



Eutrophication effects differ among functional groups in vernal pool invertebrate communities

Randall Robert Kido · Jamie M. Kneitel

Received: 9 June 2020 / Revised: 30 January 2021 / Accepted: 6 February 2021 / Published online: 2 March 2021
© The Author(s), under exclusive licence to Springer Nature Switzerland AG part of Springer Nature 2021

Abstract Environmental productivity and biodiversity are two intricately linked ecological concepts. One commonly observed pattern is a unimodal curve with biodiversity peaking at intermediate levels of productivity. While unimodal relationships are by no means universal, biodiversity declines at high productivity levels (eutrophication) raise serious concerns given anthropogenic nutrient inputs into waterbodies. One habitat that may be particularly vulnerable to eutrophication are California vernal pools. These ephemeral wetlands provide habitat to numerous threatened or endangered invertebrate taxa. To investigate the impact of eutrophication on invertebrates, experimental mesocosms were setup in the California State University, Sacramento Arboretum. Thirty mesocosms were inoculated with vernal pool soil and divided into five nutrient treatments in each of six blocks. Data was collected over a 21-week hydroperiod to quantify changes over a vernal pool hydroperiod. Nutrient addition increased mesocosm turbidity,

chlorophyll-a, and phosphorus levels, in addition to causing larger shifts in dissolved oxygen and pH. While nutrient enrichment increased abundances of small crustaceans, there were no effects on taxa richness, or abundances of large branchiopods or insects. While these findings improve our understanding of bottom-up effects in vernal pools, questions remain over long-term nutrient inputs and interactions with other human activities.

Keywords Active and passive dispersers · Crustaceans · Insects · Class Branchiopoda · Mesocosms · Species richness

Introduction

Environmental productivity has long been recognized as a major factor in structuring global biodiversity by way of bottom-up effects (Connell & Orias, 1964; Leigh, 1965). Despite the great variation in productivity–biodiversity patterns across habitats and taxa, one commonly exhibited pattern is a unimodal relationship between productivity and biodiversity (Abrams, 1995; Dodson et al., 2000). In this scenario, biodiversity is low in unproductive habitats, peaks at intermediate productivity levels, and declines at very high levels of productivity. Although unimodal productivity–biodiversity relationships are by no means

Handling editor: Dani Boix

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10750-021-04554-5>.

R. R. Kido (✉) · J. M. Kneitel
Department of Biological Sciences, California State University, Sacramento, 6000 J Street, Sacramento, CA 95819-6077, USA
e-mail: randallkido@csus.edu

universal, their occurrence in vascular plants and aquatic systems (Dodson et al., 2000; Mittelbach et al., 2001) shows that this is an important pattern for understanding how biodiversity interacts with environmental productivity.

The eutrophication of previous nutrient-limited systems has raised serious concerns about effects on global biodiversity (Carpenter et al., 1998). Adverse effects appear to be especially common in aquatic systems, where nutrient inputs oftentimes lead to “regime shifts” in which formerly clear, macrophyte-dominated systems rapidly transform into turbid, algae-dominated habitats (Scheffer & Carpenter, 2003). Alongside these effects, numerous studies have found changes to productivity can greatly alter community composition. Cultural eutrophication has been implicated in declines of many organisms (Pace, 1986), including crustaceans (Gannon & Stemberger, 1978; Straile & Geller, 1998) and sensitive aquatic insect taxa (Wang et al., 2000; Gong & Xie, 2001; Timm et al., 2006; Epele & Miserendino, 2015). These deleterious effects coupled with ecological theory hypothesizing decreased biodiversity at high levels of productivity suggest anthropogenic nutrient inputs pose a serious risk to global biodiversity, especially in aquatic systems.

Although cultural eutrophication is increasingly ubiquitous across aquatic ecosystems (Smith, 2003), excessive nutrient inputs may be particularly damaging to seasonal (also referred to as temporary or ephemeral) wetlands, including vernal pools. In California, vernal pools are ephemeral, rain-fed water bodies found across the state (Keeley & Zedler, 1998). Owing to their reliance on highly variable climatic conditions, including rainfall, vernal pools are prone to rapid changes in water quality, including pH, temperature, and dissolved oxygen levels (Keeley & Zedler, 1998; Kneitel et al., 2017). In terms of overall productivity, vernal pools are typically classified as oligotrophic (unproductive) water bodies (Keeley & Zedler, 1998).

In addition to these abiotic characteristics, vernal pools are habitats for numerous endemic organisms, which have specialized life histories adapted to ephemeral aquatic conditions. Many crustacean taxa found in these habitats persist via resting eggs (sometimes referred to as cysts) capable of withstanding desiccating conditions (passive dispersers; Mozley, 1944; Colburn et al., 2007). One crustacean group

of particular note are large branchiopods (Orders Anostraca, Notostraca, Spinicaudata, Laevicaudata, and Cyclestherida), which tend to be restricted to temporary waterbodies (Keeley & Zedler, 1998). Many species are vernal pool endemics and federally endangered, including the California tadpole shrimp (*Lepidurus packardii* Simon, 1886) and vernal pool fairy shrimp (*Branchinecta lynchi* Eng, Belk & Eriksen, 1990). In contrast, small crustaceans such as water fleas (Order Cladocera), copepods (Subclass Copepoda), and ostracods (Class Ostracoda) occupy diverse freshwater habitats as well as temporary wetlands (Colburn et al., 2007; Kneitel 2018). In addition to emergent crustacean taxa that persist via resting eggs, vernal pools are also colonized by actively dispersing taxa from other wetland habitats such as aquatic insects (active dispersers; Colburn et al., 2007).

The presence of endemic vernal pool species combined with current adverse human activities makes California vernal pools a key conservation priority (King, 1998; Van den Broeck et al., 2015, Calhoun et al., 2017). Currently, it is estimated only 3–10% of historic habitat remains (King, 1998; Gerhardt & Collinge, 2003), and several studies have suggested remaining vernal pool habitat is threatened by anthropogenic nutrient inputs commonly found in the intensely cultivated California Central Valley (King, 1998). Kneitel & Lessin (2010) found nutrient inputs and subsequent algal blooms resulted in reduced native plant diversity in a mesocosm experiment. Similarly, Croel & Kneitel (2011) also found this pattern of declining plant diversity due to cattle waste addition. In field observations, large branchiopods, crustaceans, and insects exhibit reduced species richness or occurrence in pools associated with livestock grazing and anthropogenic development in Spain (Miracle et al., 2008), Morocco (Van den Broeck et al., 2015, 2019), and Argentina (Epele & Miserendino, 2015). This pattern of shifting diversity suggests that nutrient inputs and other associated anthropogenic activities may have negative effects on vernal pool biodiversity as these systems move towards higher productivity.

Overall, eutrophication studies and ecological theory suggest anthropogenic nutrient inputs potentially pose a threat to vernal pool invertebrate communities. While there has been documentation of these effects on zooplankton and insects in permanent freshwaters,

studies in ephemeral wetlands and associated organisms remain rare (Mittelbach et al., 2001, Boix et al., 2008). Such understudied organisms include large branchiopods, which include numerous federally threatened or endangered species. Further, few studies compare taxa with different traits (e.g., dispersal mode: passive and active dispersers). In light of literature highlighting the dangers of anthropogenic nutrient inputs to global biodiversity (Carpenter et al., 1998), how eutrophication affects temporary waterbodies and certain invertebrate taxa inhabiting them remains an area of concern.

The objective of this study was to quantify how nutrient additions influence invertebrate community composition in California vernal pools. To this end, the study measured water quality conditions, taxa richness, and taxa abundances across a gradient of nutrient treatments. Effects on dispersal-size groups were also assessed. These included different dispersal strategies and body sizes: large branchiopods (passive dispersers), small crustaceans (passive dispersers), and aquatic insects (active dispersers). In addition, two trophic levels were assessed, prey (primary consumers) and top predators. Lastly, the study also attempted to explain the mechanisms by which nutrient additions structure vernal pool invertebrate communities. Based on previous studies, nutrient addition was expected to have two main effects on vernal pool invertebrate communities. First, taxa richness was expected to exhibit a unimodal curve with highest diversity at intermediate nutrient treatments due to containing both low and high nutrient specialists. Second, high nutrient mesocosms were expected to contain communities of primarily colonizing insects adapted to late hydroperiod conditions as opposed to early emergent crustacean species, many of which are sensitive to lowered oxygen levels typical of eutrophic waters (Colburn et al., 2007). Community similarity was also investigated to detect changes to invertebrate composition not readily apparent based on taxa richness alone, such as the replacement of sensitive taxa by those adapted to eutrophic conditions, or extirpation of early emergent species by colonizing predators (Pace, 1986; Straile & Geller, 1998; Colburn et al., 2007).

Methods

This study was conducted at the California State University, Sacramento Arboretum. The design consisted of 30, 151 l oval mesocosm pools (length = 1.03 m, width = 0.36 m, height = 0.72 m, area = 0.37 m²). Mesocosms were inoculated with 7 kg of homogenized vernal pool soil (approximately 2 cm depth) from the Elder Creek Watershed and Gill Ranch (Kneitel et al., 2017) in order to introduce vernal pool seed banks and emergent crustacean resting eggs (passive dispersers). We do not know the exact trophic status of these pools, but the inoculum reflects the species composition of many oligotrophic vernal pools (King et al., 1996, Kneitel, 2018). Pools were then uniformly filled with well water, and rainfall naturally kept mesocosms at capacity for the duration of the experiment. Mesocosms were left open for colonization by active dispersers to reflect assemblages found in natural vernal pools (Colburn et al., 2007; Kneitel, 2014).

The 30 mesocosm replicates were divided into six experimental blocks to minimize confounding factors due to spatial variation, and subsequent analysis found block effects did not occur across most biotic variables. Each experimental block contained five aqueous NaNO₃ and KH₂PO₄ nutrient treatments, with treatment regimens composed of no nutrient additions (control), 0.25 mg/l nitrate 0.5 mg/l phosphate (low), 0.5 mg/l nitrate 1 mg/l phosphate (medium), 1.5 mg/l nitrate 3 mg/l phosphate (high), and 2.5 mg/l nitrate 5 mg/l phosphates (very high). Nutrients were added every 2 weeks and were based on Kneitel & Lessin (2010), in which treatments over 0.5 mg/l nitrogen and 1 mg/l phosphate elicited algal growth, and treatments over 2 mg/l nitrogen and 4 mg/l phosphate resulted in increased aquatic phosphorus levels. Mesocosms were then filled by December 2, 2018, via natural rainfall and supplemental well water.

After inundation, the first nutrient addition occurred on December 7, 2018, and data collection was conducted every 2 weeks for 130 days from December 21, 2018, to April 29, 2019, for ten data-collecting sessions. Measured water quality variables included temperature (°C), dissolved oxygen levels (mg/l), turbidity (μS), pH, nitrogen (NO₃), phosphorus (PO₄), and chlorophyll-a. The used instruments included an Oakton pH 450 Probe (pH and temperature), YSI

550A Meter (oxygen), and Lovibond TB 250 WL Portable Turbidimeter (turbidity) for field measurements, as well as laboratory measurements via a Trilogy Fluorometer (chlorophyll-a) and Hach 2800 (nitrogen and phosphorus).

Biotic measurements consisted of taxa richness and abundances that were collected via standardized net sweeps. Organisms were identified to lowest possible taxonomic level and returned to the mesocosm. When field identification was not feasible, a specimen was collected in 70% ethanol solution for laboratory identification and as a reference in subsequent collections. For extremely numerous small taxa, approximately 15 individuals were randomly sampled for laboratory identification. Taxa were identified using Smith (2001), Merritt et al. (2008), and Thorp & Covich (2010). Abundances of taxa were measured via grid tray, with large and rare taxa being counted as individuals per tray, while smaller, more numerous taxa were subsampled and densities per sample were calculated. Individual taxa were also grouped into three dispersal-size groups, colonizing insects (which includes all insects as well as the water mite *Hydrachna*), emergent small crustaceans, and emergent large branchiopods (Table 1). We also categorized them into trophic categories of prey (primary consumers) and predators (secondary consumers).

Water quality variables were analyzed via repeated-measures analysis of variance (rm-ANOVA) to determine time (within-subject effect), treatment (between-subject effect), and time–treatment effects. The water quality-dependent variables included temperature, dissolved oxygen saturation, dissolved oxygen,

turbidity, pH, nitrogen, phosphorus, and chlorophyll-a. Biotic variables were analyzed via repeated-measures multivariate analysis of variance (MANOVA) with follow-up rm-ANOVAs to determine time, treatment, and time–treatment effects. The biotic MANOVAs used the abundances of either the dispersal-size groups or the trophic groups. Finally, a separate rm-ANOVA was conducted to determine time and treatment effects on species richness. Due to lack of sphericity in datasets, F-values and significance for rm-ANOVAs were derived from Greenhouse–Geisser values. Other assumptions for statistical tests (normality, linearity, outliers, etc.) were not violated or otherwise did not alter statistical significance of results. To distinguish differences among nutrient treatments, post hoc Games–Howell tests were used for rm-ANOVAs. All rm-ANOVA and MANOVA tests were conducted in IBM SPSS Statistics 25 (IBM Corp 2017) and R Studio Version 1.3.1073 (R Studio Team 2020) utilizing R Version 4.0.2 (Kassambara 2019, 2020a, b; R Core Team 2020), with accompanying line graph figures created in Microsoft Excel.

Community composition among treatments was assessed using one-way permutational analysis of variance (PERMANOVA). Significant PERMANOVA results were followed up with a similarity percentages test (SIMPER) to quantify how each individual taxa contributed to differences in invertebrate community composition. Both PERMANOVA and SIMPER tests were conducted using the PAST software (Hammer et al., 2001).

Table 1 Water quality repeated-measure ANOVAs (Greenhouse–Geisser *F*-statistics) for water quality variables

Dependent variables	Time			Treatment			Time × treatment		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Temperature (°C)	1218.604	1.57	< 0.001	0.045	4	0.996	0.128	6.3	0.993
Dissolved oxygen (mg/l)	127.92	4.13	< 0.001	0.113	4	0.977	3.021	16.51	< 0.001
Dissolved oxygen saturation (%)	46.329	2.97	< 0.001	0.056	4	0.994	2.237	11.87	0.018
Turbidity (NTU)	279.87	2.24	< 0.001	3.77	4	0.016	3.49	8.96	0.002
pH	23.031	3.66	< 0.001	0.446	4	0.774	2.506	14.65	0.004
Nitrogen (NO ₃ mg/l)	161.76	2.41	< 0.001	1.27	4	0.308	1.39	9.63	0.209
Phosphorus (PO ₄ mg/l)	52.8	2.62	< 0.001	86.5	4	< 0.001	10.5	10.47	< 0.001
Chlorophyll-a (mg/l)	56.08	3.83	< 0.001	6.71	4	< 0.001	1.65	15.32	0.073

Bold denotes a significant result

A Pearson correlation was conducted (SPSS Statistics 25, IBM Corp, 2017) to determine pairwise correlations between individual water quality variables and individual group abundances as well as taxa richness. In addition to pairwise correlations, datasets were organized via principal components analysis to visualize correlations between variable sets. All principal components ordinations were created in JMP Pro 14 (SAS Institute, 2018) and were composed of two charts, one detailing how mesocosm treatments correlate with one another on two principal components axes, and one illustrating correlations between individual dependent variables. This was applied to two ordinations: water quality variables and biotic variables.

Results

The rm-ANOVAs found time, treatment, and time–treatment effects on water quality variables (Table 1). Nutrient addition treatments increased levels of turbidity, phosphorus, and chlorophyll-a (Table 1). Further, time–treatment effects resulted in nutrient additions causing extreme temporal swings in dissolved oxygen and pH, as well as larger peaks to turbidity and phosphorus during the later hydroperiod (Table 1).

Dissolved oxygen declined over time after an initial increase (Table 1), and there was a time–treatment interaction indicating nutrient additions resulted in more extreme dissolved oxygen shifts (Table 1). This was most apparent in very high nutrient mesocosms, where oxygen levels initially peaked above lower nutrient treatments 5 weeks after inundation (over 25% more saturation than the control) before collapsing 15 weeks after inundation (14% less saturation than the control) and subsequently leveling out (Fig. 1a). This pattern of extreme swings due to time–treatment interactions was also observed for pH (Fig. 1b). In addition, nutrient inputs also increased turbidity, with very high nutrient mesocosms having on average 52% greater turbidity compared to lower nutrient treatments (Fig. 1c). There was also a time–treatment interaction on turbidity, with more pronounced nutrient induced turbidity in later stages of the mesocosm hydroperiod. For example, 21 weeks after inundation, there was an 80% turbidity increase in high nutrient treatments compared to controls.

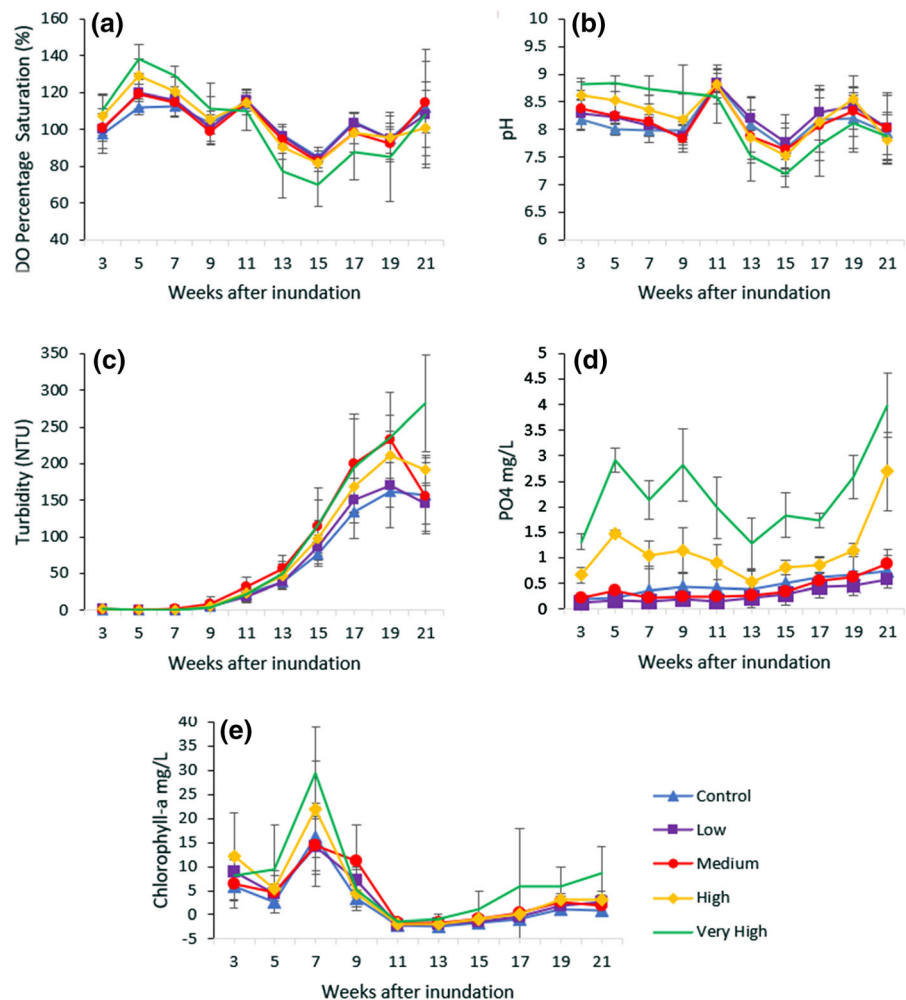
While nitrogen levels varied over time, with large increases in nitrogen levels across all treatment levels after week 13, there was no nutrient effect or time–treatment interaction on nitrogen levels (Table 1). In contrast, phosphorus levels increased with both time and treatments (Table 1), with high and very high nutrient treatments having elevated phosphorus levels at every time period after inundation (Fig. 1d). When compared to control treatments, very high nutrient treatments had on average almost 400% greater phosphorus levels. Phosphorus levels also responded to the time–treatment interaction (Table 1), with high and very high nutrient treatments having higher phosphorus levels during the later hydroperiod, peaking at 4 and 2.7 mg/l, respectively, 21 weeks after inundation compared to less than 1 mg/l PO_4 in control treatments (Fig. 1d). These patterns were also found in chlorophyll-a levels, with treatment and time–treatment interactions causing very high treatments to have more chlorophyll-a compared to control and low treatments, with very high treatments having on average 200% greater chlorophyll-a levels compared to control mesocosms (Table 1; Fig. 1e).

Ordination of water quality variables into two principal components indicated a clear temporal factor to the experiment, with different time periods sorted mainly along component 1 of the axis (Appendix S1). In contrast to the mainly time dependent component 1, component 2 was mainly associated with effects due to nutrient treatments. Component 2, accounting for 21.3% of water quality variation, was most strongly associated with phosphorus levels and to a lesser extent chlorophyll-a, both of which were affected by nutrient treatments (Appendix S1).

Over the course of the experiment, 27 taxa were recorded, of which four were large branchiopods, six were other small crustacean taxa and 17 were insects (Table 2). Furthermore, 16 taxa were classified as prey taxa while 11 were classified as predator taxa (Appendix S1). Taxa richness increased over time but was not affected by either treatment or the time–treatment interaction (Table 3).

Large branchiopod abundance was not affected by either nutrient treatments or time–treatment interactions (Table 3). Several species exhibited abundance peaks, with *B. lynchi* and *Lindieriella occidentalis* Dodds 1923 peaking at Week 7, and *Cyzicus californicus* Packard 1883 peaking later during Weeks 19–21 (Fig. 2a). In contrast, tadpole shrimp (*L. packardii*)

Fig. 1 Plots of mean **a** dissolved oxygen saturation, **b** pH, **c** turbidity, **d** phosphate, and **e** chlorophyll-a in the five nutrient treatments over time. Error bars represent standard deviation



displayed no clear temporal peaks, appearing at low abundances throughout the mesocosm hydroperiod. Additionally, large branchiopod abundances were positively correlated with turbidity and phosphorus levels (Table 4).

Small crustacean abundance exhibited a hump-shaped pattern over time with a peak at week 17 (Fig. 2b). Nutrient treatments had an effect on small crustacean abundances, with very high treatments having consistently higher abundances (Fig. 2b). Time-treatment interactions were also found with higher small crustacean abundances only appearing in nutrient-enriched treatments after week 11, and there was a 119% increase in very high nutrient treatments compared to control treatments after 17 weeks (Fig. 2b). Small crustacean abundances were

positively correlated with phosphorus and chlorophyll-a levels (Table 4).

Insect abundances were not influenced by nutrient treatments or time-treatment interactions (Table 3). Instead, insect abundances only changed with time, with insect abundances increasing rapidly by Week 21 (Fig. 2c). In addition, insect abundances were positively correlated with temperature and negatively correlated with dissolved oxygen levels (Table 4).

The effects of nutrient inputs on predator and prey categories were consistent with the 3 dispersal-size groups. Repeated-measures MANOVA found time, treatment, and time-treatment interactions on the predator and prey trophic groups. Follow-up rm-ANOVA found prey taxa (comprised primarily of small crustaceans) were affected by treatment and time-treatment effects, with very high nutrient

Table 2 Identified mesocosm taxa, divided by dispersal-size and trophic group

Taxonomic groups	Dispersal-size group	Trophic group
<i>Branchinecta lynchi</i>	Large Branchiopods (passive Dispersers)	Prey
<i>Lindieriella occidentalis</i>		Prey
<i>Lepidurus packardii</i>		Predator
<i>Cyzicus californicus</i>	Small Crustaceans (Passive Dispersers)	Prey
<i>Bosmina</i> sp.		Prey
<i>Daphnia</i> sp.		Prey
<i>Simocephalus</i> sp.		Prey
<i>Candona</i> sp.		Prey
<i>Eucypris</i> sp.		Prey
<i>Leptodiptomous</i> sp.		Prey
Chironomidae	Insects (Active Dispersers)	Prey
<i>Hydrodites</i>		Predator
<i>Tropisternus</i>		Predator
<i>Heterosternuta</i>		Predator
<i>Graptocorixa</i>		Prey
<i>Berosus</i>		Predator
<i>Laccophilus</i>		Predator
<i>Agabus</i>		Predator
<i>Pachydus</i>		Predator
<i>Nebrioporus</i>		Predator
<i>Neoporus</i>		Predator
<i>Cymatia</i>		Prey
<i>Hydrachna</i>		Predator
<i>Culex</i>		Prey
<i>Anopheles</i>		Prey
Ephydriidae		Prey
Ceraptogonidae		Prey

Table 3 Biotic MANOVA (Wilk's Lambda F -statistic) and univariate component (Greenhouse–Geisser F -statistic) for biotic variables

	Time			Treatment			Time \times treatment		
	Wilk's λ	df	P	Wilk's λ	df	P	Wilk's λ	df	P
MANOVA	24.257	27	< 0.001	3.829	12	< 0.001	1.586	108	< 0.001
Dependent variables	F	df	P	F	df	P	F	df	P
Large Branchiopods (individuals/cm ²)	7.12	3.958	< 0.001	1.51	4	0.229	1.19	15.83	0.289
Small Crustaceans (individuals/cm ²)	43.711	3.224	< 0.001	12.563	4	< 0.001	2.695	12.897	0.003
Insects (individuals/cm ²)	29.378	1.106	< 0.001	1.041	4	0.406	0.887	4.424	0.493
Taxa Richness	79.420	4.919	< 0.001	0.07	4	0.991	0.803	19.676	0.705

Bold denotes a significant result

treatments having on average 75% greater prey taxa abundances compared to control mesocosms. In contrast, predator taxa (comprised of insects and

tadpole shrimp) were unaffected by nutrient inputs, similar to our large branchiopod and insect findings (Appendix S1).

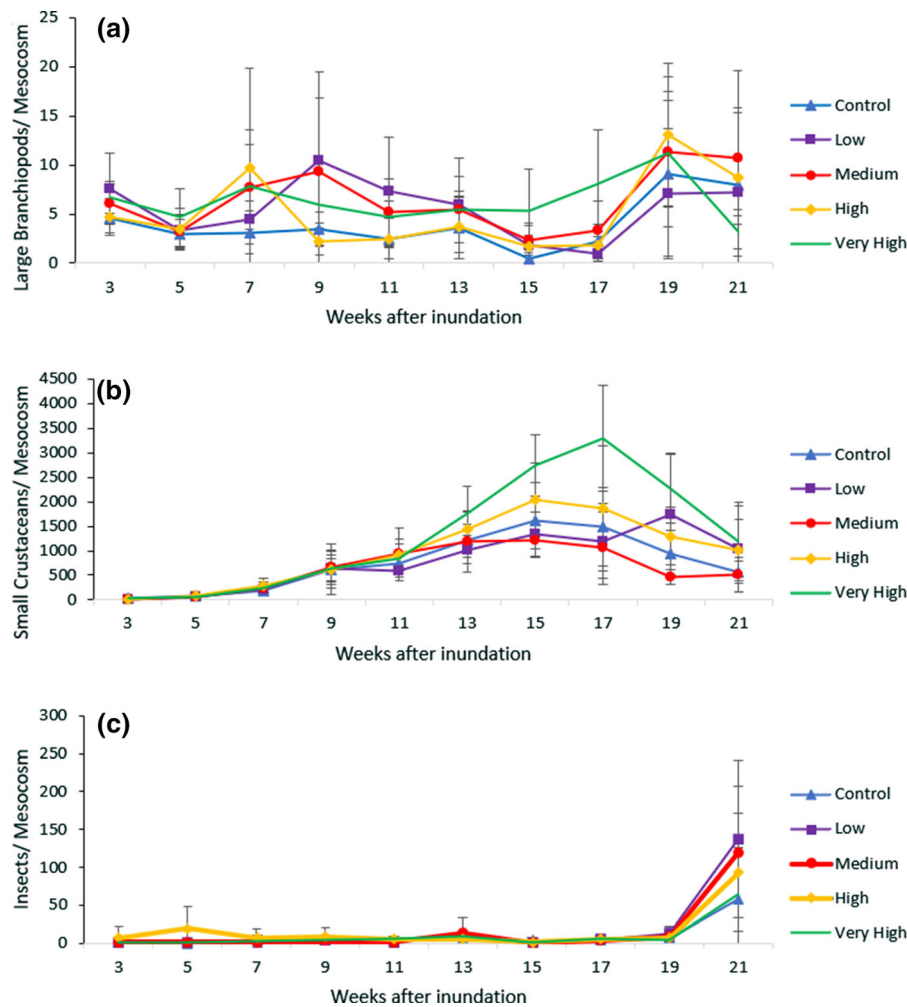


Fig. 2 Mean abundances/mesocosm of **a** large branchiopods, **b** small crustaceans, and **c** insects in the five nutrient treatments over time. Error bars represent standard deviation

Table 4 Pearson correlations between water quality and biotic variables

	Taxa Richness <i>r</i>	Large Branchiopod <i>r</i>	Small Crustacean <i>r</i>	Insect <i>r</i>
Temperature	0.121	0.191	− 0.034	0.539
DO%	− 0.178	− 0.308	0.147	− 0.497
DO	− 0.15	− 0.263	0.17	− 0.513
Turbidity	0.311	0.463	0.087	0.28
pH	− 0.026	− 0.262	0.24	− 0.292
Nitrogen	0.410	0.34	0.203	0.014
Phosphorus	0.147	0.222	0.637	− 0.166
Chlorophyll-a	0.127	0.364	0.481	0.15

Bold denotes a significant *P*-value ($P \leq 0.05$)

Invertebrate community composition was also affected by nutrient treatments (PERMANOVA: $F = 8.78$, $P < 0.001$). Pairwise comparisons among

treatments found the lowest community similarities between very high and less enriched nutrient regimens, (Table 5). SIMPER analysis indicated

Table 5 Community similarity PERMANOVA *F*-values

Treatment	1	2	3	4	5
1		0.07418	1.139	2.424	31.56
2	0.07418		1.251	1.188	16.43
3	1.139	1.251		6.387	55.44
4	2.424	1.188	6.387		8.359
5	31.56	16.43	55.44	8.359	

Bold denotes a significant difference ($P < 0.05$) in mesocosm invertebrate communities

differences in community composition were almost entirely driven by differences in small crustacean abundances, accounting for over 90% of variability in all pairwise comparisons (Table 6).

Ordination of biotic variables exhibited a strong temporal structure, with early mesocosms and late mesocosms separating to opposite ends of the Component 1 axis, which accounted for 38.5% of variation in invertebrate communities (Fig. 3). This pattern was mainly driven by the separation between early hydroperiod fairy shrimp and later hydroperiod taxa such as small crustaceans, insects, and clam shrimp. In contrast, component 2, accounting for 23.5% of variation, appeared to be driven by several factors, most notably the dissociation of small crustacean and tadpole shrimp-dominated mesocosms from insect-dominated mesocosms (Fig. 3).

Discussion

Nutrient enrichment altered mesocosm water quality conditions, but richness remained similar across treatments, not supporting the hypothesis that biodiversity would peak at intermediate levels of productivity. Furthermore, while changes to community composition due to nutrient additions were detected, effects were restricted to increased small crustacean abundances.

The effect of time on all water quality variables and separation of mesocosms across time in the ordination are consistent with previous characterizations of vernal pools as prone to large shifts in water quality conditions (Keeley & Zedler, 1998; Boix et al., 2008; Kneitel et al., 2017). Nevertheless, nutrient additions also increased turbidity, phosphorus, and chlorophyll-

a levels. These trends aligned with previous studies of “regime shifts” from oligotrophic to eutrophic conditions associated with nutrient inputs (Scheffer & Carpenter, 2003; Timm et al., 2006). Another important aspect was how nutrient addition timing influenced water quality variables. Nutrient inputs resulted in more extreme dissolved oxygen shifts, indicative of hypoxic (low-oxygen) conditions caused by eutrophication driven organic matter accumulation and decomposition (Carpenter et al., 1998). Nutrient effects causing more extreme pH shifts were also consistent with pH increases due to carbon fixation (Hinga, 1992; Keeley & Zedler, 1998) and subsequent acidification due to decomposition of organic matter (Cai et al., 2011). Finally, the timing of nutrient inputs also indicated a regime shift toward more turbid conditions may take time to manifest in temporary waterbodies (Scheffer & Carpenter, 2003). Overall, while dramatic shifts in water conditions over time are well attested to in vernal pools (Keeley & Zedler, 1998; Kneitel & Lessin, 2010), nutrient inputs in this experiment created a more eutrophic state.

Chlorophyll-a, which increased with nutrient addition, was our only measure of primary production. This, in concert with the increase in secondary production (small crustaceans) in response to added nutrients indicated evidence for strong bottom-up effects on abundance, but no effects on diversity (Dodson et al., 2000; Mittelbach et al., 2001). Consumer–prey interactions were also present, but these top-down effects appeared to be weak. Primary (chlorophyll-a) and secondary (small crustaceans) production both increased with nutrient addition without any evidence of suppression by the trophic level above. Bottom-up and top-down effects in ephemeral waters are commonly found (e.g., Ali et al., 1996; Mokany et al., 2008), but are strongly regulated by the hydro-regime and abiotic conditions (Wellborn et al., 1996; Magnusson & Williams, 2006). Therefore, changes in our hydro-regime may interact with these interactions and resulting richness and abundance patterns.

Our results were consistent with studies under similar environmental conditions to vernal pool habitats. Mittelbach et al. (2001) found the majority of systems ($n = 5$) at the local scale (< 5 km) displayed no pattern of animal biodiversity with productivity. Similar findings were reported by Rosset et al. (2014), with variable, non-unimodal responses to productivity

Table 6 Community dissimilarity SIMPER tests comparing treatments to control treatment

A: Simper control - low	Total dissimilarity 23.53	Cumulative dissimilarity %	Direction of abundance change
Taxa			
<i>Bosmina</i>	7.056	29.86	+
<i>Simocephalus</i>	6.967	59.34	+
<i>Leptodiptomous</i>	2.532	70.06	+
<i>Eucypris</i>	2.387	80.16	–
<i>Daphnia</i>	2.321	89.98	–
<i>Candona</i>	1.115	94.7	+
<i>Culex</i>	0.7492	97.87	+
Other insects	0.2386	98.88	–
<i>B. lynchi</i>	0.1355	99.45	+
<i>C. californicus</i>	0.1018	99.88	–
<i>L. occidentalis</i>	0.02277	99.98	+
<i>L. packardi</i>	0.00544	100	+
B: Simper control-medium	Total dissimilarity 27.23	Cumulative dissimilarity %	Direction of abundance change
Taxa			
<i>Simocephalus</i>	7.146	26.24	–
<i>Bosmina</i>	6.132	48.77	–
<i>Daphnia</i>	5.018	67.2	+
<i>Leptodiptomous</i>	3.291	79.28	+
<i>Eucypris</i>	3.127	90.77	–
<i>Candona</i>	1.217	95.24	–
<i>Culex</i>	0.7435	97.97	+
Other insects	0.2374	98.84	–
<i>C. californicus</i>	0.1408	99.36	+
<i>B. lynchi</i>	0.1065	99.75	+
<i>L. occidentalis</i>	0.06046	99.97	+
<i>L. packardi</i>	0.007463	100	+
C: Simper control - high	Total dissimilarity 25.94	Cumulative dissimilarity %	Direction of abundance change
Taxa			
<i>Simocephalus</i>	7.162	27.61	+
<i>Bosmina</i>	5.575	49.11	+
<i>Daphnia</i>	5.115	68.83	+
<i>Eucypris</i>	3.23	81.28	+
<i>Leptodiptomous</i>	2.326	90.25	+
<i>Candona</i>	1.402	95.65	+
<i>Culex</i>	0.6566	98.19	+
Other insects	0.2486	99.14	–

Table 6 continued

C: Simper control - high	Total dissimilarity 25.94	Cumulative dissimilarity %	Direction of abundance change
<i>C. californicus</i>	0.1216	99.61	+
<i>L. occidentalis</i>	0.05036	99.81	+
<i>B. lynchi</i>	0.04161	99.97	–
<i>B. lynchi</i>	0.008279	100	+
D: Simper control - very high	Total dissimilarity 37.91	Cumulative dissimilarity %	Direction of abundance change
Taxa			
Simocephalus	13.14	34.67	+
Bosmina	6.512	51.85	+
Leptodiaptomous	6.175	68.13	+
Eucpyris	5.89	83.67	+
Daphnia	4.13	94.57	+
Candona	1.428	98.33	+
Culex	0.2664	99.04	+
Other insects	0.1804	99.51	–
<i>C. californicus</i>	0.08935	99.72	+
<i>B. lynchi</i>	0.05814	99.9	+
<i>L. occidentalis</i>	0.03382	99.99	+
<i>L. packardii</i>	0.003391	100	+

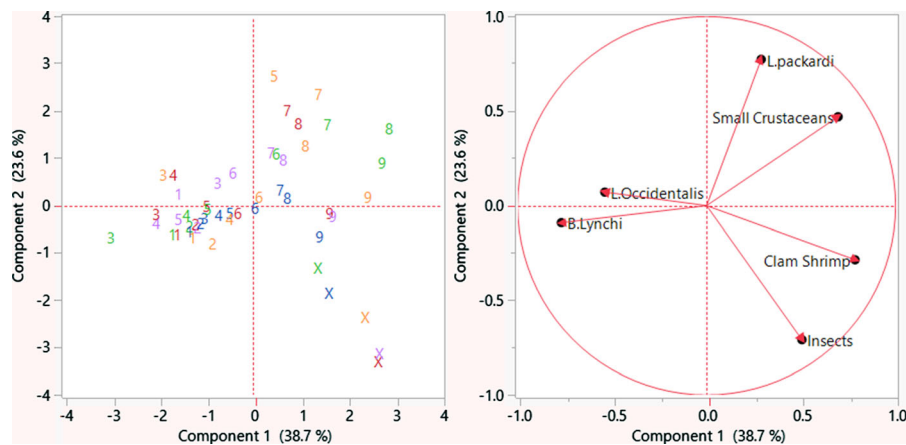


Fig. 3 Biotic principal components analysis in which the left chart illustrates average of each mesocosm treatments across two principal components during the 10 sampling periods (5 nutrient treatments per session \times 10 sampling dates = 50 data points in total). Color indicates treatment type (Blue = Control, Purple = Low, Red = Medium, Orange = High, Green = Very

High) and number indicates data collecting session (1 = 3 weeks after inundation, 2 = 5 weeks after indication, ... X = 21 weeks after inundation). The right chart illustrates how individual biotic variables associated across the two principal components

among invertebrate taxa in small temperate waterbodies, suggesting that these habitats may not be as

impaired by eutrophication. In addition, Dodson et al. (2000) found lakes under short-term nutrient

enrichment (< 3 years) had non-unimodal productivity–biodiversity relationships compared to unimodal patterns in other lake surveys. They attributed these discrepancies to productivity–biodiversity mechanisms acting on different timescales. These studies suggest biodiversity in ephemeral, small-scale habitats react differently to changes in productivity compared to larger, more permanent aquatic habitats such as lakes (Dodson et al., 2000; Stomp et al., 2011; Rosset et al., 2014) and marine habitats (Irigoin et al., 2004). Therefore, spatial and temporal scales may prove to be important factors when assessing productivity–diversity relationships (e.g., Chase & Leibold, 2002).

Nutrient treatment effects also differed among the three dispersal-size groups. Large branchiopods and small crustaceans, both passive dispersers, were positively correlated with chlorophyll-*a*, which increased nutrient additions. However as a whole, large branchiopods in our mesocosms were unaffected directly by nutrient addition; this was similar to a previous mesocosm experiment (Kneitel et al., 2017), but in contrast to field studies where these taxa were adversely affected by associated disturbances (Mittelbach et al., 2001; Angeler et al., 2008; Epele & Miserendino, 2015; Van den Broeck et al., 2015, 2019). These results suggest that nutrient enrichment alone does not influence vernal pool large branchiopods. Instead, it is plausible that declines in field studies were due to anthropogenic activities accompanying eutrophication, such as livestock disturbance (Miracle et al., 2008; El Madihi et al., 2017; Van den Broeck et al., 2019) or chemical runoff (Peltzer et al., 2008). In addition, the lack of treatment effect on large branchiopods illustrates that these organisms (emergence and individuals) remained robust to water quality changes driven by eutrophication. Nevertheless, the divergent findings between field studies and mesocosm experiments warrants further research on how anthropogenic activities associated with eutrophication may affect large branchiopods and aquatic ecosystems.

While several studies have focused on adverse effects of eutrophication on aquatic insects (Wang et al., 2000; Timm et al., 2006; Buxton et al., 2020), our results found insect abundances and species richness were primarily related to hydroperiod. This pattern had previously been noted by Boix et al. (2008), which found insect richness in Mediterranean waterbodies was more sensitive to arrival of spring

conditions later in the hydroperiod compared to crustacean fauna. Colonizing vernal pool insects were not influenced by system productivity. Moreover, predatory invertebrate taxa did not respond to treatments, while the smaller prey taxa increased with nutrient addition.

In contrast to large branchiopods and insects, small crustacean taxa were affected by treatments, with consistently higher abundances in nutrient-enriched mesocosms. This result is consistent with previous studies showing increased zooplankton biomass in more productive habitats due to bottom-up effects, namely increased food concentrations (Pace, 1986; Balla & Davis, 1995; Straile & Geller, 1998). However, nutrient enrichment did not lead to previously attested changes in small crustacean taxa richness due to eutrophication, such as extirpation of calanoid copepods or novel small crustacean colonization (Pace, 1986; Straile & Geller, 1998; Jeppesen et al., 2000). Instead, all observed small crustacean taxa increased in density with increasing nutrient enrichment and positively correlated with increases in chlorophyll-*a* and phosphorus. The taxa in this group are distinguished by their smaller body size and higher population growth rates (Hildrew et al., 2007). This suggests that these traits are important when considering community responses to eutrophication in vernal pools. These combined traits may have been involved with their expeditious response to nutrient inputs compared to organisms with larger bodies and those with active dispersal. This result also indicates small crustaceans may be an effective indicator of eutrophic conditions (e.g., Gannon & Stemberger, 1978; Nevalainen & Luoto, 2017). Finally, the increase in this taxonomic group due to nutrient inputs indicates that prey taxa lower in the trophic web may respond differently to eutrophication in comparison to larger predatory taxa.

Water quality conditions of mesocosm pools were changed by nutrient enrichment, effects on invertebrate taxa were variable. Nevertheless, these results are far from an exhaustive assessment of nutrient enrichment in vernal pools. First, it is possible that our nutrient addition treatments only captured a segment of the theoretical unimodal curve. Second, anthropogenic eutrophication is oftentimes accompanied by other environmental alterations, such as invasive species (Liu et al., 2009), changes to predation patterns (predominantly by fish) (Straile & Geller,

1998; Wang et al., 2000; Liu et al., 2009), or chemical runoff (Peltzer et al., 2008). These associated anthropogenic activities may explain why certain taxa such as large branchiopods and insects were negatively affected by nutrient inputs in field studies (Wang et al., 2000; Timm et al., 2006; Miracle et al., 2008; Van den Broeck et al., 2015). Another area of future research is whether nutrient inputs into ephemeral wetlands manifest differently over longer time scales (i.e., across hydroperiods), as previous studies have indicated community alterations due to nutrient inputs may take time to manifest (Dodson et al., 2000; Sand-Jensen et al., 2017).

Acknowledgements We thank Tim Davidson, Brett Holland, and two reviewers for useful comments that greatly improved the clarity of the manuscript. We also appreciate Kayleigh Lampe and Dalton Burroughs for assistance with the sampling.

Author contributions All authors contributed to study conception and material preparation. Funding acquisition was performed by JK. Data collection and analysis were performed by RK. The first draft of the manuscript was written by RK, with all authors contributing to subsequent versions. All authors read and approved the final manuscript.

Funding This research was supported by CSUS Department of Biological Sciences Delisle Research Fund and National Science Foundation Grant DEB 1354724 to JMK.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval This study was sampled under USFWS Permit TE192702 to JMK.

References

- Abrams, P. A., 1995. Monotonic or unimodal diversity–productivity gradients: what does competition theory predict? *Ecology* 76: 2019–2027.
- Angeler, D. G., O. Viedma, S. Sánchez-Carrillo & M. Alvarez-Cobelas, 2008. Conservation issues of temporary wetland Branchiopoda (Anostraca, Notostraca: Crustacea) in a semiarid agricultural landscape: what spatial scales are relevant? *Biological Conservation* 141: 1224–1234.
- Ali, A. J., S. S. S. Sarma, G. Murugan & H. J. Dumont, 1996. Effect of zooplankton type and abundance on prey consumption by the fairy shrimp, *Streptocephalus proboscideus* (Anostraca: Crustacea). *Hydrobiologia* 319: 191–202.
- Balla, S. A. & J. A. Davis, 1995. Seasonal variation in the macroinvertebrate fauna of wetlands of differing water regime and nutrient status on the Swan Coastal Plain, Western Australia. *Hydrobiologia* 299: 147–161.
- Boix, D., S. Gascón, J. Sala, A. Badosa, S. Brucet, R. López-Flores, M. Martinoy, J. Gifre & X. D. Quintana, 2008. Patterns of composition and species richness of crustaceans and aquatic insects along environmental gradients in Mediterranean waterbodies. *Hydrobiologia* 597: 53–69.
- Buxton, M., R. N. Cuthbert, T. Dalu, C. Nyamukondiwa & R. J. Wasserman, 2020. Cattle-induced eutrophication favours disease-vector mosquitoes. *Science of The Total Environment* 715: 136952.
- Cai, W., W. Huang, M. C. Murrell, J. C. Lehrter, S. E. Lohrenz, W. Chou, W. Zhai, J. T. Hollibaugh, Y. Wang, P. Zhao, X. Guo, K. Gundersen, M. Dai & G. Gong, 2011. Acidification of subsurface coastal waters enhanced by eutrophication. *Nature Geoscience* 4: 766–770.
- Calhoun, A. J. K., D. M. Mushet, K. P. Bell, D. Boix, J. A. Fitzsimons & F. Isselin-Nondedeu, 2017. Temporary wetlands: challenges and solutions to conserving a “disappearing” ecosystem. *Biological Conservation* 211B: 3–11.
- Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley & V. H. Smith, 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications* 8: 559–568.
- Chase, J. M. & M. A. Leibold, 2002. Spatial scale dictates the productivity–biodiversity relationship. *Nature* 416: 427–430.
- Colburn, E. A., S. C. Weeks & S. K. Reeds, 2007. Diversity and ecology of vernal pool invertebrates. In Calhoun, A. J. K. & P. G. deMaynadier (eds), *Science and Conservation of Vernal Pools in Northeastern North America*. CRC Press, Boca Raton: 105–126.
- Connell, J. H. & E. Orias, 1964. The ecological regulation of species diversity. *The American Naturalist* 98: 399–413.
- Croel, R. C. & J. M. Kneitel, 2011. Cattle waste reduces plant diversity in vernal pool mesocosms. *Aquatic Botany* 95: 140–145.
- Dodson, S., S. E. Arnott & K. L. Cottingham, 2000. The relationship in lake communities between primary productivity and species richness. *Ecology* 81: 2662–2679.
- Epele, L. B. & L. M. Miserendino, 2015. Environmental quality and aquatic invertebrate metrics relationships at Patagonian wetlands subjected to livestock grazing pressures. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0137873>.
- El Madihi, M., L. Rhazi, M. Van den Broeck, M. Rhazi, A. Waterkeyn, E. R. Saber, S. Bouahim, M. Arahou, A. Zouahri, A. Guelmami & S. D. Muller, 2017. Plant community patterns in Moroccan temporary ponds along latitudinal and anthropogenic disturbance gradients. *Plant Ecology & Diversity* 10: 197–215.
- Gannon, J. E. & R. S. Stemberger, 1978. Zooplankton (especially crustaceans and rotifers) as indicators of water quality. *Transactions of the American Microscopical Society* 97: 16–35.
- Gerhardt, F. & S. K. Collinge, 2003. Exotic plant invasions of vernal pools in the Central Valley of California, USA. *Journal of Biogeography* 30: 1043–1052.

- Gong, Z. & P. Xie, 2001. Impact of eutrophication on biodiversity of the macrozoobenthos community in a shallow Chinese lake. *Journal of Freshwater Ecology* 16: 171–178.
- Hammer, Ø., D. A. T. Harper & P. D. Ryan, 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4: 9.
- Hildrew, A. G., D. G. Raffaelli & R. Edmonds-Brown (eds), 2007. *Body Size: The Structure and Function of Aquatic Ecosystems*. Cambridge University Press, Cambridge.
- Hinga, K. R., 1992. Co-occurrence of dinoflagellate blooms and high pH in marine enclosures. *Marine Ecology Progress Series* 86: 181–187.
- Corp, I. B. M., 2017. SPSS Statistics for Windows, Version 25. Armonk, New York.
- Irigoin, X., J. Huisman & R. P. Harris, 2004. Global biodiversity patterns of marine phytoplankton and zooplankton. *Nature* 429: 863–867.
- Jeppesen, E., J. P. Jensen, M. Søndergaard, T. Lauridsen & F. Landkildehus, 2000. Trophic structure, species richness, and biodiversity in Danish lakes: changes along a phosphorus gradient. *Freshwater Biology* 45: 201–208.
- Kassambara, A., 2019. datarium: Data Bank for Statistical Analysis and Visualization. R package version 0.1.0. <https://CRAN.R-project.org/package=datarium>
- Kassambara, A., 2020. ggpubr: 'ggplot2' Based Publication Ready Plots. R package version 0.4.0. <https://CRAN.R-project.org/package=ggpubr>
- Kassambara, A., 2020. rstatix: Pipe-Friendly Framework for Basic Statistical Tests. R package version 0.6.0. <https://CRAN.R-project.org/package=rstatix>
- Keeley, J. E. & P. H. Zedler, 1998. Characterization and global distribution of vernal pools. In Witham, C. W., E. T. Bauder, D. Belk, W. R. Ferren Jr. & R. Ornduff (eds), *Ecology, Conservation, and Management of Vernal Pool Ecosystems – Proceedings from a 1996 Conference*. California Native Plant Society, Sacramento: 1–14.
- King, J. L., 1998. Loss of diversity as a consequence of habitat destruction in California vernal pools. In Witham, C. W., E. T. Bauder, D. Belk, W. R. Ferren Jr. & R. Ornduff (eds), *Ecology, Conservation, and Management of Vernal Pool Ecosystems – Proceedings from a 1996 Conference*. California Native Plant Society, Sacramento: 119–123.
- King, J. L., M. A. Simovich & R. C. Brusca, 1996. Species richness, endemism and ecology of crustacean assemblages in northern California vernal pools. *Hydrobiologia* 328: 85–116.
- Kneitel, J. M., 2014. Inundation timing, more than duration, affects the community structure of California vernal pool mesocosms. *Hydrobiologia* 732: 71–83.
- Kneitel, J. M., 2018. Occupancy and environmental responses of habitat specialists and generalists depend on dispersal traits. *Ecosphere* 9: e02143. <https://doi.org/10.1002/ecs2.2143>.
- Kneitel, J. M. & C. L. Lessin, 2010. Ecosystem–phase interactions: aquatic eutrophication decreases terrestrial plant diversity in California vernal pools. *Oecologia* 163: 461–469.
- Kneitel, J. M., N. Samiilenko, L. Rosas-Saenz & A. Nerida, 2017. California vernal pool endemic responses to hydroperiod, plant thatch, and nutrients. *Hydrobiologia*. <https://doi.org/10.1007/s10750-017-3174-7>.
- Leigh, E. G., 1965. On the relation between the productivity, biomass, diversity, and stability of a community. *Proceedings of the National Academy of Sciences of the United States of America* 53: 777–783.
- Liu, G., Z. Liu, Y. Li, F. Chen, B. Gu & J. M. Smoak, 2009. Effects of fish introduction and eutrophication on the cladoceran community in Lake Fuxian, a deep oligotrophic lake in southwest China. *Journal of Paleolimnology* 42: 427–435.
- Magnusson, A. K. & D. D. Williams, 2006. The roles of natural temporal and spatial variation versus biotic influences in shaping the physicochemical environment of intermittent ponds: a case study. *Archiv für Hydrobiologie* 165: 537–556.
- Merritt, R. W., K. W. Cummins & M. B. Berg (eds), 2008. *An Introduction to the Aquatic Insects of North America*, 4th ed. Kendall Hunt Publishing Company, Dubuque, Iowa.
- Miracle, M., M. Sahuquillo & E. Vicente, 2008. Large branchiopods from freshwater temporary pools of Eastern Spain. *Verhandlungen des Internationalen Verein Limnologie* 30: 501–505.
- Mittelbach, G. G., C. F. Steiner, S. M. Scheiner, K. L. Gross, H. L. Reynolds, R. B. Waide, M. R. Willig, S. I. Dodson & L. Gough, 2001. What is the observed relationship between species richness and productivity? *Ecology* 82: 2381–2396.
- Mokany, A., J. T. Wood & S. A. Cunningham, 2008. Effect of shade and shading history on species abundances and ecosystem processes in temporary ponds. *Freshwater Biology* 53: 1917–1928.
- Mozley, A., 1944. Temporary ponds, a neglected natural resource. *Nature* 154: 490.
- Nevalainen, L. & T. P. Luoto, 2017. Relationship between cladoceran (Crustacea) functional diversity and lake trophic gradients. *Functional Ecology* 31: 488–498.
- Pace, M. L., 1986. An empirical analysis of zooplankton community size structure across lake trophic gradients. *Limnology and Oceanography* 31: 45–55.
- Peltzer, P. M., R. C. Lajmanovich, J. C. Sánchez-Hernandez, M. C. Cabagna, A. M. Attademo & A. Bassó, 2008. Effects of agricultural pond eutrophication on survival and health status of *Scinax nasicus* tadpoles. *Ecotoxicology and Environmental Safety* 70: 185–197.
- R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Rosset, V., S. Angélibert, F. Arthaud, G. Bornette, J. Robin, A. Wezel, D. Vallod & B. Oertli, 2014. Is eutrophication really a major impairment for small waterbody biodiversity? *Journal of Applied Ecology* 51: 415–425.
- RStudio Team, (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA. <http://www.rstudio.com/>.
- Sand-Jensen, K., H. H. Bruun & L. Bastrup-Spohr, 2017. Decade-long time delays in nutrient and plant species dynamics during eutrophication and re-oligotrophication of Lake Fure 1900–2015. *Journal of Ecology* 105: 690–700.
- SAS Institute Inc., 2018. JMP® Pro Version 14.3. Cary, North Carolina.

- Scheffer, M. & S. R. Carpenter, 2003. Catastrophic regime shifts in ecosystems: linking theory to observation. *Trends in Ecology and Evolution* 18: 648–656.
- Smith, D. G., 2001. *Pennak's Freshwater Invertebrates of the United States: Porifera to Crustacea*, 4th ed. Wiley, New York.
- Smith, V. H., 2003. Eutrophication of freshwater and coastal marine ecosystems. *Environmental Science and Pollution Research* 10: 123–139.
- Straile, D. & W. Geller, 1998. Crustacean zooplankton in Lake Constance from 1920 to 1995: response to eutrophication and re-oligotrophication. *Advances in Limnology* 53: 255–274.
- Stomp, M., J. Huisman, G. G. Mittelbach, E. Litchman & C. A. Klausmeier, 2011. Large-scale biodiversity patterns in freshwater phytoplankton. *Ecology* 92: 2096–2107.
- Thorp, J. H. & A. P. Covich (eds), 2010. *Ecology and Classification of North American Freshwater Invertebrates*, 3rd ed. Elsevier, Burlington.
- Timm, H. T., Möls & T. Timm, 2006. Effects of long-term non-point eutrophication on the abundance and biomass of macrozoobenthos in small lakes of Estonia. *Proceedings of the Estonian Academy of Science* 55: 187–198.
- Van den Broeck, M., A. Waterkeyn, L. Rhazi & L. Brendonck, 2015. Distribution, coexistence, and decline of Moroccan large branchiopods. *Journal of Crustacean Biology* 35: 355–365.
- Van den Broeck, M., L. Rhazi, A. Waterkeyn, M. El Madihi, P. Grillas, J. M. Kneitel & L. Brendonck, 2019. Livestock disturbances in Mediterranean temporary ponds: a mesocosm experiment with sheep manure and simulated trampling. *Freshwater Biology* 64: 856–869.
- Wang, S., Z. Gong & P. Xie, 2000. Environmental factors and the succession of aquatic insects in a shallow Chinese lake. *Bulletin of Environmental Contamination and Toxicology* 64: 701–707.
- Wellborn, G. A., D. K. Skelly & E. E. Werner, 1996. Mechanisms creating community structure across a freshwater habitat gradient. *Annual Review of Ecology and Systematics* 27: 337–363.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Hydrobiologia is a copyright of Springer, 2021. All Rights Reserved.