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Patterns of *Pseudacris sierra* Abundances within a Commercial Rice Ecosystem: Advancing Sustainable Rice Solutions

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***Abstract.*—Ecosystem services (ES) are the natural services provided by organisms and ecosystems that benefit humanity and have been proposed as a strategy to more sustainably produce agricultural goods by reducing the externalized environmental costs of agricultural production. Identifying how specific cultivation practices affect species that inhabit agricultural ecosystems has the potential to establish modified agricultural practices that promote ES. Rice fields are dynamic agricultural ecosystems that can support a high degree of biodiversity and can serve as critical wetland habitats for a diversity of species, including anuran amphibians. We sought to determine how conventional and organic rice cultivation practices within the Central Valley of California influence anuran populations among commercially operating farms. In addition, we tested whether behavioral mechanisms could possibly be shaping abundance patterns between types of rice cultivation. The Sierran treefrog (*Pseudacris sierra*) was the most common anuran detected among surveyed California rice fields, and there were significantly more *P. sierra* detected in organic relative to conventional rice fields. Interestingly, *P. sierra* abundances varied across individual fields, possibly because of adjacent field effects, vegetation heterogeneity, or behavioral factors. In a dichotomous choice test, we observed that male frogs spent more time on substrate soaked with water sourced from organic fields, while females were indifferent, suggesting that male *P. sierra* may be actively seeking organic rice fields for reproduction. These findings may be used as the initial steps to develop strategies that promote anurans and their intrinsic ES within rice agricultural ecosystems, advancing sustainable rice cultivation.**

Keywords. Sustainability, ecosystem service, agriculture, rice, amphibian, organic

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Introduction

Agricultural landscapes encompass over a third of earth's surface (NOAA, 2012), of which ~9% are rice fields (Maclean et al., 2002). The demand for rice has been steadily increasing 1.1% per year (OECD-FAO, 2022), likely making rice agricultural ecosystems, i.e., agroecosystems, permanent landscape fixtures. Because there is a need to produce rice globally, finding solutions that protect, restore, and promote sustainable rice agroecosystems, will support the United Nations sustainability goals (United Nations, 2015).

A potential pathway toward sustainable rice cultivation is the promotion of ecosystem services (ES), defined as the natural processes provided by species and ecosystems that sustain and enhance human life (Brauman & Daily, 2014). When appropriately managed, rice agroecosystems can support high levels of biodiversity (Dermiyati & Niswati, 2014; Edirisinghe & Bambaradeniya, 2010; Propper et al., 2023a). Anuran amphibians often inhabit rice fields, contributing ES such as pest regulation and serving as sentinels of environmental health (Hocking & Babbitt, 2014; Hopkins, 2007; Lukanov et al., 2024; Shuman-Goodier et al., 2019). Supporting anuran communities within commercial rice fields creates a "win–win" scenario, where farmers benefit from anuran-derived ES, while habitat is simultaneously provided for this globally imperiled group (Luedtke et al., 2023). Within the United States, there are two primary forms of rice cultivation, designated either as organic or conventional.

Organically produced crops are "grown and processed using no synthetic fertilizers or pesticides" (USDA

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Organic Farming, 2023) and are intended to enhance "agroecosystem health, including biodiversity, biological cycles, and soil biological activity" (Dabbert et al., 2004). In contrast, conventional rice cultivation is characterized by the use of synthetic pesticides, herbicides, and fertilizers (NRC, 2010). Supporting anuran populations within rice fields has the potential to enhance the "win-win" scenario by increasing their ES contribution. With this in mind, this study had two main objectives. First, we investigated if and why anuran abundances differed between organic and conventional rice fields in California. During the 2021 field season, we observed higher anuran abundances in organic fields compared to conventional fields. Building on these findings, we designed a second a priori study to investigate whether behavioral factors underlie the observed abundance patterns. Together, these results provide a foundation for future research aimed at characterizing how anuran abundance patterns contribute to ES within commercial rice fields. Such insights can ultimately support biodiversity conservation and inform the development of more sustainable rice production strategies.

Methods

Field site and timeline

Fieldwork was conducted in commercial rice fields located in the Sacramento Valley of California, near the city of Richvale, from May through July in both 2021 and 2022. In California, rice is harvested once per year. Fields are typically seeded in early April and harvested in mid-September (California Rice, 2024). After an initial 30-day flooded period, organic rice fields are drained for approximately 30 days to reduce weed pressure and then reflooded, whereas conventional rice fields remain continuously flooded. During this study, organic fields were drained from June 15 to July 12 in 2021 and from June 11 to July 7 in 2022. Fields 33 and 43-45 were not surveyed in 2021 but were surveyed in 2022. Table 1 presents the number of surveys each field received, while supplemental table 1 provides the exact survey dates for each field. California rice fields are generally left fallow during the winter but may be reflooded to provide habitat for waterfowl (Brouder & Hill, 1995). The fields surveyed in this study were left fallow between the 2021 and 2022 growing seasons. The collaborating farmers requested anonymity and are therefore referred to by their corresponding field numbers (Fig. 1).

Anuran surveys

Auditory and visual encounter surveys were conducted along transects at the edges of organic and conventional rice fields five times per week. Field surveys commenced after sunset, which occurred between 20:45 and 21:15 across both survey years. To minimize confusion, we designated "fields" by the number of "checks" intersected

Table 1. The number of surveys conducted per field across 2021 and 2022 field seasons. Fields 33 and 43-45 were not surveyed in 2021.

Field	Field Type	# of Surveys 2021	# of Surveys 2022
61-64	Conventional	8	6
411-413	Organic	6	6
32	Conventional	5	8
33	Organic	N/A	8
43-45	Conventional	N/A	4
42	Organic	7	6

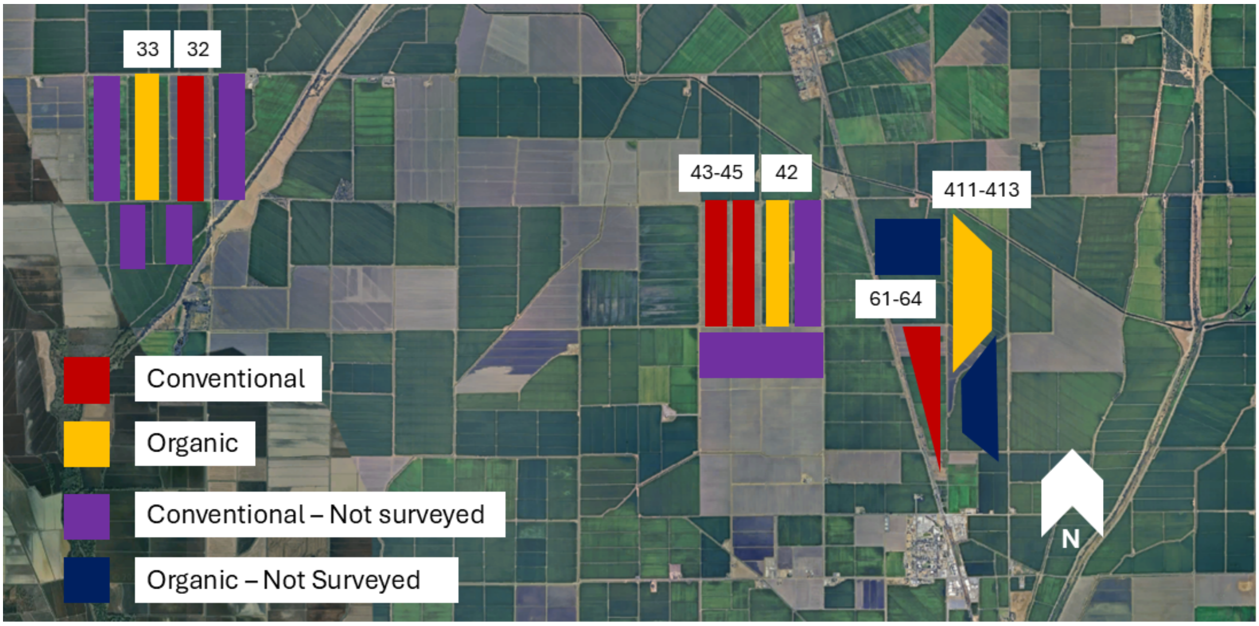

Fig 1. General locations of surveyed commercial rice fields. Red polygons indicate conventionally farmed fields that were surveyed, while yellow polygons indicate organically cultivated fields that were surveyed. Purple polygons represent conventional fields that were not surveyed, and dark blue polygons represent organic fields that were not surveyed. Numbers above each polygon correspond to field identifiers. Fields 43–45 and 33 were not surveyed in 2021.

by each transect. For example, Field 411–413 included the adjacent checks 411, 412, and 413. Survey transects ranged from 85 m to 120 m in length. To standardize results, anuran detection data were relativized across transects and transformed to counts per 100 m. Surveys were conducted in pairs, with each researcher recording the number of anurans detected within five meters on their respective half of the transect. Environmental data, including air temperature (°C) and relative humidity (%), were recorded prior to the start of each survey (Supplemental Table 1). Sierran treefrogs (*Pseudacris sierra*) were the sole anuran species used to statistically compare abundance patterns between rice field types because, aside from the rare occurrence of two American bullfrogs (*Lithobates catesbeianus*), they were the most consistently detected anuran across the surveyed rice fields.

Behavior study

A circular choice arena, 25 cm in diameter, was constructed to test the hypothesis that *P. sierra* could differentiate between water sourced from conventional versus organic rice fields. Each half of the arena floor contained a separate paper towel saturated with water collected from either field type. At the end of each behavioral trial, the paper towels were discarded, and newly saturated towels were used at the start of the next trial. Choice arenas were rotated to ensure that each half did not consistently face the same cardinal direction.

During the 2022 field season, fourteen individual *P. sierra* were captured across separate nights (eight males and six females). Each frog was immediately placed into the circular choice arena, which was sealed with a transparent lid and then positioned inside a larger opaque, dark plastic box. Following a ten-minute habituation period, a small night-vision camera was mounted atop the larger opaque box and positioned to face the arena (Fig. 2). The camera recorded for 45 minutes. After the recording period, each frog was released at its original capture site. All video recordings were uploaded to a computer and visually analyzed to quantify the total time each captured individual spent on the organic relative to the conventional side of the choice arena.

Statistical analysis

Environmental variable analysis

Two separate Poisson GLMs were used to assess if air temperature or relative humidity differed either between years or field types. In both cases, either air temperature or relative humidity were the response variables and year or field type (organic or conventional) were the explanatory factors (see equation below). The *check_distribution* function in the package *performance* was utilized to check each models' distribution (Lüdecke et al., 2021). All analyses were performed using the statistical software R (R. Core Team, 2024).

glm(air_temperature ~ field type, family = gaussian, data = environmental data 2021

P. sierra abundance analysis

A zero-inflated linear model, with a Poisson distribution, was used to compare *P. sierra* counts per 100m among field types (organic or conventional) and across rice growth stages (tillering and booting) using the *zeroinfl* function within the *pscl* r package (Zeileis et al., 2008). To normalize differences between transect lengths, we converted raw *P. sierra* observations to counts per 100m, by dividing the number of observed *P. sierra* by 100m. In addition, *P. sierra* abundances were compared only when both organic and conventional rice fields were flooded. We acknowledge that individual fields likely contributed to abundance differences between field types, but the factor was not included in the final model because of the limited number of surveyed fields both in 2021 (n = 4) and in 2022 (n = 6).The final model incorporated *P. sierra* counts per 100m as the response variable and field type and rice growth stage as main effects (see equation below).

zeroinfl(*count_100m ~ field_type + growth_stage, data = data_year, dist = 'poisson'*)

A dispersion statistic was also calculated to the fitted Poisson GLM both for data from 2021 and 2022 using the following equation:

sum(*residuals*(*model, type="pearson"*)*^2*) */ model$df.residual*

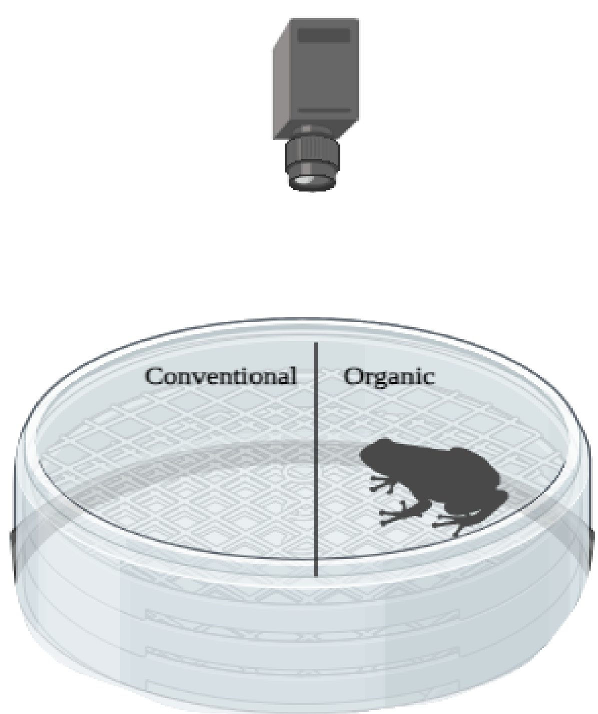

Fig 2. Visual representation of the choice arena for the behavioral experiment. Each half of the arena floor contained paper towels saturated with water collected either from organic or conventional rice fields. Choice arenas were placed within an opaque box (not shown) with a video camera affixed above and were rotated to ensure that each half did not consistently face the same cardinal direction.

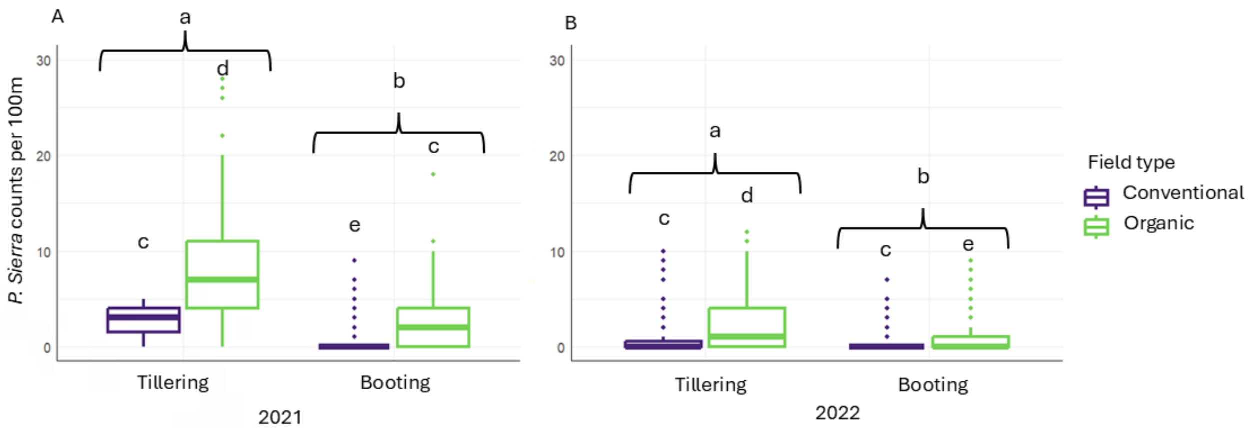

Fig 3. *P. sierra* abundances were assessed across the tillering and booting stages of rice growth both in 2021 and 2022 field seasons. Letters above each box plot represents statistically significant differences, calculated using pairwise contrasts with Tukey HSD adjustments. *P. sierra* counts differed between 2021 (**A**) and 2022 (**B**). More *P. sierra* were detected during the tillering stage (a) relative to the booting stage (b) across both years. In 2021 (**A**), *P. sierra* counts did not differ between conventional fields (c) in the tillering stage and organic fields in the booting stage (c). In 2022 (**B**), *P. sierra* counts were similar between conventional fields across both growth stages (c).

A post hoc analysis was executed using the *emmeans* function within the *emmeans* r package, which performs pairwise contrasts (z-tests) with p-value adjustment via Tukey's Honest Significant Difference to correct for the multiple comparisons between groups (Lenth, 2024).

P. sierra behavior analysis

The distribution of time spent on either half of the choice arena was normally distributed and equally varied across all captured frogs and among male or female populations. As a result, a Student's t-test was used to assess if captured *P. sierra* spent more time on either organic or conventional halves of the choice arena.

Results

Environmental variable analysis results

Air temperature and relative humidity, both followed gaussian distributions and differed between survey years (p_{air} temperature = 0.035; prelative humidity < 0.0001). Collected environmental variables were consequently compared within each survey year independently. Air temperature in 2021 and 2022 did not differ between field types (p2021 = 0.52; p2022 = 0.378). Relative humidity in 2021 was similar between field types (p = 0.641) but differed between field types in 2022 (p = 0.0006).

P. sierra abundance results:

More *P. sierra* were detected in organic relative to conventional rice fields both in 2021 (p = 0.030) and in 2022 (p = 0.028). The was also an interactive effect between rice growth stage and field type on *P. sierra* counts in 2021 (p = 0.0003) but not in 2022 (p = 0.918). More *P. sierra* were detected during the tillering growth stage relative to booting (p = 0.006; Fig. 3). The dispersion statistic of the Poisson GLM fitted to 2021 *P.sierra* count data was 2.254, suggesting moderate overdispersion, while the 2022 dispersion statistic was 1.250, indicating mild overdispersion.

P. sierra behavior results

Across all captured *P. sierra*, there was no significant difference in time spent on either side of the arena (p = 0.181). However, male frogs spent significantly more time on the organic half of the choice arena (p = 0.004), whereas females showed no preference (p = 0.331; Fig. 4).

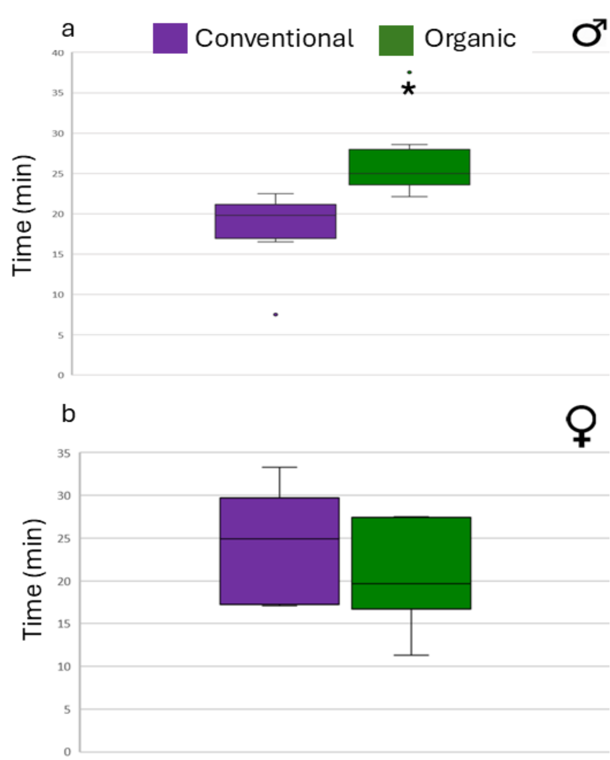

Fig 4. Time spent on either organic or conventional halves of the choice arena among captured *P. sierra* males (**a**) or among captured females (**b**). The asterisks (*) represents statistically significant differences (p < 0.05).

Discussion

We found distinct abundances of Sierran treefrogs (*Pseudacris sierra*) between organic and conventional California rice fields. Hundreds of *P. sierra* were detected, indicating that the surveyed fields served as suitable habitat for thousands of individuals. The collected environmental variables generally did not appear to drive abundance differences between field types. However, relative humidity did differ between field types in 2022, suggesting that further testing across additional growing seasons is needed to disentangle the effects of climate on *P. sierra* abundances. Nevertheless, we hypothesize that several factors may be shaping *P. sierra* abundances among California rice fields, including adjacent field effects, vegetation heterogeneity, and behavioral mechanisms.

Fields adjacent to surveyed plots appeared to influence *P. sierra* abundances. For example, more *P. sierra* were detected in conventional field 61–64 than in any other surveyed organic field, with the exception of field 411–413. Notably, field 61–64 was surrounded by organic fields, which may have served as source habitats in this context. Conversely, organic field 42 had the lowest *P. sierra* abundances, possibly because it was bounded by conventional fields. These patterns suggest that individual fields may function as source–sink environments within California, although further testing is warranted. Additionally, we hypothesize that vegetation dynamics may be another factor contributing to greater *P. sierra* abundances within organic rice fields.

Organic rice fields are typically more heterogeneous than conventional fields and are often heavily intermixed with weeds (Uno et al., 2021). Such heterogeneous environments at the individual field scale could, in theory, support higher *P. sierra* abundances (Sirami et al., 2019; Wan et al., 2023). At the landscape scale, Li et al. (2018) found that the amount of forest within an agricultural matrix was positively associated with frog abundances. Thus, increasing heterogeneity at both field and landscape scales may enhance *P. sierra* populations in California rice fields. In addition, *P. sierra* may actively avoid habitats with higher pesticide concentrations, further contributing to differences in abundance between organic and conventional rice fields.

Pesticide residues have been shown to decrease species abundances across taxa (Beaumelle et al., 2023; Tassin de Montaigu & Goulson, 2023). We conducted a behavioral choice experiment to determine whether *P. sierra* that inhabit rice fields could distinguish between conventional and organic water sources. Male *P. sierra* spent more time on paper towels saturated with water from organic rice fields, whereas females demonstrated no preference. We hypothesize that male *P. sierra* are able to detect pesticides among different water sources and could be selecting for better quality habitat (Ortiz-Ross et al., 2020). Male *P. sierra*, like many anuran species (Byrne & Keogh, 2007; Méndez-Tepepa et al., 2023), call and attract females to oviposition sites within organic rice fields and could thereby increase overall *P. sierra* abundances among organic rice fields. Other male frog species have also been shown to select higher-quality habitats. For example, male Phytotelma frogs (*Kurixalus eiffingeri*) preferred breeding in taller bamboo stumps containing more water, while females showed no site-based preference (Poo et al., 2024). There is also evidence to support the idea that anurans are able to detect pesticides in the environment. For instance, male Strawberry poison frogs (*Oophaga pumilio*) were observed using olfactory cues to avoid areas treated with a glyphosate-based herbicide (Farabaugh & Nowakowski, 2014). We acknowledge the limitations of our findings, as only 14 individuals (8 males, 6 females) were used in the behavioral experiment, and therefore recommend more extensive testing to determine if behavioral factors are shaping *P. sierra* abundance dynamics among California rice fields.

There is likely not a single factor shaping *P. sierra* abundances among California rice fields, but rather a suite of complex interactions. These factors may include adjacent field effects, vegetation heterogeneity at both field and landscape scales, and potentially behavioral influences. Supporting higher *P. sierra* abundances within California rice fields could enhance their ES-potential, as native frog species have been shown to consume a variety of rice pests (Shuman-Goodier et al., 2019). Collectively, these findings provide a foundation for testing how distinct rice farming practices influence anuran-derived ES. Such insights may ultimately inform cultivation strategies that simultaneously support biodiversity and ES within rice fields, thereby promoting more sustainable rice production.

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