EFFECTS OF *PSEUDACRIS REGILLA* TADPOLE DENSITY ON A CALIFORNIA VERNAL POOL COMMUNITY

A Thesis

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Ian Anderson

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Abstract

of

EFFECTS OF *PSEUDACRIS REGILLA* TADPOLE DENSITY ON A CALIFORNIA VERNAL POOL COMMUNITY

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Biotic interactions between herbivores and primary producers are believed to play a major role in determining community composition within many ecological communities. How differences in herbivore abundance alter the relative importance of these interactions is not well understood, especially in seasonal wetland systems. This study investigated the effects of varying *Pseudacris regilla* (pacific chorus frog) larval densities on California vernal pool aquatic communities. It was hypothesized that with increasing tadpole density, macrophyte abundance, biomass and richness would decrease; while algae and zooplankton abundance, biomass and richness increase.

The experimental mesocosm design consisted of five tadpole density treatments, and was arranged in a 5 x 5 randomized block design. Each density treatment was replicated five times. Macrophytes, algae and invertebrates were identified to the lowest possible taxonomic unit, and the biomasses of each community were quantified. Turbidity, dissolved oxygen, nitrate, orthophosphate, temperature, conductivity and pH were measured monthly throughout the experiment. Treatment effects were determined using both multivariate and univariate analysis of variance techniques. To detect

differences in community composition, multivariate ordination analyses (ANOSIM, SIMPER, nMDS) were conducted.

Increasing densities of *P. regilla* tadpoles had a large impact on the vernal pool macrophyte community. Plant abundance, diversity, biomass, percent cover and species richness were all negatively impacted by increasing tadpole densities. Macrophyte community structure was also found to be significantly different in the presence of tadpoles due to the fact that *Downingia bicornuta*, *Ranunculus aquatilis*, and *Gratiola ebracteata* were predominately isolated to mesocosms were *P. regilla* was absent. In addition, periphyton biomass was shown to be facilitated by increasing tadpole densities in the lower treatment ranges. Aquatic macroinvertebrates and phytoplankton communities, on the other hand, were not significantly affected by increasing tadpole densities. This study demonstrates the impact of *P. regilla* larvae on the primary producers within California vernal pool systems. Understanding the impact of these abundant consumers, and the mechanisms that drive the importance of their direct and indirect effects are essential for the conservation of vernal pools and the threatened species associated with them.

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INTRODUCTION

Global biodiversity has been declining due to human overexploitation and habitat destruction for decades (Millenium Ecosystem Assessment, 2005). Despite the intervention of the global community, human pressures on biological diversity continue to increase (Butchart et al., 2010). Loss of biodiversity affects the functioning of ecosystems and the goods and services they provide (Daly et al., 2000). It can also negatively affect the stability of biological communities through alterations of biotic interactions (Yachi & Loreau, 1999; Bruno et al., 2003). To better understand some of the possible effects diversity losses can have on a biological community, a greater understanding of biotic interactions and the role they play in structuring communities is needed (Thompson, 1996; Price, 2002).

Biotic interactions between pairs of species play an important role in structuring species composition and abundance within many ecological systems (Wootton, 1994). These biotic interactions can be classified as direct, involving pair-wise interactions between species, such as interference competition, mutualisms, and predator-prey interactions (Wootton, 1994), or indirect, which occur when the interaction between two species is mediated by a third (Miller & Kerfoot, 1987; Strauss, 1991; Menge, 1995). Examples of indirect interactions are exploitative competition (Colwell & Fuentes, 1975), trophic cascades (Carpenter et al., 1985), apparent competition (Holt, 1977), indirect mutualisms (Dethier & Duggins, 1984), and interaction modifications (Crowder & Cooper, 1982).

The importance of indirect interactions and their role in structuring ecological communities was first demonstrated by Hairston et al. (1960), who hypothesized that the world is green because predators regulate the abundance of producers indirectly through the consumption of herbivores. This trophic cascade hypothesis has been supported by many studies and has been shown to have positive and negative effects on organisms within both terrestrial and aquatic communities (Paine, 1980; Carpenter et al., 1985; Power, 1990; Marquis & Whelan, 1994).

Indirect interactions have been shown to occur in a variety of terrestrial communities, but tend to have a greater impact in aquatic ecosystems (Shurin et al., 2002). In a subtidal marine community, Schmitt (1987) demonstrated apparent competition between two marine mollusks. Increases in the abundance of bivalves lead to the decline of grazing gastropods due to an increase in invertebrate predators. Carpenter et al. (1985) showed that lakes could be turned from an algae dominated turbid state to a clear water state, through the manipulation of a top predator. Dodson (1970) performed an observational study in small permanent ponds and detected an indirect mutualistic relationship between larval salamanders (*Ambystoma tigrinum)* and phantom midges (*Chaoborus*). Larval salamanders were observed to feed on large predatory copepods, which indirectly benefited the phantom midge population.

Indirect effects have been demonstrated to have a profound impact on the structure and function of many ecological communities. Most of the research in this area has focused primarily on terrestrial or permanent aquatic systems. There is however, a

lack of research addressing the possible impact of indirect effects on temporary freshwater ecosystems, including vernal pool communities (Wilbur, 1997).

Vernal pools are precipitation-fed seasonal wetlands that cycle through four stages: a wetting phase, an aquatic phase, a water-logged terrestrial phase, and a drought phase (Keeley and Zedler, 1998). In California, winter rains initiate the wetting phase by providing precipitation to fill the pools. Vernal pools can remain inundated until late April to early May, depending on pool size and the amount of rainfall. As the pools begin to fill, the aquatic phase begins. During this phase, specialist invertebrate species lying dormant as cysts emerge. Some species of aquatic plants also begin to emerge during the wetting phase (Bauder, 1987), whereas others require inundation to initiate germination (Keeley, 1988). At the end of spring, the rains cease, pools dry, and the permanent invertebrate residents return to their resistant life stages in order to survive the water-logged terrestrial phase and the extreme conditions of the drought phase (Wiggins et al., 1980).

Due to their temporary nature and isolation from other bodies of water, vernal pools are free from large predators (i.e., fish) often associated with more permanent waters (Williams, 1987). Invertebrate predators are common within these temporary pools and can have a large influence on the composition and abundance of organisms within the aquatic community. Typically, invertebrate predators are absent early in the aquatic phase and increase in frequency towards the end of the aquatic phase (Schneider and Frost, 1996). It is generally during this time of reduced predation pressure that amphibian species that exploit vernal pools begin to colonize (Skelly, 1996).

Vernal pools in the California Central Valley have historically supported four amphibian species: california tiger salamander (*Ambystoma californiense*), western spadefoot toad (*Spea hammondii*), western toad (*Anaxyrus boreas*), and the pacific chorus frog (*Pseudacris regilla*). However, recent estimates of the abundance and distribution of these amphibian species in the Sacramento Valley vernal pool region illustrate that most of these species are in steep decline or virtually extirpated from this area (Collins et al., unpublished data; Fisher and Shaffer, 1996). Of these amphibian species, pacific chorus frogs are the only one that is not in decline. Today, it is often the most abundant, if not the only amphibian species, found within California's vernal pools (Zedler, 1987; T. Collins, personal communication, September 27, 2011; Collins et al., unpublished data).

In the Sacramento Valley vernal pool region, pacific chorus frog adults typically begin to breed in late winter to early spring depending on the amount of rainfall (Collins et al., unpublished data; L-A. Spencer-Hartle, personal communication, September 15, 2011; personal observation). Eggs are deposited in clusters of 9-80 eggs on submerged vegetation or on the bottom of the pool (Stebbins, 1985; Hollingsworth and Roberts, 2001). Pacific chorus frog adults have extended reproductive periods, and females are able to produce three or more clutches per season (Perrill and Daniel, 1983). It takes from five days to five weeks for the embryos to develop and hatch, depending on external temperatures (Nussbaum et al., 1983; Leonard et al., 1993).

After hatching, *P. regilla* larvae require pools with a hydroperiod of at least 5 weeks to support successful metamorphosis (Morey, 1998). Wassersug (1976) considered

P. regilla to be non-discriminatory suspension feeders that feed primarily on green algae, blue-green algae, protozoa, bacteria, diatoms, organic and inorganic debris. Although population levels of *P. regilla* larvae tend to fluctuate from year-to-year and from site-to-site, mean densities are often between 0-100 individuals/m (Zedler, 1987; Kupferburg, 1997, 1998; M. Benard, personal communication).

Because tadpoles often occur at relatively high densities in California vernal pools (Zedler, 1987) and have high rates of ingestion, they may be the top primary consumer within this ecosystem (Dickman, 1968; Morin et al., 1990; Lamberti, 1992). Given the oligotrophic nature of vernal pool systems, food resources are a limiting factor. This can lead to increased competition among conspecifics and other herbivores.

Species that tend to have strong direct effects in ecosystems are more likely to exhibit strong indirect effects in these same systems (Strauss, 1991). Alford (1989) demonstrated that tadpole density can affect the growth rates of larval newts through competitive interactions between tadpoles and zooplankton, which are the newt's prey. Tadpoles reduced zooplankton populations, and in turn reduced the growth rate and survival of larval newts. Although this type of indirect competitive interaction may be important in shaping vernal pool communities, current research is lacking.

Pacific chorus frog tadpoles may also affect vernal pool communities through bioturbation (Francois et al., 1997). As tadpole density increases, bioturbation can result in increased turbidity levels due to the suspension of sediment within the water column (Osborne and McLachlan, 1985; Zedler, 1987), which can indirectly affect both biotic and abiotic elements of an ecosystem. Bioturbation has been shown to alter the rate of

carbon mineralization and oxygen exchange at the sediment water interface, which can stimulate the growth of benthic microbial communities (Mermillod-Blondin et al., 2004). Increased turbidity due to bioturbation can also result in a reduction in light penetration, which can negatively affect aquatic macrophytes (Scheffer et al., 1993; Croel and Kneitel, 2011) and phytoplankton populations (Lougheed et al., 1998). Bioturbation can also increase species richness of vernal pool crustaceans by exposing buried cysts to cues that initiate hatching (Brendonck, 1996; Croel and Kneitel, 2011). On the other hand, there is some evidence that bioturbating species can negatively affect crustacean species richness due to their consumption of dormant cysts (Albertsson and Leonardsson, 2001; Persson and Rosenberg, 2003; Viitasalo, 2007). The emergence of zooplankton due to bioturbation will most likely depend on the organism performing the disturbance (Widdicombe and Austin, 1999; Gyllstrom et al., 2008). There appear to be no studies to date that have examined the possible impact pacific chorus frog tadpoles may have on the composition and abundance of vernal pool zooplankton.

Pacific chorus frog tadpoles can have a negative impact on aquatic plant growth, biomass, and diversity as tadpole density increases (Anderson, 2011, unpublished data). Tadpoles foraging on epiphytic algae attached to aquatic plants indirectly damaged the leaves, and in some instances uprooted the entire plant. The establishment of new plants was prevented by the foraging activity at the sediment-water interface, which killed young seedlings. The destruction of aquatic plants can indirectly lead to increased turbidity levels due to the resuspension of bottom materials that were once held in place by plants (Carpenter & Lodge, 1986; Engelhardt & Ritchie, 2001), and through algae

blooms that can result from increased nutrient availability (Scheffer et al., 2001; Declerck et al., 2007). The destruction of aquatic plants can also indirectly affect zooplankton through a reduction in habitat (Hosper, 1989), or reduce feeding ability due to soil particles clogging feeding filters under turbid conditions (Gerritsen & Porter, 1982).

The effects of increasing pacific chorus frog tadpole density on plant growth and turbidity levels are similar to those seen in other studies that have examined the impact of the common carp (*Cyprinus carpio)* on benthic communities (Parkos et al., 2003; Lougheed et al., 2004; Miller & Crowl, 2006). Common carp were witnessed mechanically damaging and uprooting aquatic plants during foraging activities (Kerfoot & Sih, 1987; Hinojosa-Garro & Zambrano, 2004), increasing turbidity through sediment resuspension (Roberts et al., 1995; Hamilton & Mitchell, 1997), preventing plant reestablishment (Hootsman et al., 1996; Hootsman, 1999), and negatively affecting aquatic invertebrate composition, richness, and diversity (Zambrano et al., 2001; Miller & Crowl, 2006). This type of density-mediated affect can have negative impacts on biodiversity and ecosystem functioning through a combination of direct and indirect interactions (Carpenter & Lodge, 1986; Jones et al., 1994; Engelhardt & Ritchie, 2002).

Pacific chorus frog tadpoles can potentially have a strong direct and ultimately indirect impact on the vernal pool ecosystem depending on larval densities. The extent of this impact and the role it may play in structuring the vernal pool community has not been addressed in past research. It is important to determine what possible effects fluctuating tadpole densities could have on California's vernal pool systems, and the endemic species that rely on them.

The main objective of this study is to examine the impact of varying pacific chorus frog larvae density on vernal pool plant, algae and aquatic invertebrate community structure. I hypothesize that as tadpole density increases, macrophyte biomass, abundance, and species richness will decrease, whereas zooplankton, periphyton and phytoplankton abundance, richness and biomass will increase.

MATERIALS AND METHODS

Study animals

Pacific chorus frog egg masses were collected from ephemeral grassland pools found in and around the city of Winters, California. This was the only anuran species included in the study because it is native to the region, occurs in high abundances, and is generally the only anuran species found in California vernal pools (Fisher & Shaffer, 1995). Egg masses were transported to California State University of Sacramento and housed in plastic containers (62.5 L) that contained de-chlorinated tap water (50 L) and a layer of vernal pool soil (0.5 cm). Vernal pool soil was acquired from the Gill Ranch Conservation Bank, Sacramento CA. The presence of vernal pool soil in the container served to prevent a haemorrhagic condition from developing in the gut after hatching (Brattstrom & Warren, 1955). To ensure that eggs were exposed to natural environmental conditions, the containers were stored outdoors in the California State University Sacramento Arboretum. After hatching, pacific chorus frog larvae were randomly assigned to treatment groups.

Experimental design

Twenty-five ovate plastic containers (0.6 m length, 0.5 m width, 0.3 m deep, total volume 56.8 L) were used as experimental mesocosms. Mesocosms were placed outdoors in the California State University Sacramento Arboretum, and arranged in a randomized 5x5 block design on 26 December 2011. To ensure a homogenous cyst and seed bank, vernal pool soil was crushed and thoroughly mixed using a cement mixer. Each

mesocosm received 9.1 kg of soil, which resulted in a soil layer roughly 3.8 cm deep. To reduce the loss of vertebrate and invertebrate study organisms during high rain periods (when the mesocosms could possibly overfill), fine aluminum screening (182.9 cm in length, 6.4 cm wide, with an aperture size of 1 mm x 1 mm) was attached to the perimeter of the mesocosm opening using a non-toxic silicon adhesive. Four microscope slides (7.6 cm x 2.5 cm x 1 mm) were attached (using non-toxic silicon adhesive) to the walls of the mesocosm to provide a measurable substrate area for the growth of periphyton. Two slides were attached to the north-facing wall of the mesocosm, while the other two were attached to the south-facing wall. Each slide was attached 3.8 cm above the soil-water interface.

Mesocosms were left exposed to natural environmental conditions and allowed to accumulate with water from rainfall throughout the winter and early spring of 2012. Due to unseasonably dry conditions, mesocosms only contained 7.7 cm of water by the end of January 2012. To simulate wetter environmental conditions, each mesocosm was filled using well water on February 1, 2012. A layer of bubble wrap was placed on the water surface to prevent the suspension of sediment particles while the mesocosms were being filled.

To coincide with natural colonization times (Leonard et al., 1993; Livezey, 1953; Anderson, 2011 unpublished data, Lu-Anne Spencer-Hartle, personal communication), pacific chorus frog tadpoles were introduced to mesocosms on 8 February 2012. Tadpoles were randomly assigned to one of 5 density treatment groups: (i) 0 tadpoles; (ii)

8 tadpoles (0.14 tadpole/L); (iii) 16 tadpoles (0.28 tadpole/L); (iv) 24 tadpoles (0.42 tadpole/L); or (v) 32 tadpoles (0.56 tadpole/L). Each treatment was replicated 5 times.

Water chemistry

Water chemistry was measured on a monthly basis (February, March and April 2012) until the end of the experiment in May 2012. An Oakton pH/CON 300 meter was used to measure pH, conductivity, and water temperature. Water samples (30 mL) were collected from each mesocosm and used to quantify turbidity and nutrient levels and in the laboratory. Turbidity levels were measured using a LaMotte 2020i turbidity meter. Nitrate ($N0_3^-$) and orthophosphate ($P0_4^{3-}$) concentrations (mg/L) were quantified by using a HACH DR 2800 spectrophotometer. A cadmium reduction (Hach method 8171) was used to estimate nitrate concentrations, and ascorbic acid (Hach method 8048) was used to estimate orthophosphate concentrations.

Aquatic invertebrate abundance and biomass

To determine the composition and abundance of the aquatic invertebrate community, a 20 cm x 15 cm, 500 μm aquarium net was gently passed through each mesocosm for 1 minute. Individuals were transferred from the net to a holding container for identification and enumeration. Individuals from orders Anostraca and Notostraca were identified to species. All other taxa were identified to class or order (e.g., Copepoda, Ostracoda, Cladocera, and Conchostraca).

While in the holding container, twenty individuals from each taxon were selected to determine mean dry weight biomass (mg) (EPA Great Lakes National Program Office,

2003). In order to preserve specimens for subsequent analysis, nondestructive indirect biomass estimation techniques were utilized (Baguley et al., 2004). Digital photos were taken of each individual in the chamber using a Dino-Lite AM-2011 Digital Microscope, and individuals were measured (mm) using ImageJ 1.45s software. Cladoceran length was determined by measuring from the top of the head to the end of the carapace. Copepod length was determined by measuring from the top of the head to the base of the caudal spines (Bottrell et al., 1976). Both *Branchinecta lynchi* and *Linderiella occidentalis* lengths were determined by measuring from the top of the head to the base of the tail (Bertilsson et al., 2003). Lengths were averaged for each taxon within each mesocosm.

Mean individual length was used to estimate biomass through the use of previously established dry weight-length relationships. Due to the coarse level of taxonomic identification, pooled equations were used for both cladocerans and copepods (Bottrell et al., 1976). Biomass of *Branchinecta* and *Linderiella* was determined using a regression equation developed for the related anostracan *Streptocephalus* (Mitchell, 1991; Bertilsson et al., 2003). Zooplankton identified as Conchostraca or Ostracoda were omitted from the biomass analysis due to lack of published length-weight regression equations. Mean individual biomass was multiplied by abundance to estimate total biomass of each taxon within a mesocosm.

Phytoplankton, periphyton and macrophyte community measurements

Phytoplankton samples were collected in late April 2012. In order to sample the phytoplankton community throughout the water column, an integrated water sampler was used (Straskraba and Javornicky, 1973). The integrated water sampler was composed of a flexible plastic tube (63.5 cm in length, diameter of 2.5 cm) with a cord (70 cm of 12 lb monofilament fishing line) attached to one end of the plastic tubing. The sampler was inserted into the mesocosm, and lowered to the sediment water interface. A plug was then inserted into the opening of the tube, and the water sample was collected. This procedure was repeated once per mesocosm. Water column samples were stored in opaque jars to prevent degradation.

Samples were preserved in a Lugol's iodine solution to prevent further degradation and aid in the algal sedimentation process (Bellinger & Sigee, 2010). Lugol's iodine was prepared by dissolving 15 g of potassium iodide and 5 g of iodine in 98 ml of distilled water. Glacial acetic acid (2 mL) was added to the solution and stirred for one minute. Lugol's iodine solution (3.75 mL) was added to 100 ml of each phytoplankton water sample. Samples were stored in the dark on a vibration-free surface for 72 hours to allow all of the algae to sediment. At the end of the waiting period, a transfer pipette was used to siphon off the top 80 mL of algae-free solution. The remaining 20 mL was re-suspended using a glass stir rod, and transferred to a 30 mL opaque high-density polyethylene container. Samples were stored at 4° C for one month, and then shipped to BSA Environmental Services for further analysis.

Phytoplankton samples were analyzed by the membrane filtration technique (McNabb, 1960; Beaver et al., 2012). Common taxa abundance was estimated by random field counts, while rarer taxa abundances were quantified by scanning a filter transect. A Leica DMBL compound microscope was used for enumerating filtered samples. At least 400 natural units (unicells, colonies, and filaments) were counted and identified to the lowest possible taxonomic level. Biovolume calculations were based on measurements of ten organisms per taxon, and estimated using solid geometric shape formulas that most closely resembled cell shape.

Periphyton samples were collected in late April 2012. Three microscope slides (2 from the south facing wall, and one from the north facing wall) were carefully removed from each mesocosm. Periphyton on the slides was harvested by brushing the surface of the slide and rinsing the brushed material with deionized water into opaque HDPE containers. Sample slurries were filtered onto precombusted and preweighed Whatman GFF filters (4.7 cm diameter, aperture size 0.7 micron) and dried in a Lab-Line oven at 40°C for 48 hours until a constant mass was reached. Filters were weighed using a Mettler AT201 balance, and periphyton biomass (g) was determined for each sample. Biomass measurements from each mesocosm were averaged to determine the overall periphyton biomass (g/cm).

Callitriche marginata floating rosettes were quantified once on March 26, and May 25, 2012. Counts of individual plants were not possible due to high turbidity, and high levels of cover created by floating rosettes in the zero tadpole density treatments. Assessment of the plant community was conducted on June 25, 2012, when standing

water was no longer visible on the sediment surface. Plants were identified to species when possible, and their abundances were quantified by visual observation. After complete desiccation of mesocosm sediment, and the completion of each plant's life cycle, above ground plant biomass (g) was measured by clipping each plant at the sediment level and drying them in an oven at 60°C to a constant mass. Plants were collected for biomass determination on June 11, 2012

Statistical analyses

To determine if there were significant differences in water chemistry measurements between treatments, and to determine if there were interactions between water chemistry variables, a multivariate analysis of variance (MANOVA: using IBM SPSS Statistics v20) was conducted. Conductivity, pH, water temperature (°C), dissolved oxygen, turbidity, nitrate ($N0_3^-$) and orthophosphate ($P0_4^{3-}$) were the dependent variables. Preliminary assumption testing was conducted to check for linearity (scatterplot matrix), normality (Mahalanobis distances), univariate and multivariate outliers, homogeneity of variance-covariance matrices (Box's M Test of Equality of Covariance Matrices), and multicollinearity (Correlations tested among dependent variables). If assumptions were violated, dependent variables were either log or square root-transformed to alleviate the problem.

Several diversity indices were calculated using taxonomic data sets from the plant, invertebrate, and phytoplankton community measurements. Richness was calculated by summing the number of taxonomic units (or lowest possible taxonomic classification) within each mesocosm. To quantify the relative abundance of rare and

common taxa within each mesocosm (evenness), Pielou's evenness (Pielou, 1966) and Shannon-Wiener indices (Shannon & Weaver, 1949) were calculated. To determine if significant differences in diversity indices, rosette abundance counts, and macrophyte cover existed between treatment groups, a mixed model two-factor analysis of variance (ANOVA) was conducted. Tadpole density was used as the fixed factor, and each mesocosm block was treated as a random factor. Tukey HSD tests were used for post-hoc comparisons. All ANOVA and MANOVA analyses were conducted using IBM SPSS Statistics v20.

To determine if significant treatment differences existed in the abundance and biomass of the plant, invertebrate and phytoplankton communities, multivariate ordination analyses were used (PRIMER version 5.1.2). Abundance and biomass data were square-root transformed to reduce the weight of common species, and a Bray-Curtis distance matrix was calculated to measure the similarity of community compositions between mesocosms. To visualize patterns of similarity among mesocosms, non-metric multidimensional scaling (NMDS) was conducted based on the similarity values of species composition. Subsequently, the effect of tadpole density treatments on community composition (using Bray-Curtis distances) was tested using an analysis of similarity (ANOSIM: PRIMER-E) test (Clarke, 1993). When ANOSIM revealed significant differences between groups, a similarity percentage (SIMPER: PRIMER-E) test was used to identify the species that contributed most to the differences observed among treatment groups.

RESULTS

Water chemistry

Tadpole density treatments did not have a significant effect on any of the water chemistry variables (pH, conductivity, temperature, turbidity, nitrate and orthophosphate) collected during the February, March and April sampling periods (Table 1).

Aquatic invertebrate community measurements

A total of 11 invertebrate taxa were found in the mesocosms (see Appendix A). Invertebrate species abundance and composition was not significantly different among tadpole density treatments over the two sampling dates (February: ANOSIM, R = 0.006, $P = 0.448$; March: ANOSIM, R = 0.057, $P = 0.234$). Furthermore, no significant differences in invertebrate biomass were observed among density treatments (February: ANOSIM, R = 0.081, $P = 0.448$; March: ANOSIM, R = 0.036, $P = 0.211$). Invertebrate richness, diversity and evenness were also not significantly different among density treatments (Table 2).

Phytoplankton and periphyton community measurements

A total of 50 phytoplankton taxa were identified within the mesocosms (see Appendix B). Phytoplankton density (cells/L) and biovolume (µm /L) were not significantly different among tadpole density treatments (ANOSIM, R = 0.11, $P= 0.06$; R = 0.091, $P = 0.10$, respectively).

Table 1. MANOVA results of water chemistry variables.

Sampling period	Pillai's Trace	*F*	*df*	Error *df*	*P*-Value
February	0.952	1.187	20	76	0.289
March	0.737	0.858	20	76	0.638
April	1.111	1.461	20	76	0.122

Table 2. ANOVA results of tadpole density treatment effects on invertebrate richness, Shannon-Wiener diversity and Pielou's evenness. *df* = 4; error *df* = 15

Variable	*F*	*P*
Richness		
February	0.722	0.590
March	0.898	0.490
Diversity		
February	0.641	0.641
March	0.474	0.755
Evenness		
February	0.495	0.740
March	0.336	0.836

There were no significant differences in phytoplankton richness among density treatments ($F_{4, 16} = 0.919$, $P = 0.477$). Furthermore, there were no significant differences in phytoplankton abundance and Shannon-Wiener indices among density treatments ($F_{4, 16} = 0.740$, $P = 0.578$; $F_{4, 16} = 0.273$, $P = 0.273$, respectively). There were also no significant differences in Pielou's evenness values among treatments ($F_{4, 16} = 2.788$, $P = 0.062$).

A significant difference in periphyton biomass was observed among tadpole density treatments (Figure 1; $F_{4, 16} = 3.762$, $P = 0.024$). Mean periphyton biomass in the zero tadpole density treatments was significantly lower than the 0.42 tadpoles/L treatment (Table 3). Periphyton levels were nearly 5 times lower in mesocosms without tadpoles.

Macrophyte community measurements

In the March sampling, abundance of *Callitriche marginata* floating rosettes differed significantly among tadpole density treatments (Figure 2; $F_{4, 16} = 13.87$, $P < 0.001$). Mean rosette abundance in the zero tadpole density treatments was significantly higher compared to the 0.14 tadpoles/L, 0.28 tadpoles/L, 0.42 tadpoles/L, and 0.56 tadpoles/L density treatments (Table 3). Floating rosettes were 4.5 times more abundant in the zero tadpole density treatment compared to the other treatments.

In the May sampling, abundance of *C. marginata* floating rosettes differed significantly among density treatments (Figure 3; $F_{4, 16} = 16.528$, $P = 0.01$).

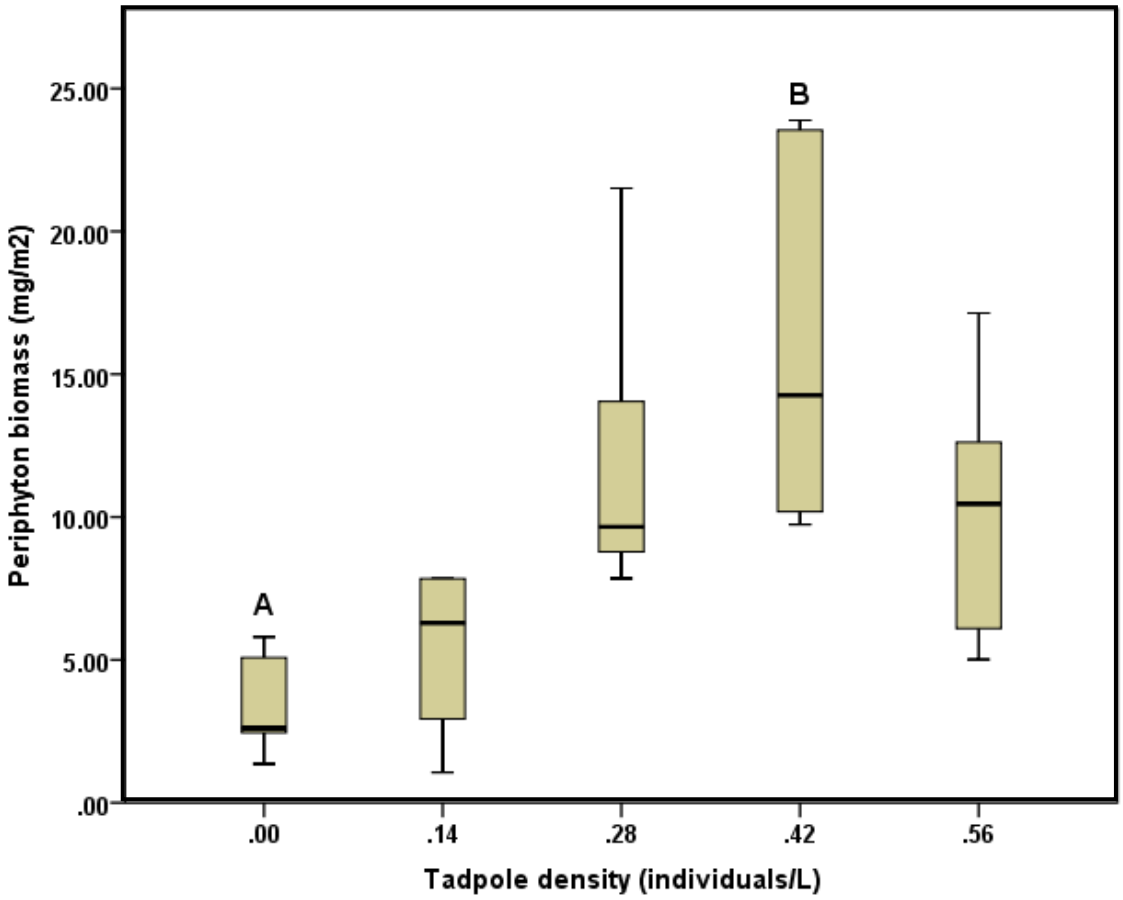

Figure 1. Comparison of mean (± 95% CI whiskers) periphyton biomass across tadpole density treatments. The median is illustrated by the horizontal line within the box. The lower quartile value is at the lower end of the box, and the upper quartile value is at the upper end of the box. Means with different uppercase letters are significantly different at $P < 0.05$.

Table 3. ANOVA results and means (± SE) for periphyton biomass (mg/m), and March *Callitriche marginata* rosette abundance (individuals) across the five tadpole density treatments. Different superscript letters indicate significant differences ($P < 0.05$) among means for each dependent variable.

	Density treatment (tadpoles/L)	Mean (± SE)
Periphyton biomass (mg/m)	0	3.46 (± 0.84) ^{A}
	0.14	7.30 (± 3.01)
	0.28	12.37 (± 2.52)
	0.42	16.33 (± 3.12) ^{B}
	0.56	10.27 (± 2.21)
	$F_{4, 16} = 3.762$, $P = 0.024$	
March rosette abundance (individuals)	0	75.20 (± 15.10) ^{A}
	0.14	13.80 (± 2.94) ^{B}
	0.28	14.40 (± 1.81) ^{B}
	0.42	16.80 (± 2.44) ^{B}
	0.56	15.40 (± 2.11) ^{B}
	$F_{4, 16} = 13.87$, $P < 0.001$	

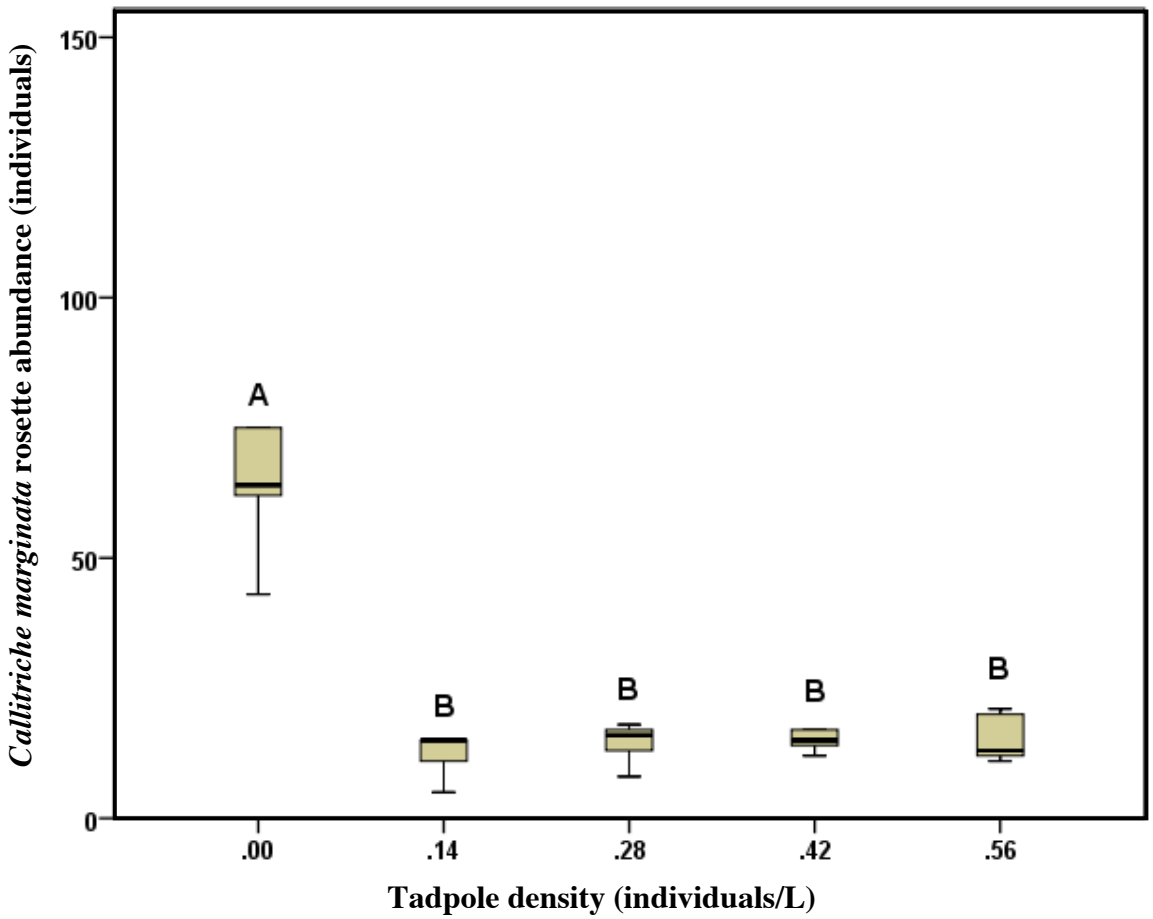

Figure 2. Comparison of mean *C*. *marginata* rosette abundance (± 95% CI whiskers) under the different tadpole density treatments (March 2012). The median is illustrated by the horizontal line within the box. The lower quartile value is at the lower end of the box, and the upper quartile value is at the upper end of the box. Means with different uppercase letters are significantly different at $P < 0.05$.

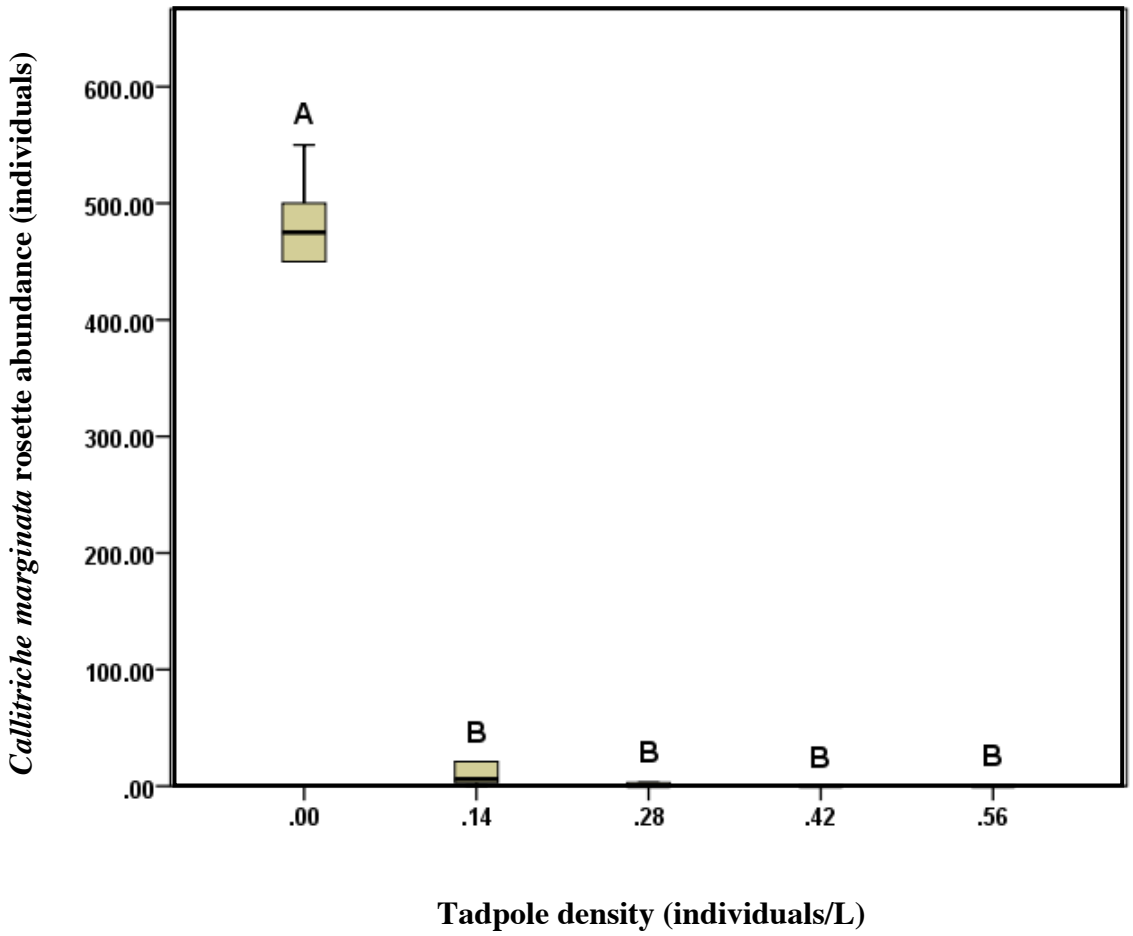

Figure 3. Comparison of mean *C*. *marginata* rosette abundance (± 95% CI whiskers) under various tadpole density treatments (May 2012). The median is illustrated by the horizontal line within the box. The lower quartile value is at the lower end of the box, and the upper quartile value is at the upper end of the box. Means with different uppercase letters are significantly different at $P < 0.05$.

Mean rosette abundance in the zero tadpole density treatments was significantly higher compared to the 0.14 tadpoles/L, 0.28 tadpoles/L, 0.42 tadpoles/L, and 0.56 tadpoles/L density treatments (Table 4). Rosettes within the zero tadpole density treatment were 20-465 times more abundant compared to the other treatment groups.

Percent macrophyte cover of emergent and floating aquatic vegetation differed significantly among density treatments ($F_{4, 16} = 23.357$, $P < 0.001$; Figure 4). Mean percent macrophyte cover in the zero tadpole density treatments was significantly greater compared to the 0.14 tadpoles/L, 0.28 tadpoles/L, 0.42 tadpoles/L, and 0.56 tadpoles/L density treatments (Table 4). Zero tadpole density treatments had 10-23 times the percent macrophyte cover than that found in the other treatment groups.

A total of 8 macrophyte species were measured in the mesocosms (see Appendix C). Based on macrophyte abundance and composition data collected in the May sampling period, a significant difference was observed in macrophyte community structure among tadpole density treatments (ANOSIM, R = 0.258, $P = 0.001$; Figure 5). According to the ANOSIM pairwise tests, macrophyte communities with no tadpoles differed significantly from all other treatment densities (Table 5). SIMPER analysis indicated that *Downingia bicornuta, Ranunculus aquatilis,* and *Gratiola ebracteata* were the highest contributors to the dissimilarities observed between communities (Table 6).

There was a significant difference in macrophyte biomass among tadpole density treatments ($F_{4, 16} = 7.199$, $P = 0.002$; Figure 6). Mean plant biomass (g) in the zero tadpole density treatments was significantly greater compared to the 0.14 tadpoles/L, 0.28 tadpoles/L, 0.42 tadpoles/L, and 0.56 tadpoles/L density treatments (Table 4).

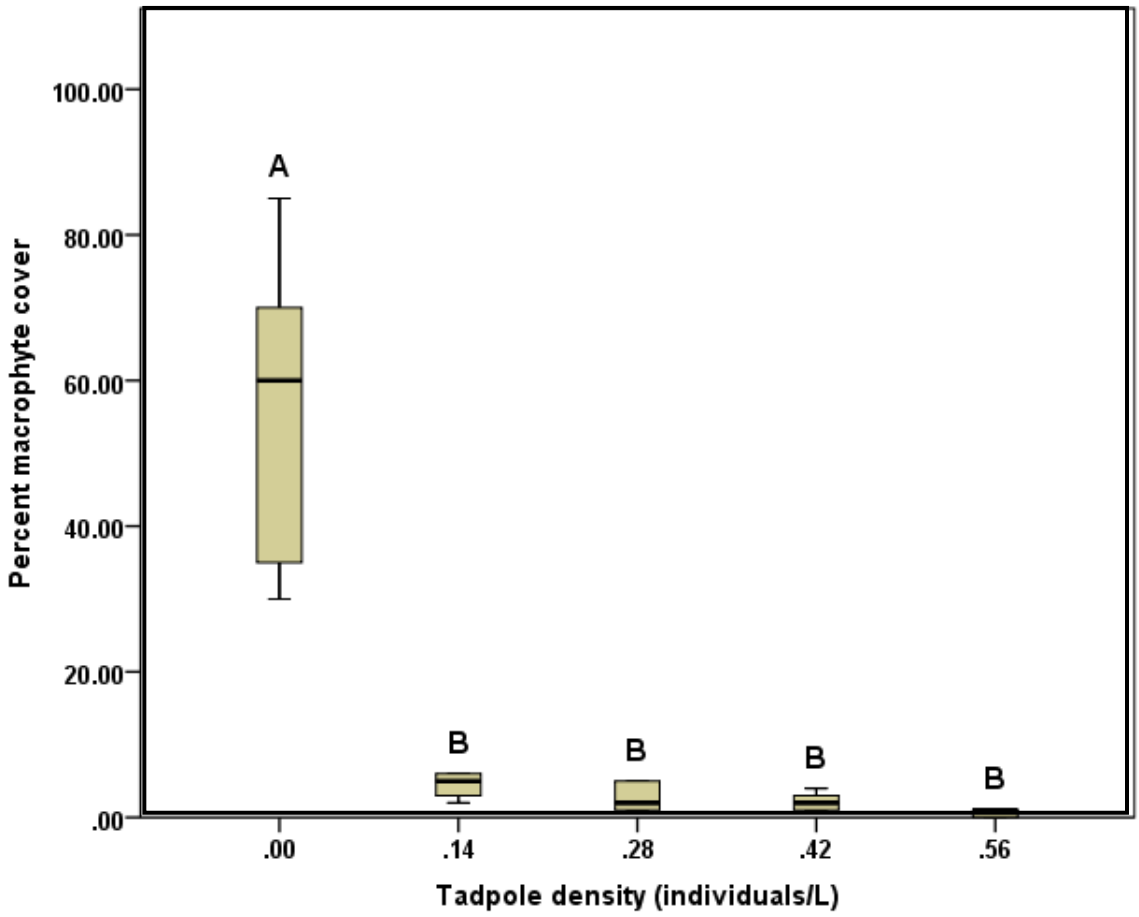

Figure 4. Comparison of mean percent macrophyte cover (± 95% CI whiskers) under various tadpole density treatments. The median is illustrated by the horizontal line within the box. The lower quartile value is at the lower end of the box, and the upper quartile value is at the upper end of the box. Means with different uppercase letters are significantly different at $P < 0.05$.

Table 4. ANOVA results and means (± SE) for May *Callitriche marginata* rosette abundance, percent macrophyte cover, and macrophyte biomass (g) within five tadpole density treatments. Different superscript letters indicate significant differences ($P < 0.05$) among means for each dependent variable.

	Density treatment (tadpoles/L)	Mean (± SE)
May rosette abundance (individuals)	0	465 (± 33.17) ^{A}
	0.14	23 (± 15.92) ^{B}
	0.28	2.4 (± 1.75) ^{B}
	0.42	1.20 (± 1.20) ^{B}
	0.56	0 (± 0) ^{B}
	$F_{4, 16} = 16.528$, $P = 0.01$	
Percent macrophyte cover	0	56.00 (± 10.42) ^{A}
	0.14	5.60 (± 1.75) ^{B}
	0.28	2.80 (± 0.92) ^{B}
	0.42	2.20 (± 0.58) ^{B}
	0.56	2.40 (± 1.91) ^{B}
	$F_{4, 16} = 23.357$, $P < 0.001$	
Macrophyte biomass (g)	0	29.03 (± 1.86) ^{A}
	0.14	5.44 (± 2.16) ^{B}
	0.28	2.39 (± 0.28) ^{B}
	0.42	3.42 (± 0.91) ^{B}
	0.56	3.41 (± 1.57) ^{B}
	$F_{4, 16} = 7.199$, $P = 0.002$	

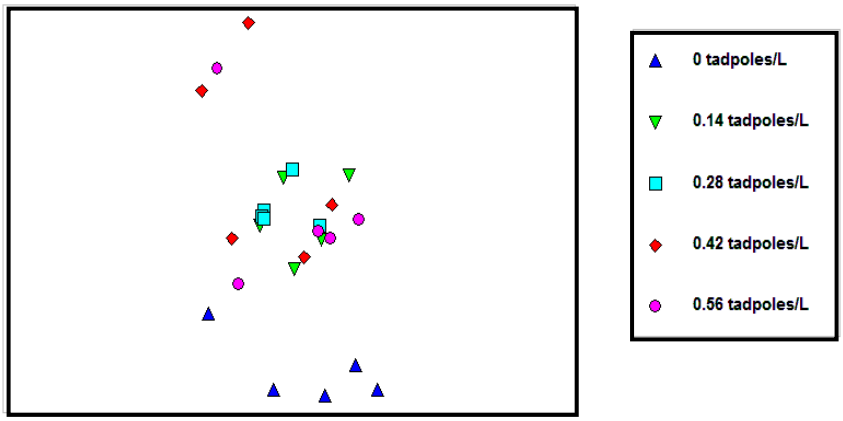

Figure 5. Non-metric multidimensional scaling (NMDS) ordination of macrophyte count data collected in May 2012. The 2D solution exhibited a final stress of 0.09.

Table 5. Statistical results of the ANOSIM pairwise tests. There were 126 possible and actual permutations in the analysis.

Treatments	**R Statistic**	***P*-value**
0/L vs. 0.14/L	0.824	0.008
0/L vs. 0.28/L	0.848	0.008
0/L vs. 0.42/L	0.604	0.008
0/L vs. 0.56/L	0.472	0.008

Table 6. SIMPER analysis of macrophyte species abundance and composition between the five tadpole density treatments. Mean abundance of each macrophyte species in the specified density treatment is given. Contribution % refers to the percentage difference explained by the mean abundance of a given macrophyte species.

	Mean abundance	Mean abundance	Contribution %
	0/L	**0.14/L**	
Species			
D. bicornuta	4.00	0.00	21.34
R. aquatilis	5.20	0.00	20.43
G. ebracteata	4.20	0.00	19.90
	0/L	**0.28/L**	
D. bicornuta	*	0.00	21.60
R. aquatilis	*	0.00	20.71
G. ebracteata	*	0.00	20.12
	0/L	**0.42/L**	
D. bicornuta	*	0.00	18.96
R. aquatilis	*	0.00	18.24
G. ebracteata	*	0.00	17.62
	0/L	**0.56/L**	
D. bicornuta	*	0.00	19.66
R. aquatilis	*	0.20	18.47
G. ebracteata	*	0.20	18.08

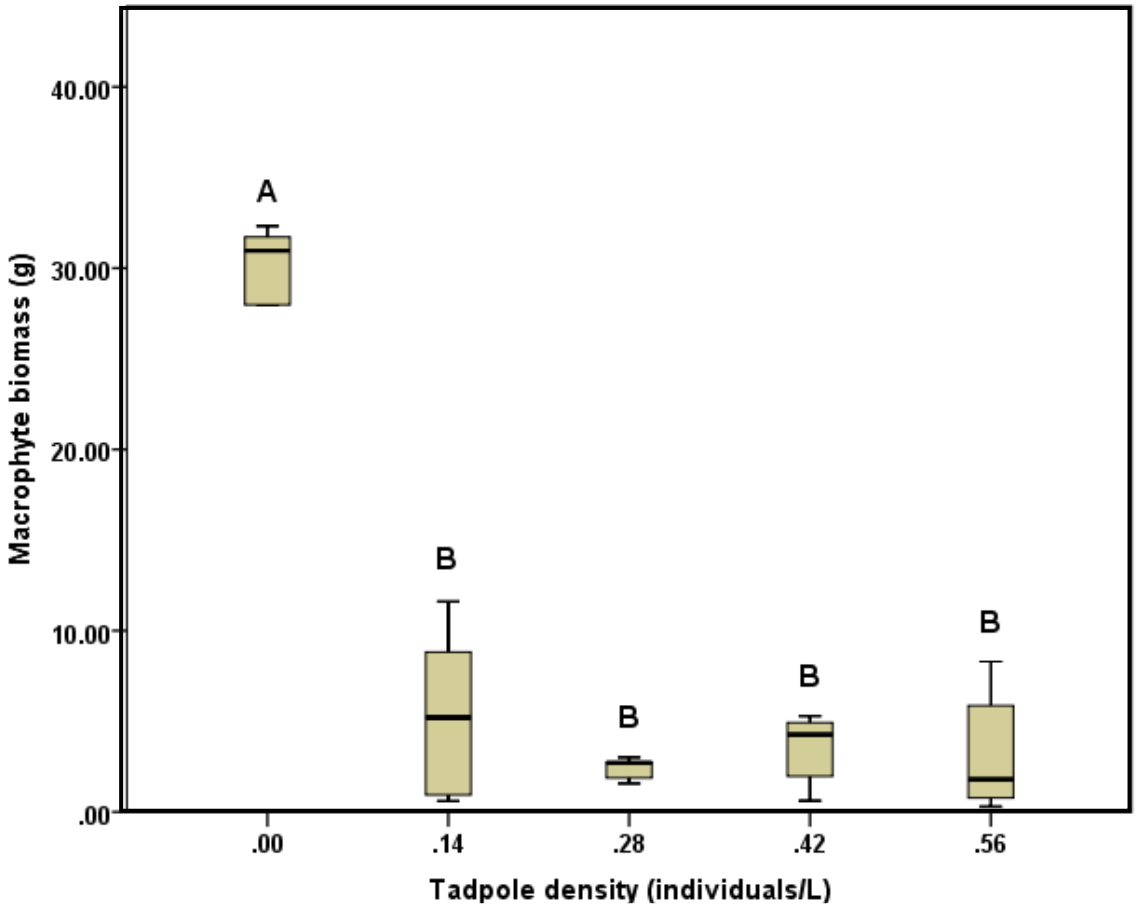

Figure 6. Comparison of mean macrophyte biomass (± 95% CI whiskers) under various tadpole density treatments. The median is illustrated by the horizontal line within the box. The lower quartile value is at the lower end of the box, and the upper quartile value is at the upper end of the box. Means with different uppercase letters are significantly different at $P < 0.05$.

Macrophyte biomass in the zero tadpole density treatment was 5-8 times higher than the other treatment groups.

Macrophyte species richness differed significantly among tadpole density treatment groups ($F_{4, 16} = 11.401$, $p < 0.001$; Figure 7). Mean plant richness within the zero tadpole density treatments was significantly higher compared to the 0.14 tadpoles/L, 0.28 tadpoles/L, 0.42 tadpoles/L, and 0.56 tadpoles/L density treatments (Table 7). Macrophyte species richness was 2-3 times higher in the zero tadpole density treatments compared to the other treatment groups.

Macrophyte community diversity (Shannon-Wiener index) was significantly different among the tadpole density treatment groups ($F_{4, 16} = 14.439$, $P < 0.001$; Figure 8). Mean macrophyte diversity within the zero tadpole density treatments was significantly higher compared to the 0.14 tadpoles/L, 0.28 tadpoles/L, 0.42 tadpoles/L, and 0.56 tadpoles/L density treatments (Table 7). Macrophyte diversity was 2-3 times higher in the zero tadpole density treatments compared to the other treatment groups.

Macrophyte and algae interactions

There was a significant negative relationship between periphyton biomass (mg/m) and total macrophyte biomass (Figure 9). A linear regression model accounted for 38.8 % (adjusted R) of the total variance ($F_{1, 23} = 16.207$, $P = 0.001$). There were no significant relationships found between macrophyte biomass and phytoplankton density, or between macrophyte biomass and phytoplankton biovolume ($P > 0.05$).

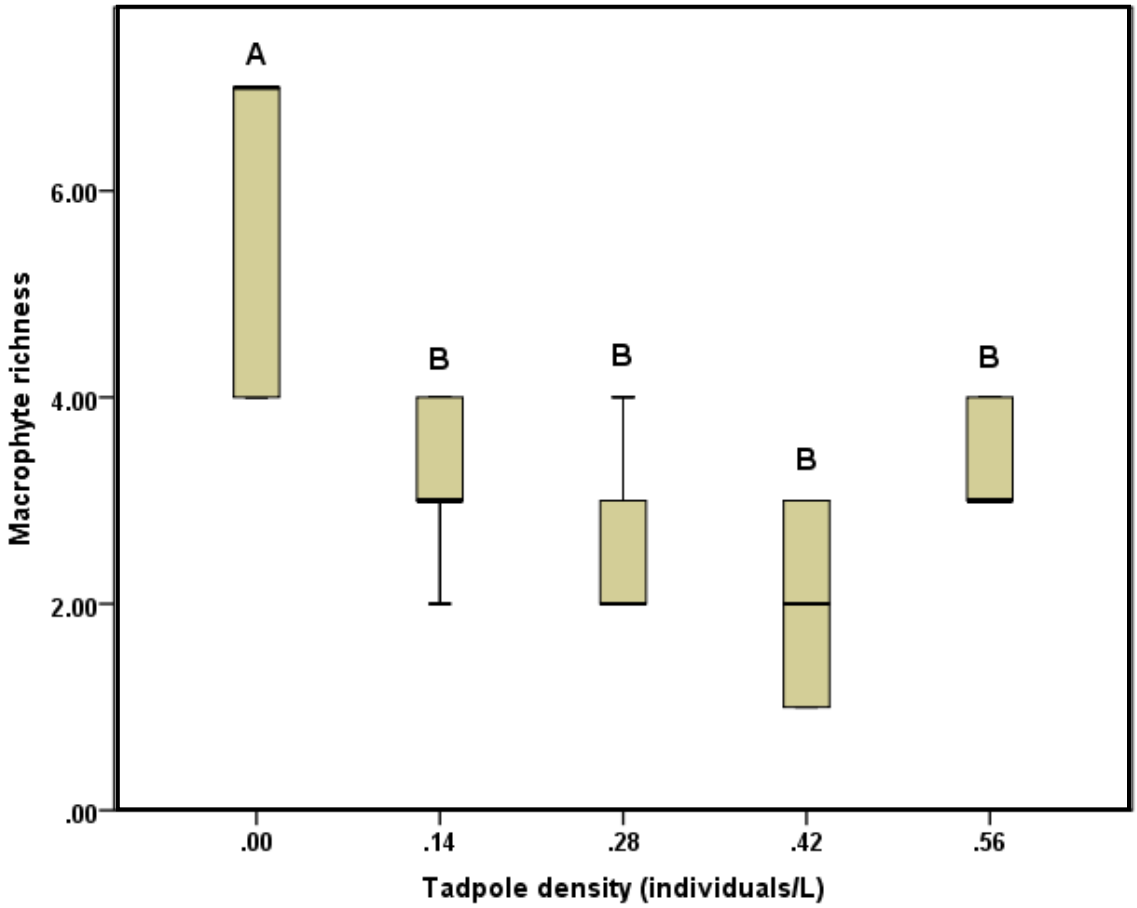

Figure 7. Mean macrophyte richness (± 95% CI whiskers) under various tadpole density treatments. The median is illustrated by the horizontal line within the box. The lower quartile value is at the lower end of the box, and the upper quartile value is at the upper end of the box. Means with different uppercase letters are significantly different at $P < 0.05$.

Table 7. ANOVA results and means (± SE) for macrophyte species richness and macrophyte diversity within five tadpole density treatments. Different superscript letters indicate significant differences ($P < 0.05$) among means for each dependent variable.

	Density treatment (tadpoles/L)	Mean (± SE)
Macrophyte species richness	0	5.80 (± 0.73) ^{A}
	0.14	3.20 (± 0.37) ^{B}
	0.28	2.60 (± 0.40) ^{B}
	0.42	2.00 (± 0.45) ^{B}
	0.56	3.00 (± 0.55) ^{B}
	$F_{4, 16} = 11.401$, $p < 0.001$	
Macrophyte diversity	0	1.57 (± 0.93) ^{A}
	0.14	0.87 (± 0.08) ^{B}
	0.28	0.75 (± 0.07) ^{B}
	0.42	0.50 (± 0.21) ^{B}
	0.56	0.81 (± 0.20) ^{B}
	$F_{4, 16} = 14.439$, $P < 0.001$	

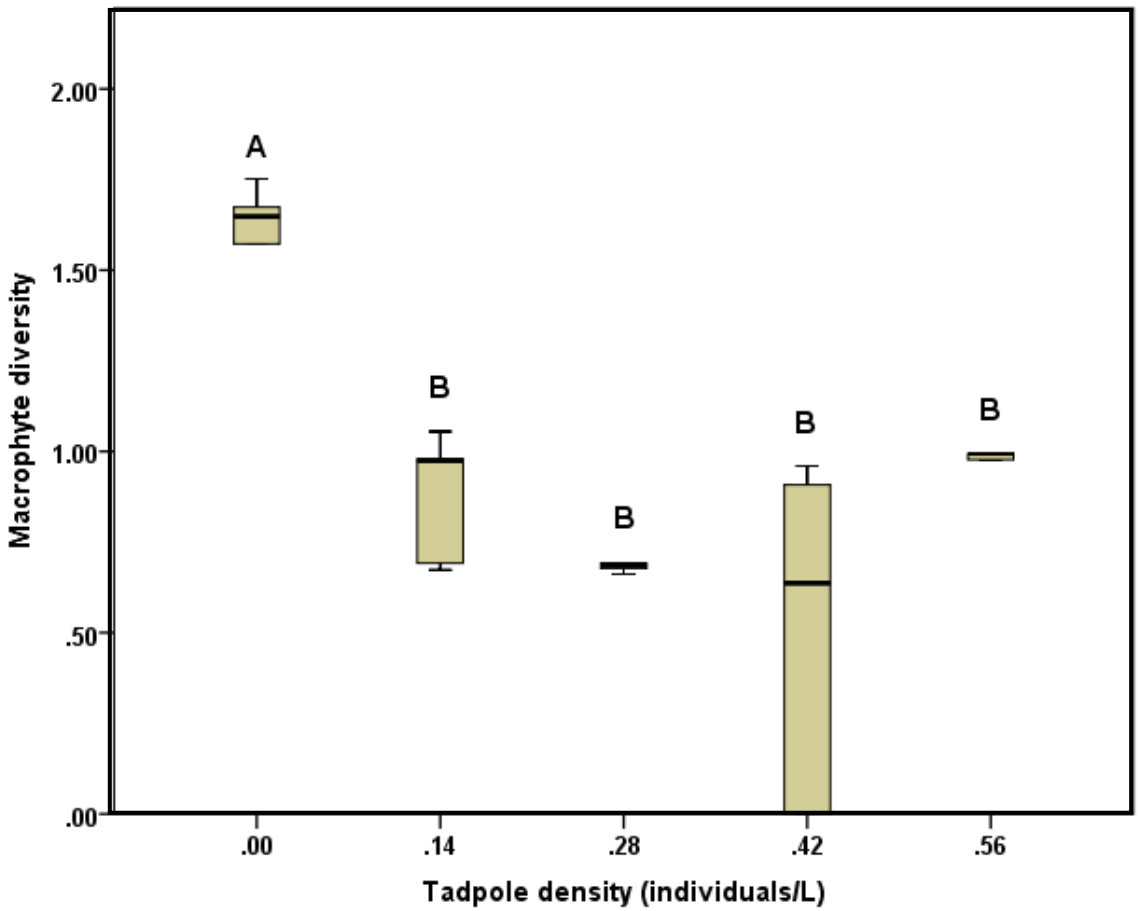

Figure 8. Comparison of mean macrophyte Shannon-Wiener diversity (± 95% CI whiskers) under various tadpole density treatments. The median is illustrated by the horizontal line within the box. The lower quartile value is at the lower end of the box, and the upper quartile value is at the upper end of the box. Means with different uppercase letters are significantly different at $P < 0.05$.

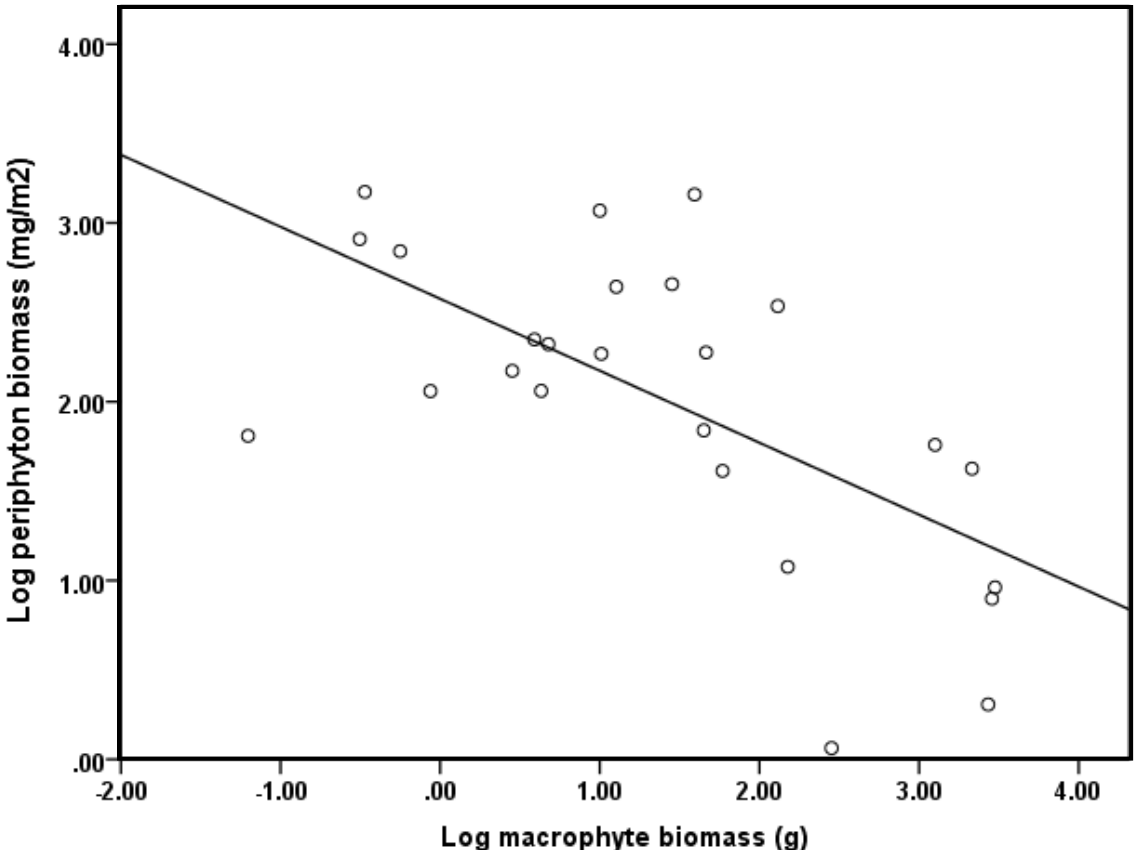

Figure 9. Regression of log periphyton biomass versus log macrophyte biomass ($F_{1, 23} = 16.207$, $R = 0.388$, $P = 0.001$).

DISCUSSION

Increasing densities of *Pseudacris regilla* tadpoles had a large impact on the vernal pool macrophyte community. Plant abundance, biomass and diversity were all negatively affected by increasing tadpole densities. Macrophyte community structure also differed in the presence of tadpoles due to the fact that *D. bicornuta*, *R. aquatilis*, and *G. ebracteata* were predominately isolated to mesocosms were *P. regilla* was absent. Periphyton biomass increased with increasing tadpole densities in the lower treatment ranges. This facilitative effect on periphyton biomass decreased in the highest density treatments, which could be attributed to tadpole consumption at higher densities. *Pseudacris regilla* tadpoles are non-discriminatory suspension and scraper feeders (Wassersug, 1975; Wagner, 1986), and were observed damaging macrophytes as they foraged. Consequently, the positive impact on periphyton was likely driven by the tadpole induced reduction in plant biomass, as well as the cycling of nutrients through herbivore feeding activity.

The presence of submerged, emergent, and floating-leaved macrophytes is crucial for the stabilization of a clear water state in shallow water bodies (Perrow et al., 1997; Scheffer, 1998; Cazzanelli et al., 2008). In the absence of macrophytes, a turbid, algae dominated equilibrium is reached (Moss et al., 1986; Hansson et al., 1998). This positive effect of macrophytes on water clarity has been attributed to various factors: macrophytes compete with algae for nutrients and light (Ozimek et al., 1990; van Donk et al., 1993), macrophytes provide refugia for planktivorous invertebrates (Paterson, 1993; Lauridsen et al., 1996), macrophytes reduce the mixing of the water column and resuspension of

seston (Barko & James, 1998), and possibly produce allelopathic substances that negatively affect phytoplankton and periphyton communities (Wium-Andersen et al., 1982; Jasser, 1995).

During this experiment, *P. regilla* tadpoles were observed to cause mechanical damage to the macrophyte community, likely due to their constant foraging of epiphytic and benthic algae. This scraping of algae damaged leaves, branches, and even uprooted smaller macrophytes. The largest impact was seen in the populations of *C. marginata*, the dominant macrophyte species within the experimental mesocosms. The floating apical rosettes of *Callitriche* spp. form a dense cover that can greatly reduce light penetration (Ministry of Technology, 1965). Mesocosms without tadpoles had significantly greater numbers of floating rosettes. As a result, mesocosms without tadpoles had the greatest percentage of cover compared to the other treatment groups. Rosettes, and the stems anchoring them to the parent plant, were very thin and fragile. Tadpoles were often observed damaging floating rosettes while foraging on the epiphytic algae and bacteria attached to them. The large difference in macrophyte biomass between treatments was primarily due to the large decrease in the *C. marginata* floating rosettes.

Given the large negative impact of *P. regilla* on the plant community, the algae community was likely released from plant mediated factors controlling their populations, and a shift to a more algae dominated equilibrium was reached. Overall, *P. regilla* tadpoles appeared to indirectly stimulate an increase in their food source, due to the foraging activities for the same food source. Resources most likely became more available to the algae population due to the modifications performed by *P. regilla*

tadpoles on the surrounding environment. This possible increase in resource availability due to environmental modifications would classify *P*. *regilla* tadpoles as an ecosystem engineer (Jones et al., 1994).

Consumer-mediated nutrient cycling could also have contributed to the increase in periphyton biomass (Vanni & Layne, 1997; Vanni, 2002). As the densities of herbivores increased (tadpoles + zooplankton), more nutrients were likely being excreted and made available to the algae populations (Vanni et al., 2002). Macrophytes are known to increase the surface area available for epiphytic algae (Blindow, 1987), and the foraging by herbivores can release the nutrients back into the water column (Vanni, 2002). This cycling of nutrients, in combination with the destruction of macrophytes, likely released the phytoplankton and periphyton from the competitive effects of the macrophyte community (Moss et al., 1986; Ozimek et al., 1990; Barko & James, 1998). The facultative effects observed in periphyton biomass, and the lack of treatment effects observed in the phytoplankton population could be due to the preference of phytoplankton as a food source. Phytoplankton and periphyton have been shown to compete for nutrients (Confer, 1972), and reducing phytoplankton levels may indirectly facilitate periphyton. The increase in periphyton biomass reached its peak in the 0.42 tadpole/L treatments, and started to decline in the 0.56 tadpole/L. This decrease in biomass likely represented the density where tadpole consumptive effects overcame the facilitative effects.

Tadpoles could potentially have an indirect effect on periphyton populations due to interactions with the zooplankton community (Whiles et al., 2010; Hamilton et al.,

2012). Changes in zooplankton community composition and/or species abundance have been shown to change consumption and/or excretion rates, which can have large effects on the algae community (Vanni & Layne, 1997; Brett & Goldman, 1996). In this experiment, there were no significant differences detected in the zooplankton communities between the various tadpole density treatments. This may be due to the coarse level of taxonomic identification used in the experiment or to the low levels of aquatic predators present within the mesocosms. If predation pressure was stronger within the mesocosms, the reduction of macrophyte refugia might have had a stronger effect on invertebrate abundance and/or community structure. Further study is required to determine the impact of *P. regilla* on vernal pool zooplankton communities.

The results of this experiment showed that *P. regilla* tadpoles have a density-dependent affect on vernal pool ecosystems. Besides the importance of their direct consumptive effects, their impacts on the aquatic and terrestrial communities are driven by various direct and indirect interactions. The ability of tadpoles to regulate macrophyte composition and abundance, alter their environmental conditions, and to facilitate the production of periphyton, demonstrates that the effect of tadpoles on the vernal pool food web is more important and complex than originally thought.

Biodiversity loss continues to be a global problem (Jenkins et al., 2003) exasperated by human induced overexploitation and habitat destruction. This loss can affect the functioning of ecological communities through the alteration of biotic interactions and biogeochemical cycling. The effect of biodiversity loss is becomes increasingly worrisome in small isolated communities with high levels of endemism,

such as California vernal pools. Furthermore, this habitat has been nearly removed from the California landscape due to human encroachment and habitat destruction. This study demonstrates that even a small change in *P. regilla* tadpole density can have a large impact on some organisms within the vernal pool aquatic community. Understanding the impact of these abundant consumers, and the mechanisms that drive the importance of their direct and indirect effects are essential for the conservation of vernal pools and the species associated with them.

APPENDIX A

Invertebrate taxa found within the vernal pool mesocosms.

Copepoda	Culicidae	Planaridae
Ostracoda	Dytiscidae	
Cladoceran	Chironomidae	
Branchinecta lynchi		
Linderiella occidentalis		
Lepidurus packardi		
Conchostraca		

APPENDIX B

Phytoplankton taxa found within the vernal pool mesocosms

Achnanthidium minutissimum	*Kirchneriella contorta*	*Rhoicosphenia curvata*
Amphora sp.	*Nitzschia acicularis*	*Scenedesmus bijuga*
Aphanocapsa incerta	*Nitzschia constricta*	*Scenedesmus dimorphus*
Characium ambiguum	*Nitzschia flexa*	*Scenedesmus quadricauda*
Closterium sp.	*Nitzschia inconspicua*	*Snowella lacustris*
Cosmarium sp.	*Nitzschia palea*	*Sphaerocystis schroeteri*
Craticula cuspidata	*Nitzschia perminuta*	*Stauroneis anceps*
Cryptomonas spp.	*Nitzschia sp.*	*Stauroneis javanica*
Dictyosphaerium pulchellum	*Oedogonium sp.*	*Staurosirella pinnata*
Encyonema minutum	*Oocystis parva*	*Stephanodiscus parvus*
Eunotia arcus	*Peridinium umbonatum*	*Surirella sp.*
Eunotia bilunaris	*Pinnularia sp.*	*Synedra ulna*
Eunotia exigua	*Planktolyngbya limnetica*	*Trachelomonas hispida*
Glenodinium palustre	*Planothidium lanceolatum*	*Trachelomonas volvocina*
Gomphonema acuminatum	*Pyramimonas tetrarhynchus*	*Ulothrix subtilissima*
Gomphonema parvulum	*Raphidiopsis curvata*	*Westella botryoides*
Hantzschia amphioxys	*Rhodomonas spp.*	

APPENDIX C

Macrophyte species found within the vernal pool mesocosms.

Callitriche marginata	*Gratiola ebracteata*
Eleocharis macrostachya	*Ranunculus bonariensis*
Downingia bicornuta	*Plagiobothrys stipitatus*
Ranunculus aquatilis	Unknown grass sp.

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