOVA indicated that adult frogs were selecting for shallow water rather than pickleweed for laying eggs.

The possibility that egg masses occurred in deeper locations can not be ruled out. Although all egg masses observed were either at the surface or within 0.1 m of the surface, the ability to locate egg masses in deeper water was impaired by poor water clarity (0.1 m to 0.25 m Secchi depth). However, during the El Niño storms of 1998, a levee cracked on one side of an artificial pond at Elkhorn Slough in Monterey County, causing the depth to drop a total of 1.2 m. I was the able to measure water depth and note the age of exposed CRLF egg masses at each 0.3 m drop in depth was the water drained from the pond. I found CRLF egg masses, clearly a few days old, attached to vegetation at depths ranging from the surface to 1.0 m below the original waterline (personal observation).

The use of free-floating and emergent vegetation as substrate for egg mass attachment has several possible ecological explanations. Hayes and Krempels (1986) found that during the breeding season almost all male CRLF call above water from shallow, vegetated water sites, presumably to announce their presence. It has been suggested that the selection of vegetation for egg mass attachment may be an artifact of the need for vegetation as a refuge from predation during reproduction (Hayes and Krempels 1986, Briggs 1987, Hayes 1984). In addition, eggs attached to free-floating vegetation were protected from desiccation as water levels receded. Also, eggs floating at the surface may benefit from higher water temperatures. Another possible interpretation for egg mass attachment to vegetation is that mating adults do not require vegetation for egg mass attachment, but attached egg masses may be more visible and thus easier for the surveyor to locate.

CRLF tadpole habitat in Pescadero Marsh, like egg mass habitat, was best described by a combination of characteristics. Tadpole habitat was characterized by the presence of pondweed and cattails, water with salinity less than 6.5 ppt, water temperatures between 15.0 C and 24.9° C, and water depths no greater than 0.75 m. Of these characteristics, the abundance of pondweed was the most important. The presence of bulrush added to the predictability of tadpole absence.

CRLF egg masses and tadpoles, but not adults, were found in the shallow, brackish-water regions of the middle of North Marsh and in the middle of East Delta Marsh. The absence of adults indicated that they briefly used these areas for reproduction and then left. Without more intensive surveys during the reproductive periods, they are unlikely to be observed. Instead, I found adult frogs most often in locations with mean water depths of 0.64 meters. Further, all sixteen plots where adult CRLF were found, but tadpoles were absent, contained deep water during the reproductive season. Areas with adult frogs only included the freshwater habitats along Pescadero and Butano creeks, a sag pond in North Butano Marsh, and Trout Pond Three (Figure 1).

Sampling for tadpoles was more reliable than sampling for egg masses in confirming reproductive habitat. I found CRLF tadpoles at all but five of the egg mass sites and at thirty additional locations where egg masses were not found. I concluded that either the eggs or the newly hatched tadpoles did not survive at the five egg mass locations, or that low tadpole densities or sampling difficulty prevented tadpole captures. Part of the increased success for locating tadpoles was due to the prolonged sampling period for tadpoles compared to the limited six-day to two-week sampling (depending on water temperature) to locate an egg mass before embryos hatch (Storm 1960). If this survey was based on plots with egg masses alone, large portions of the marsh would have been omitted as reproductive habitats, including East Delta and East Butano marshes, Trout Pond One, and the east end of North Marsh irrigation ditch, where it parallels Pescadero Creek (Figure 1).

There are several plausible explanations for the high association observed between CRLF tadpoles and pondweed (*Potamogeton* spp.). First, *Potamogeton* is known to produce high rates of oxygenation relative to most aquatic plants (Prescott 1980). The respiratory rates of frogs are known to increase with their developmental rates (Connon 1947); thus *Potamogeton* beds may aid tadpole development. Secondly, *Potamogeton* is a highly branched, rooted aquatic plant that often develops into dense mats throughout the water column, providing structural cover for CRLF tadpoles. It is likely that this structural cover provides a refuge from aquatic predators or from species such as mosquito fish, which are known to weaken CRLF tadpoles by chewing on their tails (Lawer *et al.* 1997). In addition, the *Potamogeton* or invertebrates associated with it, may serve as a food source for tadpoles. Calef (1973) observed that *R. a. aurora* tadpoles were found more often and in higher abundance in the *Potamogeton* beds of freshwater ponds and lakes than elsewhere.

The association between the presence of cattails and CRLF tadpoles is more difficult to explain. The presence of cattails was independent of water depth, the dry-down date, water temperature, and water salinity during the tadpole season. In addition, the goodness-of-fit test showed that selection for cattails as an attachment type for egg masses was less than expected; therefore, the association between the presence of cattails and tadpoles was not an artifact of egg mass habitat. It may be possible that CRLF tadpoles were foraging on algae that I observed attached to the cattail stems. Dickman (1968) noted that *Rana aurora a.* larvae feed on epiphytic filamentous green algae of the genius *Mougeotia*. CRLF tadpoles may have a similar foraging ecology. Calef (1972) suggests that water depth may indirectly be of great importance to frog larvae, as the standing crop and productivity levels of benthic algae can vary greatly with water depth. Studies on the foraging ecology of CRLF tadpoles, including investigations on the use of pondweed, periphyton on submerged cattail stems, and benthic algae as forage types, are greatly needed.

My findings indicated that there was no salinity preference by either egg masses or tadpoles, as long as salinity values did not reach the lethal levels reported by Jennings and Hayes (1990). All egg mass sites tested had relatively low salinity levels, with the highest observed level at 3.8 ppt. As a high proportion of the eggs within each mass appeared to be normal at all locations, I assumed that CRLF eggs can undergo normal development at these levels. In controlled experiments Jennings and Hayes (1990) found that CRLF embryos and larvae in pre-hatching stages within the eggs required water at salinity levels less than 4 ppt for survival, and that prolonged exposures to salinity levels of more than 4.5 ppt caused 100% mortality within the egg mass. During this study, salinity levels increased later in the season after embryos had developed and hatched into free-swimming tadpoles. During the period when tadpoles were present, I found that tadpoles occurred most often in brackish water plots with open tidal gates, but that salinity levels of 6.6 ppt or greater were associated with the absence of CRLF tadpoles. However, tadpoles showed no preference among salinity levels below 6.6 ppt. Jennings and Hayes (1990) have shown that CRLF tadpoles will die during prolonged exposure to salinity levels of 7.5 ppt or greater. With the exception of one plot, all tadpoles in this study were in plots with salinity levels below 7.5 ppt. I did locate tadpoles in a single plot with a mean salinity level of 9.9 ppt (Figure 6), but I was unable to determine their long-term survival, as my sampling was terminated with the federal listing of CRLF. However, the tadpoles in this one plot appeared lethargic, with cloudy white eyes, and they may not have survived at this salinity level. Lethal salinity concentrations for tadpoles are probably not fixed, but are likely dependent on tadpole size and relative surface area.

Adult CRLF frogs clearly preferred warmer water sites for egg deposition , even at the cost of slightly higher salinity levels for developing embryos. In this study, water temperatures adjacent to egg masses had a mean of 20.4° C, compared to the 16.8° C surface water mean at sites without egg masses. However, the cooler locations without egg masses were within known ranges for developing CRLF eggs, as Cook (1997) found healthy CRLF egg masses in water with temperatures ranging between 7.5° C and 13.7° C. Likewise, salinity levels were higher at sites with egg masses (mode of 2.3 ppt) than at sites without egg masses (mode of 0.1 ppt).

There have been no studies investigating thermal maximums or optimal water temperatures for either CRLF eggs or tadpoles. Adult CRLF die of heat exposure at 29° C (Jennings and Hayes 1990), but embryos and tadpoles may be more sensitive because of the greater relative surface-area-to-volume ratio of their smaller bodies. I found healthy CRLF frog embryos in water with temperatures as high as 21.7° C. I also found that water temperatures between 15.0° C and 24.9° C best predicted CRLF tadpole presence. Tadpoles appeared to be healthy at water temperatures of 24.9° C, suggesting their thermal maximum is even higher.

Further studies of thermal minimums and maximums specific to CRLF at each life-history stage are needed. The water temperature data in this study is limited; maximum and minimum temperatures were not recorded, nor were temperatures recorded over time. Furthermore, thermal minimums and maximums for tadpoles are not expected to be fixed, but most likely vary with tadpole size. Studies of embryo temperature tolerances may also be complicated, as temperature tolerances of embryos are known to change with age or duration of exposure (Brown 1969, Licht 1970, Zweifel 1977). For example, embryos of R. *pretiosa* and *R. a. aurora* can survive short-term exposures to temperatures that become lethal at longer exposures (Licht 1970, Zweifel 1977). In shallow areas water warms quickly and the temperature remains elevated for several hours after dusk. In such areas both embryos and tadpoles can undergo several hours of rapid development before being exposed to cold night temperatures (Licht 1970). Also, temperature tolerances change with oxygen availability (Licht 1970). Further studies specific to CRLF embryos and tadpoles are needed, where oxygen availability and daily temperature fluctuations are recorded over time at different development stages.

Even though CRLF are thought of as a cold adapted frog (Dumas 1966), the exploitation of warm-water sites has several advantages for developing embryos and tadpoles. Small changes in water temperature are known to result in large developmental effects in frog embryos (Licht 1970, Zweifel 1977) and tadpoles (Hayes *et al.* 1993). For example, northern red-legged frogs kept at a constant temperature of 15.6° C began hatching in 11 to 12 days, but eggs kept at a constant 18.3° C began hatching in 8.5 to 9 days (Storm 1960). Once embryos complete gastrulation (Gosner stage 11), development proceeds especially rapidly with only small increments of increased water temperatures, shortening the time required for development to a free-swimming tadpole (Licht 1970). Therefore, exploitation of warmer water temperatures that are close to, but less than the thermal maximum, can increase developmental rates. Increased development rates may provide several advantages. Stationary eggs and small tadpoles are much more vulnerable to predation compared to larger tadpoles. Warm-water sites in ephemeral marshes and ponds may also be advantageous for development as warmer water temperatures shorten the time before tadpoles reach metamorphosis in habitats that provide a shorter potential growing season due to late summer drying or increased salinities. CRLF breed very early in the year and may move away from water during the non-breeding season (Stebbins 1985). CRLF occurring in the foggy coastal ranges, like northern red-legged frogs, are commonly found away from water in damp, herbaceous vegetation up to 1,000 yards from water (Dumas 1966, Scott pers. comm. 1997).

Recognition of the differences in utilization of different water depths by eggs, tadpoles, and adults is important for comprehensive management efforts for this species. CRLF egg masses successfully produced tadpoles at Pescadero Marsh in water ranging between 0.1 and 1.0 m, with a mean of 0.3 m. Sixty-three percent of the sites with tadpoles had water depths less than 0.5 m, and 23.9 % of the sites with tadpoles had depths less than 0.26 m. Cook (1997) also found CRLF egg masses and tadpoles in shallow water. In addition, the exploitation of shallow-water habitats (0.075 m to 0.152 m) for reproduction has been noted for some populations of the northern subspecies, *R. a. aurora* (Storer 1925). Potential adaptations of the use of shallow-water habitats may also include the escape from predators, such as fish and large aquatic insects associated with deeper waters. I found that adult CRLFs were more likely to utilize areas of deeper water. Mean water depth for adult frogs was 0.64 m.

Jennings and Hayes (1988) found that adult CRLF in the Central Valley occurred in water at least 0.75 m deep. This has been translated to "CRLF *require* water at least 0.75 m deep" by the U.S. Fish and Wildlife Service in its listing document (Miller *et al.* 1996). This statement has been incorrectly interpreted by some biologists to be a requirement when assessing potential reproductive habitat for CRLF in the coastal ranges of California (personal observation). However, the basis for such a statement is inadequate in at least three ways. First, data for adult frogs has been extrapolated to egg mass and tadpole data. Secondly, the study from which these conclusions were derived did not look at the full spectrum of available habitats and, therefore, did not show preference or minimum depth requirements for adults. Third, findings from the warm Central Valley have been used for the cooler coastal ranges.

CRLF tadpoles were more likely to be absent when predaceous invertebrates were present (Table 11). I observed four incidents of predation by invertebrates, three by giant diving beetles and one by a dragonfly nymph. Similar predaceous invertebrates have been observed foraging on Northern red-legged frogs (Calef 1973). Water salinity, temperature, and water depth for each of these invertebrate types were within the same ranges observed for CRLF tadpoles, but some partitioning between water depths and invertebrate types occurred. Mean water depths for backswimmers (0.3 m) were shallower than that of CRLF tadpoles (0.5 m). In addition, when backswimmers were present, they were usually very abundant.

CRLF successfully reproduced in ephemeral freshwater habitats and seasonal brackishwater habitats in Pescadero Marsh that were not viable for bullfrog reproduction. Brackish-water habitats that were suitable for CRLF from February though July became too saline during the late summer for bullfrog reproduction. Adult bullfrogs were present in the upstream portions of Butano and Pescadero creeks and in farm ponds adjacent to these creeks. These adjacent agricultural ponds may be a continual source of bullfrog adults and tadpoles into the Pescadero Marsh Natural Preserve. I observed bullfrog tadpoles and juveniles only in the freshwater portion of Butano Creek in 1996. However, I heard no bullfrog calls during the night surveys and found no bullfrog egg masses. Bullfrog tadpoles usually require freshwater habitats that remain wet for a minimum of one year. Most of the study plots either dried down by late fall, or, if they were year-round sites, had lethally high salinity concentrations during the late summer or winter. There were clear associations between CRLF tadpoles and all life-history stages of Pacific treefrogs and no partitioning of habitats was detected in this study. Competitive and predatory relationships between CRLF and Pacific treefrogs are unknown.

In summary, CRLF eggs, tadpoles, and adults used habitats with different vegetation, water temperatures, water depths, and water salinities. Wildlife biologists should make management decisions that provide sufficient habitat for all life-history stages. Providing critical and optimal habitat for only a single life-history stage may result in the loss of populations.

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