Community dynamics of ephemeral systems: food web drivers, community assembly, and anthropogenic impacts of playa wetlands

BY

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Community dynamics of ephemeral systems: food web drivers, community assembly, and anthropogenic impacts of playa wetlands

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Abstract

Scientists know relatively little about the ecology of ephemeral wetlands and few attempts have been made to integrate the published ecological studies. We use playa wetlands, a common aquatic feature of many arid to semi-arid landscapes, to study what drives community dynamics and food web trophic structure (size, redundancy, and variability) in an extremely abundant aquatic habitat of the U.S. Great Plains. We conducted a multi-scale study to track playa wetland invertebrate communities and their food webs across space and time. We explored invertebrate diversity and richness and used geometric properties of stable isotope biplots (C^{13} and N^{15}) to measure trophic structure complexity.

On a large scale, playa communities are unaffected by changes in habitat size, depth, and native vegetation buffer. Artificially created waterbodies often hold communities indistinguishable from natural playas. Tilling for row crops negatively impacts playa diversity, but cattle grazing has a positive effect, perhaps reinstating functions of formerly abundant, native grazers, such as bison.

Playa food webs were more complex in insect dominated communities rather than crustacean branchiopods. Insect diversity was controlled by hydroperiod stage; therefore, the key to understanding playa trophic structure is an interaction between hydroperiod and the traits, lifespan, and trophic niche of the species present. Whether a playa was artificially created or grazed by cattle had little effect on trophic structure, yet tilling for row crops expanded the food sources available for the food web.

On a temporal scale, playa food webs initially increased in diversity, with declines at the end of the hydroperiod. Playas were organized in time rather than space. That is, food web complexity was more closely associated with identity of the taxa rather than total diversity.

Mesocosm experiments showed that habitat structure may play a role in determining food-web complexity, but the effect was overridden by the influence of hydroperiod. Mesocosm experiments revealed insect diversity to control trophic structure complexity in playa wetlands, rather than the trophically redundant crustacean branchiopods.

While playas are highly resistant and resilient habitats, they cannot survive all perturbations. Playas should be protected and have much to inform us about food web assembly and maintenance.

Acknowledgements

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Introduction

Ephemeral waterbodies are one of the most ubiquitous freshwater features of most terrestrial landscapes. They exist in many forms, such as small puddles, water filled structures of plants, snow-melt pools, depressional wetlands, large saline lakes, and vernal pools. Any natural depression or artificial container, which temporarily fills with water, can be considered an ephemeral waterbody. Despite their ubiquity, ephemeral waterbodies are often as inconsequential habitats because of short, unpredictable hydroperiods. Scientists know relatively little about the ecology of the organisms inhabiting ephemeral streams and wetlands because the primary ecological focus has been on permanent ecosystems. Consequently, there is a serious lack of integration among ecological studies in diverse ephemeral habitats.

This dissertation concentrates on playa wetlands, a common aquatic feature of many arid to semi-arid landscapes. While the southern portions of the U.S. Great Plains hold the highest concentration of playas, many other regions have similar habitats. One reason why these ecosystems have been studied less than similar habitats, such as the vernal pools of California, is their unpredictability. Many playas exist in quite arid regions, where rainfall patterns are highly isolated and sporadic. With recent drought patterns, some playas have not filled in decades. When they do fill with water, the pool may not last more than several weeks to a couple months depending on prevailing precipitation patterns. For example, field work for Chapter 3 was planned for the summer of 2012 but was delayed a year because the playas did not fill until the next spring following one of worst droughts in 10 years for Colorado. This makes it difficult for researchers to plan and carry out studies in these ecosystems. Even though playas may be key features of the landscape, understanding of their basic ecology is extremely limited. Geomorphological studies have closely looked at how playas form, how/when they fill, and their contribution to groundwater, but current ecological research on playas is mostly limited to species lists of animals that use these habitats, food production for waterfowl, and documenting how amphibians use playa wetlands. Even less literature has been devoted to the food webs of playa wetlands.

The following chapters are some of the first investigations into how playa food webs develop and are maintained. They take a multi-scale approach to track communities and food webs across space and time. Chapter 1 concentrates on a big-picture overview of how 93 playa communities across six U.S. states change with landscape characteristics, including natural and anthropogenic impacts. Chapter 2 takes a closer look at some of the same issues as Chapter 1, but it focuses on what drives and affects the size, redundancy, and variation of 21 playa food webs in Colorado. Chapter 3 uses a smaller spatial scale but expands the temporal scale of the study to follow how playa communities and food webs develop through multiple hydroperiods and seasons. Finally, Chapter 4 narrows further and explores how microhabitat features affect the food web in a controlled mesocosm experiment through multiple hydroperiods.

These four chapters create a comprehensive picture of how playa food webs develop, are maintained, and are affected by various natural and human-induced phenomena. While this is one of the first steps into understanding the ecology of playa food webs, we hope that future researchers can use this body of work as a springboard for studies investigating not only playas, but communities and food webs of ephemeral systems in general.

Chapter 1

Flexibility of ephemeral wetlands: environmental constraints and anthropogenic impacts

Abstract

Ephemeral wetlands commonly experience events that would be considered drastic disturbances in permanent ecosystems, such as elimination of all water. It is well known that survival in these harsh habitats requires flexibility in response to natural perturbations, but scientists have rarely investigated if this flexibility translates to anthropogenic stresses. Therefore, we evaluated aquatic invertebrate communities in ephemeral wetlands and identified ecological thresholds to environmental and anthropogenic constraints. We sampled the invertebrate communities of 93 ephemeral wetlands across 6 states of the North American high plains. Size, depth, and presence of a native vegetation buffer were unimportant in determining any recognizable aspect of the community. Artificial waterbody communities were indistinguishable from naturally formed wetlands. Grazing generally increased invertebrate densities and richness values and was a positive influence on invertebrate communities. These communities have great flexibility, which seems to be translating to resilience under anthropogenic forces. However, tilling for row-crop agriculture decreased invertebrate density and richness. Overall, current conservation strategies in ephemeral wetlands may need to be revised to include artificial habitats as viable, important habitats, and grazing as an essential component of ecosystems in areas now lacking large native grazers, such as bison.

Introduction

Ephemeral wetlands commonly experience events that would be drastic disturbances in permanent ecosystems. Organisms in these highly variable environments must cope with the disappearance of all water (Williams 2006), survive extreme temperature fluctuations from diel, hydroperiod, and seasonal changes (Eriksen 1966, Young and Zimmerman 1996), and adapt to osmotic changes as water levels fluctuate (Paradise and Dunson 1998), among other challenges. Specific adaptations allow residents to survive and even thrive through these seemingly demanding environmental conditions. Many crustaceans hatch from resting eggs (Wiggins et al. 1980), some amphibians burrow and aestivate (Bentley 1966), insects often opportunistically colonize (Haukos and Smith 2003), and many birds are transient and use ephemeral wetlands as resting, breeding, and feeding sites (Haukos and Smith 1992).

Playas are shallow, clay-lined, ephemeral wetlands common in arid to semi-arid regions (Evans 2010). They can be created by a combination of geomorphic, hydrochemical, or biologic processes (see Smith (2003), Gurdak and Roe (2009), and Evans (2010)). Playas can be dry over periods from a few weeks to decades, depending on precipitation and evaporation.

Along with environmental challenges, playa wetlands are often subjected to anthropogenic disturbances. Haukos and Smith (2003) reported that at least 90% of playa wetlands have been impacted by various human activities. Unfortunately, playas currently do not receive protection under federal law because of their ephemeral nature (Haukos and Smith 2003). Playa wetlands are threatened by various influences including urban expansion, road building (Haukos and Smith 2003), replacement of native grazers with cattle and sheep (Marty 2005), row crop agriculture (Hernandez et al. 2006) and subsequent sedimentation (Evans 2010), and changing climate patterns (Pyke and Marty 2005). Yet, we do not completely understand how playas are affected by these disturbances, let alone natural community constraints. To develop successful conservation and management strategies, we need to determine how playa wetland communities react to natural and anthropogenic constraints.

While it has long been known that many systems are maintained by some form of disturbance (Pickett et al. 1989, Cardinale and Palmer 2002) (e.g. fire dependent ecosystems, intertidal habitats, and seasonal wetlands), it can be easy to assume that all natural and anthropogenic disturbances are harmful to ecosystem function. While overarching tolerance limits exist, communities tolerant to disturbances such as episodic droughts and inundations have developed and are present in these situations worldwide. Indeed, these conditions may be critical to the survival of some species (e.g. resting eggs often need to be desiccated to hatch (Brendonck 1996)) or to overall community diversity, as in the role of abiotic disturbance and predation in maintaining intertidal communities (Paine 1966). Nonetheless, a disturbance threshold can be reached where communities change, cease to function effectively, or are largely replaced by a new disturbance-tolerant community. Determining environmental constraints is relatively straightforward for single organisms but more challenging for an entire community, food web, or ecosystem.

In this study, we evaluated the flexibility of the aquatic invertebrate communities of playa wetlands in the high plains regions of the North American Great Plains to identify ecological thresholds to environmental and anthropogenic constraints. We tested three questions: Is there a minimum area, minimum habitat size, or level of nutrients where disturbances to the aquatic invertebrate community are detectable? How different are communities in naturally formed playas and artificial waterbodies (wetlands formed by human construction or activities)? Are the aquatic invertebrate diversity or the abundance of aquatic invertebrates depressed or enhanced by cattle grazing, row crop agriculture, or the lack of a native vegetation buffer? In answering these questions, we sought to identify which stresses in these ephemeral wetlands are manageable and which cause a shift to a new ecosystem state. These answers will provide information as to whether the high tolerances and great resilience of these communities in their natural state translate to the types of anthropogenic stresses seen in the majority of playas today.

Methods

Study sites

We sampled 93 ephemeral wetlands (playas, rock pools, and saline lakes) in the North American states of Colorado, Montana, Nebraska, North Dakota, South Dakota, and Wyoming (Fig 1.1, Table 1.1). Many of these wetlands were in the Pawnee and Sheyenne National Grasslands. Other sites were on a mix of public and private land chosen from a database obtained from the Playa Lakes Joint Venture and the Rocky Mountain Bird Observatory. Many unmarked playas were fortuitously discovered and sampled while traveling between previously identified sampling sites.

Figure 1.1 - Map of ephemeral wetlands sampled

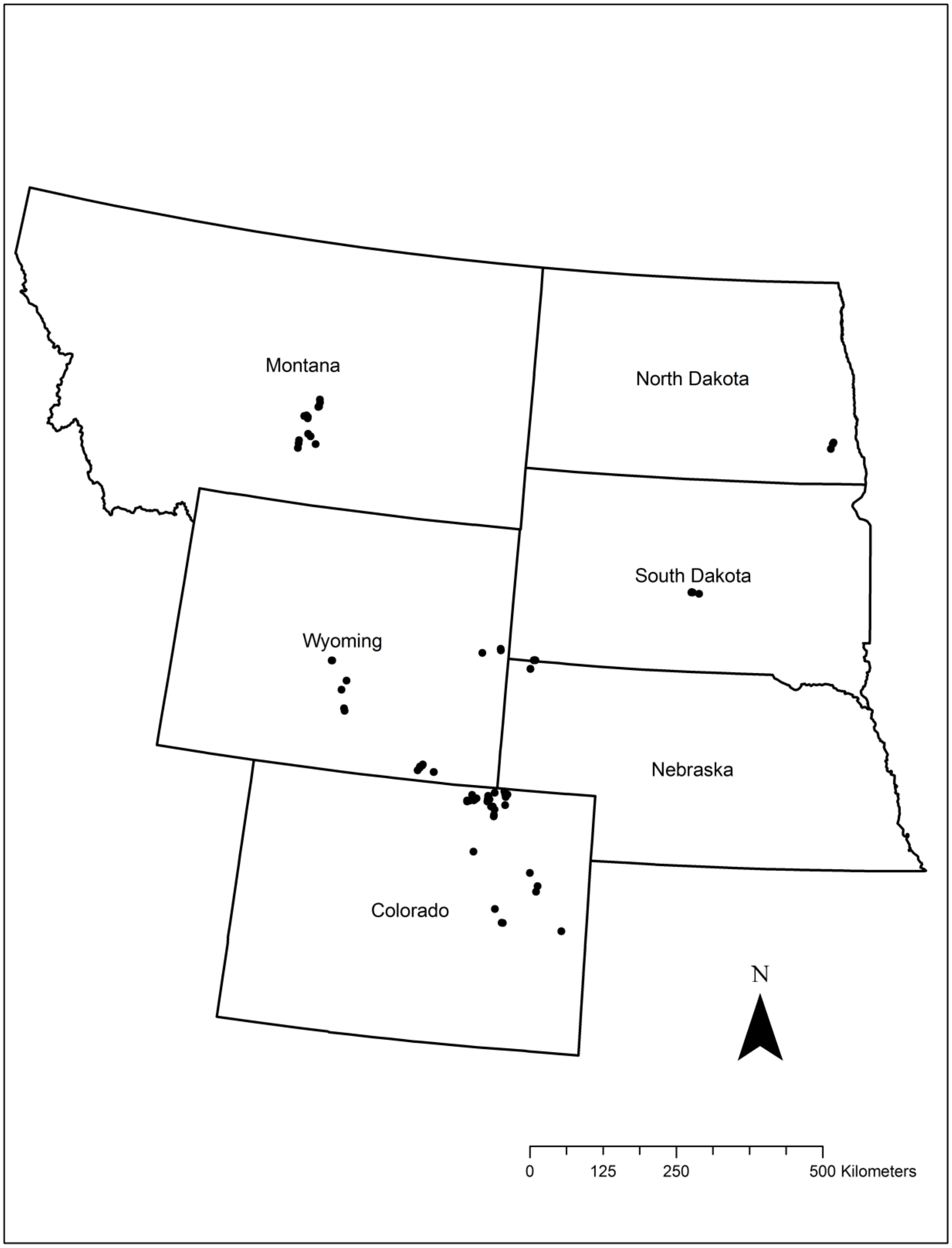
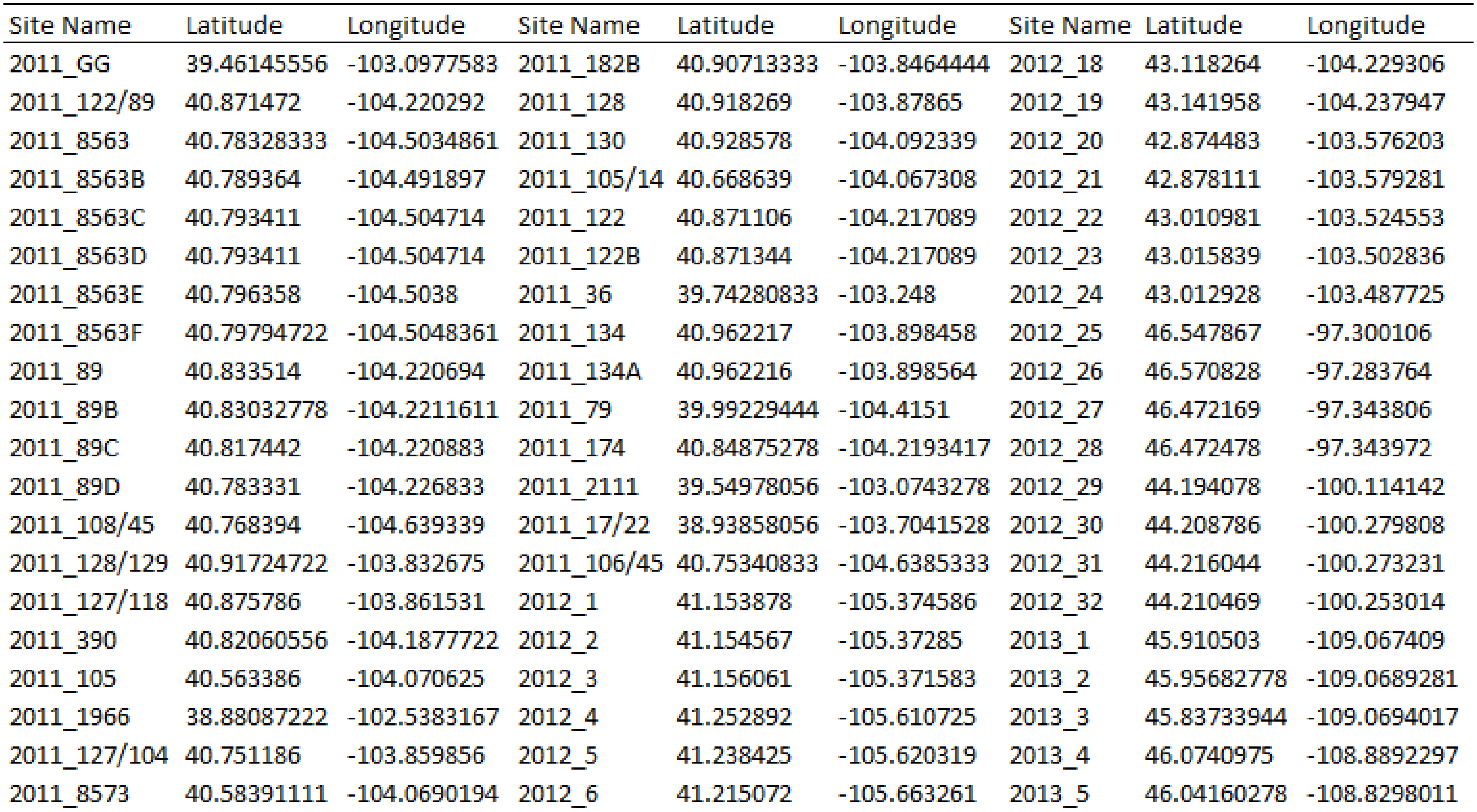
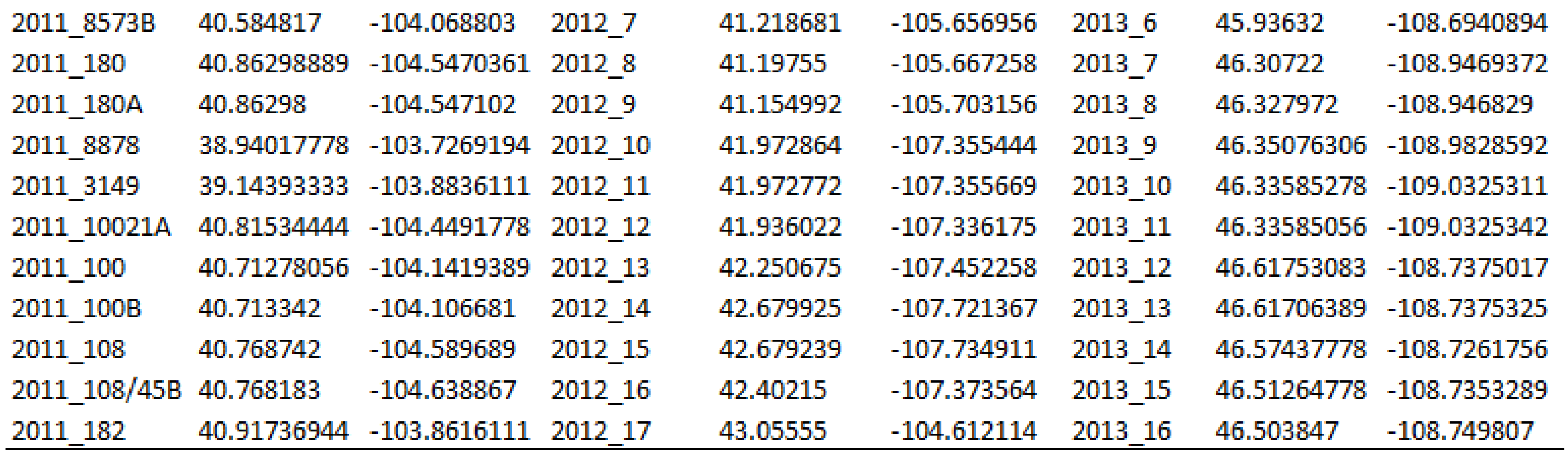


Table 1.1 – Site name, Latitude, and Longitude of study sites. Site name refers to year sampled and site name.

Columns are in groups of three and no association between different sites in the same row should be made.



Site Name	Latitude	Longitude	Site Name	Latitude	Longitude	Site Name	Latitude	Longitude
2011_GG	39.46145556	-103.0977583	2011_182B	40.90713333	-103.8464444	2012_18	43.118264	-104.229306
2011_122/89	40.871472	-104.220292	2011_128	40.918269	-103.87865	2012_19	43.141958	-104.237947
2011_8563	40.78328333	-104.5034861	2011_130	40.928578	-104.092339	2012_20	42.874483	-103.576203
2011_8563B	40.789364	-104.491897	2011_105/14	40.668639	-104.067308	2012_21	42.878111	-103.579281
2011_8563C	40.793411	-104.504714	2011_122	40.871106	-104.217089	2012_22	43.010981	-103.524553
2011_8563D	40.793411	-104.504714	2011_122B	40.871344	-104.217089	2012_23	43.015839	-103.502836
2011_8563E	40.796358	-104.5038	2011_36	39.74280833	-103.248	2012_24	43.012928	-103.487725
2011_8563F	40.79794722	-104.5048361	2011_134	40.962217	-103.898458	2012_25	46.547867	-97.300106
2011_89	40.833514	-104.220694	2011_134A	40.962216	-103.898564	2012_26	46.570828	-97.283764
2011_89B	40.83032778	-104.2211611	2011_79	39.99229444	-104.4151	2012_27	46.472169	-97.343806
2011_89C	40.817442	-104.220883	2011_174	40.84875278	-104.2193417	2012_28	46.472478	-97.343972
2011_89D	40.783331	-104.226833	2011_2111	39.54978056	-103.0743278	2012_29	44.194078	-100.114142
2011_108/45	40.768394	-104.639339	2011_17/22	38.93858056	-103.7041528	2012_30	44.208786	-100.279808
2011_128/129	40.91724722	-103.832675	2011_106/45	40.75340833	-104.6385333	2012_31	44.216044	-100.273231
2011_127/118	40.875786	-103.861531	2012_1	41.153878	-105.374586	2012_32	44.210469	-100.253014
2011_390	40.82060556	-104.1877722	2012_2	41.154567	-105.37285	2013_1	45.910503	-109.067409
2011_105	40.563386	-104.070625	2012_3	41.156061	-105.371583	2013_2	45.95682778	-109.0689281
2011_1966	38.88087222	-102.5383167	2012_4	41.252892	-105.610725	2013_3	45.83733944	-109.0694017
2011_127/104	40.751186	-103.859856	2012_5	41.238425	-105.620319	2013_4	46.0740975	-108.8892297
2011_8573	40.58391111	-104.0690194	2012_6	41.215072	-105.663261	2013_5	46.04160278	-108.8298011



2011_8573B	40.584817	-104.068803	2012_7	41.218681	-105.656956	2013_6	45.93632	-108.6940894
2011_180	40.86298889	-104.5470361	2012_8	41.19755	-105.667258	2013_7	46.30722	-108.9469372
2011_180A	40.86298	-104.547102	2012_9	41.154992	-105.703156	2013_8	46.327972	-108.946829
2011_8878	38.94017778	-103.7269194	2012_10	41.972864	-107.355444	2013_9	46.35076306	-108.9828592
2011_3149	39.14393333	-103.8836111	2012_11	41.972772	-107.355669	2013_10	46.33585278	-109.0325311
2011_10021A	40.81534444	-104.4491778	2012_12	41.936022	-107.336175	2013_11	46.33585056	-109.0325342
2011_100	40.71278056	-104.1419389	2012_13	42.250675	-107.452258	2013_12	46.61753083	-108.7375017
2011_100B	40.713342	-104.106681	2012_14	42.679925	-107.721367	2013_13	46.61706389	-108.7375325
2011_108	40.768742	-104.589689	2012_15	42.679239	-107.734911	2013_14	46.57437778	-108.7261756
2011_108/45B	40.768183	-104.638867	2012_16	42.40215	-107.373564	2013_15	46.51264778	-108.7353289
2011_182	40.91736944	-103.8616111	2012_17	43.05555	-104.612114	2013_16	46.503847	-108.749807

Field Sampling

We visited each ephemeral wetland once in the summers of 2011 (Colorado), 2012 (Nebraska, North Dakota, South Dakota, and Wyoming), and 2013 (Montana). We recorded relevant physical parameters such as size, depth, and presence and length of buffer zone (native vegetation of at least 50 m between the wetland and adjacent roads, tilling, or other anthropogenic impact). A playa was considered tilled when tilling marks proceeded into the wetland, even if tilling marks were not visible within the wetland. We qualitatively noted vegetation cover as absent (0% cover), low (1–33% cover), medium (33–67% cover), or high (67–100% cover). We did not differentiate between upland and wetland vegetation types. We used Mardel ® test strips to measure gross differences in water chemistry (in ppm): total hardness, buffering capacity, and concentrations of nitrite, nitrate, and phosphorus. Wetlands were aggregated into four types: natural playas (playas of apparently natural origin), artificial waterbodies (those created by humans: ditches, stock ponds, etc.), rock pools (rainwater pool located in rock outcroppings), and saline playas (playas with saline waters). We also recorded evidence of cattle grazing (henceforth referred to as grazed playas, however grazing from wild animals occurred on all sample sites), as well as tilling, or other habitat modification. Invertebrate samples were taken from the middle of the wetland with a 1-m net sweep (25 cm x 18 cm; 400 μm mesh), which sampled 45 L of water. If the water was less than 18 cm deep, we extended the sweep to fulfill the 45 L requirement (e.g. water 9 cm deep = 2m sweep. If the water was deeper than 18 cm, we started the sweep at the bottom and swept up evenly through 1 m to the water surface. Invertebrates were immediately preserved in 75% EtOH.

Species identifications and statistics

Invertebrates were identified in the lab using Arnett et al. (2001, 2002), Darsie and Ward (2005), Merrit et al. (2008), Thorp and Covich (2010), and literature cited therein. Large branchiopod crustaceans and several insects were identified to species, smaller crustaceans and most insects were identified to genus, and some dipterans and annelids were classified to higher taxa. We extracted various metrics (species richness; total number; mollusc number and percent; crustacean number, percent, and richness; diptera number and percent; mosquito number and percent; insect number, percent, and richness; noncrustacean number, percent, and richness; predator number and percent; nonpredator number and percent; obligate number and percent; opportunistic number and percent; and ostracod number and percent) from the dataset. We designated various invertebrate taxa as obligate or opportunistic to ephemeral wetlands based on Rogers (1998), and literature cited therein.

We conducted univariate statistics in Minitab v. 16 (Minitab Inc., State College, PA, USA), which mainly consisted of ANOVAs, two-sample t-tests, Kruskal-Wallis tests, and Mann-Whitney U tests. Due to sampling design, the wetlands had a variety of grazing, tilling and buffering states, and these predictor variables could have interacted. Where sample size allowed, we reran each statistical test while isolating the effect of each group from the other variables (e. g. tested for effects of tilling in only unbuffered wetlands, effect of buffering in only grazed wetlands, etc.). While the results of the interaction tests may have had different test statistics and p values, the overall results only diverged from the single variable analyses in one area (effect of plowing in ungrazed wetlands). Therefore, the results of the interaction analyses that did not diverge from the single variable analyses are not reported herein.

We conducted multivariate statistics in PCORD v. 6 (MJM Software Design, Geneden, OR, USA). Multi-response permutation procedure (MRPP) and nonmetric multidimensional scaling (NMS) ordination were completed on playa communities with the general procedure and starting configurations following guidelines in McCune and Grace (2002). MRPP is a multivariate statistical technique that uses multivariate data to test if groups are significantly different. The NMS ordination used the Sorensen (Bray-Curtis) distance measure, starting with six dimensions stepping down to one, 50 runs with real data, and 15 iterations to evaluate stability with a stability criterion of 0.00001. A supplied seed integer of 4483 was used for the starting configuration. The possibility of a better-than-random solution was evaluated using a Monte Carlo test with 10,000 iterations. A scree plot and final stress was used to determine the number of dimensions to use in the final test. Stability of each ordination was evaluated by using the plot of stress vs. iteration number and by the final instability.

Results

General results

A variety of aquatic macroinvertebrates were collected from the study playas. A summary of the specific results are presented in Table 1.2. Each value represents the average number of each taxon in that wetland condition. However, no association between the sets of rows should be perceived due to overlap among wetland conditions (grazing, buffering, etc.). Species richness varied between 0 and 21 species per wetland, with an average of 8.6 species. Invertebrate density ranged between 0 and 66.3 invertebrates * L^{-1}, with an average of 8.58 invertebrates * L^{-1}.

Table 1.2 – Mean number of various taxa per sweep sample grouped by different wetland types and conditions. One sweep = 45L of water sampled. Data are truncated into the shown invertebrate groups for ease of display. The “other invertebrates” group includes mites (Acari), amphipods (*Hyalella*), crayfish (*Orconectes immunis*), and collembola (*Podura aquatica*).

Mollusca	Gastropoda		1.46	0.91	1.68	0.74	1.28	1.23	0.28	1.44
Crustacea	Anostraca	*Artemia franciscana*	1.2	0	0	1.78	0	2.81	0	0.92
		Thamnocephalus platyurus	1.83	1.58	1.67	1.83	2.10	0.81	0.86	1.90
		Branchinecta packardi	0.34	1.75	0.39	1.37	1.14	0	0	0.97
		Branchinecta coloradensis	3.16	0	3.71	0	2.88	0	0	2.44
		Branchinecta constricta	0.77	0	0.90	0	0	1.81	0	0.60
		Branchinecta gigas	0.05	0	0.06	0	0.04	0	0	0.04
		Branchinecta lateralis	0.43	0	0.50	0	0.39	0	0	0.33
		Branchinecta lindahli	0.85	0	0.48	0.66	0.78	0	0	0.66
		Branchinecta paludosa	0	7.72	4.75	0	3.69	0	0	3.13
		Branchinecta readingi	6.26	0	7.35	0	5.70	0	0	4.84
		Branchinecta serrata	0.59	0	0.40	0.37	0.54	0	0	0.46
		Streptocephalus	23.22	27.80	23.06	27.01	30.25	10.77	5.86	28.16
	Notostraca	*Triops*	1.13	1.14	0.87	1.48	1.36	0.56	0.68	1.22
	Laevicaudata	*Lynceus brachyurus*	0.39	0.97	0.44	0.78	0.65	0.44	0.18	0.67
		Lynceus brevifrons	0.02	0	0.02	0	0.01	0	0	0.01
	Spinicaudata	*Eulimnadia sp.*	1.48	6.66	1.72	5.22	1.84	6.92	0	3.84
		Leptestheria compleximanus	0.93	0.13	0.86	0.39	0.87	0.08	0.14	0.74
		Cyzicus sp.	0.28	0	0.33	0	0.25	0	0	0.22
		Eocyzicus digueti	0.23	0	0.27	0	2.09	0	0	0.18
	microcrustaceans		317.80	326.50	357.60	274.10	350.30	245	174	346.80

Insecta	Ephemeroptera		0.94	0.06	423.00	0.92	0.38	1.31	1.79	0.44
	Odonata		0.59	0.11	0.35	0.52	0.29	1.64	0.50	0.41
	Hemiptera	Corixidae	2.06	2.45	2.94	1.24	2.41	1.64	1.32	2.35
		Notonectidae	1.55	1.06	1.10	1.74	0.84	2.79	2.04	1.27
	Coleoptera	Hydrophilidae	2.02	1.73	1.47	2.49	1.81	2.19	2.96	1.73
		Dytiscidae	1.01	1.00	1.08	0.92	1.06	0.87	1.00	1.01
		Other Beetles	0.61	0.44	0.70	0.35	0.59	0.44	0.29	0.60
	Diptera	Chironomidae	4.23	4.31	2.57	6.40	2.46	8.88	9.54	3.32
		Culicidae	5.42	5.03	5.53	4.98	3.54	9.77	9.07	4.61
		Ephydridae	0.39	0.13	0.32	0.27	0.26	0.39	0.36	0.29
		Other Diptera	0.51	0.03	0.62	0	0.48	0	0.07	0.39
	Other invertebrates		0.92	0.67	0.95	0.68	0.50	1.69	0.54	0.89

Community composition by wetland type

Many invertebrate metrics in natural playas (henceforth “Nat”) and artificial waterbodies (Art) significantly differed from rock pools (Rock) and saline playas (Salt). We sampled 50 Nat, 32 Art, 4 Rock, and 7 Salt. Nat and Art wetlands generally had drastically higher numbers of invertebrates and different proportions of invertebrates when compared to Rock and Salt wetlands (Table 1.3). No invertebrate metrics showed any significant differences between Nat and Art. Multi-Response Permutation Procedure (MRPP) showed that overall, the communities differed significantly among wetland types ($A = 0.031$, $p < 0.0001$). Pairwise comparisons of the MRPP showed that Nat and Art were indistinguishable, whereas both the Rock and Salt communities were significantly distinct from all other types (Table 1.4). Non-metric Multidimensional Scaling (NMS) also showed the same trend. Figure 1.2A shows a cloud of points that occupy the central portion of the NMS plot. This focal area mainly contains Nat and Art, whereas axis 2 clearly separates the rock and salt groups from the rest. The NMS solution had a final stress of 11.03826 in three dimensions (Kruskal’s rule of thumb states stress of ~10 is satisfactory (McCune and Grace 2002)). The final instability was 0.00221 after 91 iterations.

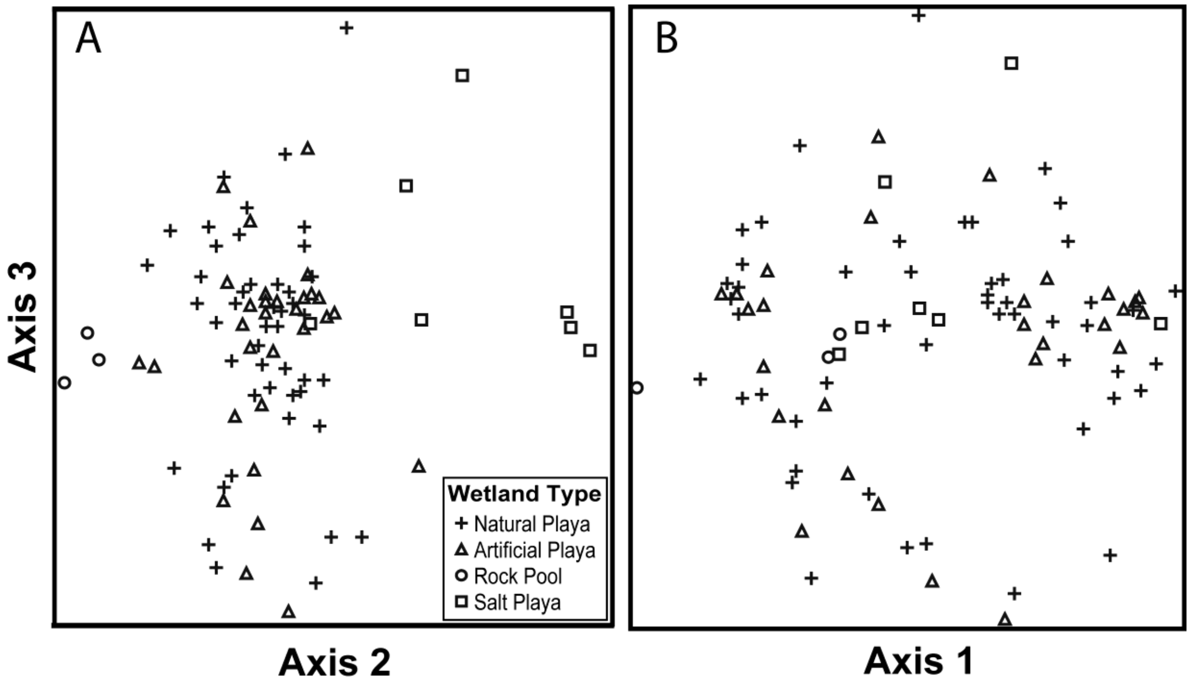
Table 1.3 – Significant results of individual invertebrate metrics grouped by type (natural playa (Nat), artificial waterbody (Art), rock pool (Rock), saline playa (Salt)). All tests had 3 degrees of freedom. Test column –KW refers to Kruskal-Wallace test. Nat, Art, Rock, Salt columns refer to either means of each group for ANOVA tests, and medians for KW tests.

Variable	Test	Test Statistic	p value	Nat	Art	Rock	Salt
species richness	KW	H = 24.86	< 0.001	10	9	1.5	2
# of invertebrates	ANOVA	F = 8.4	< 0.001	170	147	9.5	12
# of crustaceans	KW	H = 11.16	0.011	147	132	9	5
crustacean richness	KW	H = 17.83	< 0.001	4.5	4	1	1
# of insects	ANOVA	F = 5.07	0.003	2.4	2.3	0.52	1.2
insect richness	KW	H = 14.92	0.002	4.5	4	0.5	1
# of ostracods	KW	H = 9.76	0.021	2.5	0	0	0
# obligate	ANOVA	F = 2.82	0.043	4.6	4.2	1.96	2.7

Table 1.4 – MRPP results for natural playas (Nat), artificial waterbodies (Art), rock pools (Rock), and saline playas (Salt). A = chance-corrected within-group agreement.

	Nat	**Art**	**Rock**
Art	A = -0.004997, p = 1.0		
Rock	A = 0.035739, p = 0.002	A = 0.055609, p < 0.001	
Salt	A = 0.021875, p < 0.001	A = 0.0303383, p = 0.004	A = 0.145006, p = 0.001

Figure 1.2 - Non-metric multidimensional scaling plot of ephemeral wetland communities. Axis 2 clearly separates the wetland types

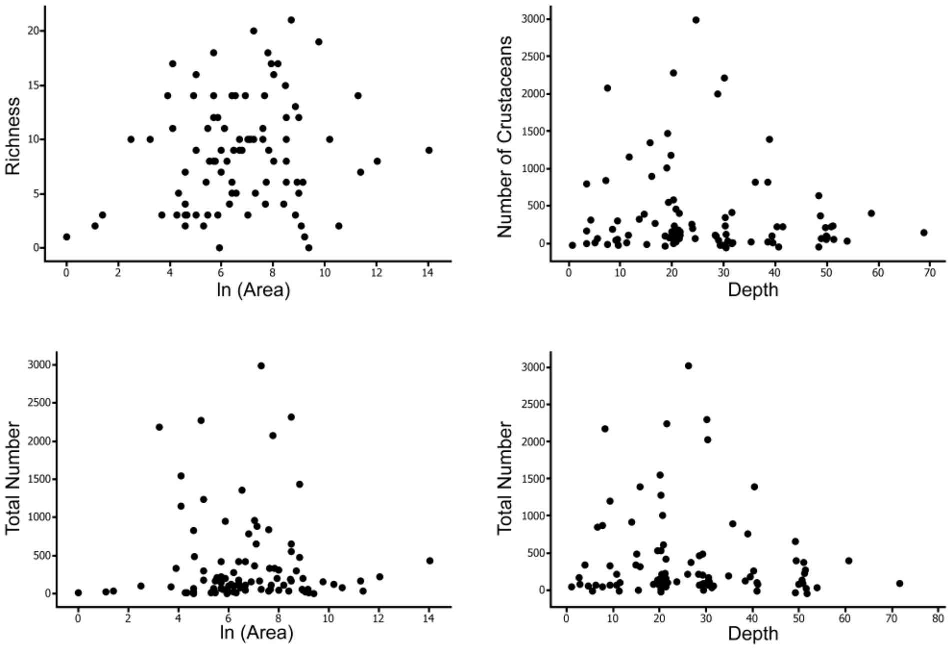


Environmental Constraints

Physical Parameters

The area of the 93 sampled wetlands ranged from 1 m^2 to 1,247,400 m^2, and averaged 19,708 m^2. Wetland depth ranged from 2–70 cm, and averaged 26.12 cm. Many invertebrate metrics showed a relatively consistent, albeit non-significant, non-linear pattern with wetland area and depth. Metrics generally peaked in the central portion of the scatterplot (e.g. Fig. 1.3), with smaller or zero metric values at the extremes of the area and depth continua.

Figure 1.3 - Selected invertebrate metrics



Vegetation

Vegetation cover was only weakly associated with crustacean richness (ANOVA, $F = 2.55$, $df = 3$, $p = 0.064$). The low vegetation cover group had the highest mean crustacean richness, with 6.233 species, whereas wetlands without vegetation, medium plant cover, and high vegetation cover averaged 3.8, 4.4, and 3.9 species, respectively. Densities of other invertebrates and invertebrate groups (e.g. insect, diptera, mosquitos, predators, non-predators, taxa obligate to ephemeral wetlands, etc.) showed no significant association with vegetation cover.

Nutrients and Chemical Parameters

Nitrite ($NO_{2)}$ was significantly higher in ungrazed wetlands (Kruskal-Wallace test, $W = 701.5$, $p = 0.0045$), with the median values being 0 and 1 ppm in grazed and ungrazed playas, respectively. Nitrate (NO_3) was also found to be at higher levels in ungrazed wetlands (Kruskal-Wallace test, $W = 711.5$, $p = 0.0002$) with the median values being 0 and 40 ppm in grazed and ungrazed playas, respectively. Alkalinity was negatively correlated with invertebrate richness ($r = -0.338$, $p = 0.025$) and insect richness ($r = -0.35$, $p = 0.02$). Values of pH were negatively correlated with species richness ($r = -0.398$, $p = 0.007$) and ln(# of insects) ($r = -0.368$, $p = 0.013$). No significant associations were found between the nutrient and chemical parameters and all other invertebrate metrics.

Anthropogenic impacts

Native Vegetation Buffer

No significant effects of vegetation buffering were apparent in the 52 buffered and 41 unbuffered wetlands we sampled. All individual invertebrate metrics showed no differences in buffered compared to unbuffered wetlands. However, MRPP showed a small difference in invertebrate communities (A = 0.00846, p = 0.01272).

Grazing

Many individual invertebrate metrics differed significantly in grazed and ungrazed wetlands (Table 1.5). Among the 67 grazed wetlands and 26 ungrazed wetlands we sampled, species richness metrics and numbers were generally higher in grazed wetlands. Grazed playas had significantly higher numbers and proportions of species obligate to ephemeral wetlands, and significantly lower numbers and proportions of species that are opportunist to ephemeral wetlands (Table 1.5). MRPP showed a small difference in communities (A = 0.00976, p = 0.0072) between grazed and ungrazed playas.

Table 1.5 - Significant results of individual invertebrate metrics grouped by grazing. Test column – 2T refers to a 2-sample T-test, MWU refers to a Mann-Whitney U test. P values for Mann-Whitney U tests have been adjusted for ties. Grazed and Ungrazed columns refer to either means of each group for 2T, and medians for MWU tests. Means for ln(#crustaceans) and ln(#obligate) reflect means of data before natural log transformation. Sample size for grazed was 67 and ungrazed 26.

Variable	Test	Test Statistic	df	p value	Grazed	Ungrazed
species richness	2T	2.73	38	0.009	9.42	4.5
Ln(# of invertebrates)	2T	2.03	38	0.05	418.9	301
ln(# of crustaceans)	2T	2.63	40	0.012	403.3	271
% crustaceans	2T	2.84	34	0.008	83%	58.8%
crustacean richness	MWU	3612	-	0.001	5	2
% diptera	2T	-2.55	30	0.016	5.3%	18.6%
% insects	2T	-2.91	31	0.007	13.3%	36.5%
ln(# obligate)	2T	2.79	39	0.008	396.4	266
% obligate	2T	2.5	35	0.017	78%	53.7%
% opportunists	MWU	2942	-	0.0332	0%	0%

Tilling

We found significant differences in the individual invertebrate metrics between 14 tilled (wetland area tilled or row crops directly abutting wetland) and 79 untilled wetlands we sampled. Untilled wetlands generally had higher numbers of invertebrates and higher species richness (Table 1.6). MRPP showed that untilled wetlands had significantly different communities as a whole compared to tilled wetlands ($A = 0.01142$, $p = 0.00383$). Interaction tests found that tilling had no effect on species richness (2-sample T test, $T_{23} = 0.0$, $p = 1.0$) or crustacean richness (Mann-Whitney U test, $W = 727$, $p=0.425$).

Table 1.6 - Significant results of individual invertebrate metrics grouped by tilling. Test column – 2T refers to a 2-sample T-test, MWU refers to a Mann-Whitney U test. P values for Mann-Whitney U tests have been adjusted for ties. Tilled and untilled columns refer to either means of each group for 2T, and medians for MWU tests. Means for ln(#obligate) reflect means of data before natural log transformation.

Variable	Test	Test statistic	df	p value	Tilled	Untilled
species richness	2T	-2.02	20	0.057	6.5	8.97
# invertebrates	MWU	405	-	0.0067	51	165
# crustaceans	MWU	371	-	0.0021	23	159
% crustaceans	2T	-3.28	15	0.005	46%	81.50%
crustacean richness	MWU	354	-	0.0011	1.5	4
% insects	2T	2.91	14	0.011	46.40%	15.10%
ln(# obligate)	2T	-3.73	16	0.002	171.00	393.40
% obligate	2T	-4.08	16	0.001	31%	78.00%

Discussion

Environmental Constraints

Wetland Type

We expected natural playas and artificial waterbodies to group separately from the rock pools and saline playa groups because previous studies have shown that rock pools often show low diversity, require organisms to be highly stress tolerant (Jocque et al. 2010), and are controlled mainly by physical factors (McLachlan and Cantrell 1980). Furthermore, our rock pools were quite small (median = 3.5 m^2). The saline playas were much larger, but such environments are characterized by more challenging chemical environments and are associated with lower diversity (La Barbera and Kilham 1974, Green 1986, Hammer 1986, Hammer 1993). As our data indicate, rock pools and saline playas have community that are characterized by lower invertebrate richness (crustacean, non-crustacean, and insect richness values) and invertebrate densities in many categories. While crustaceans, insects, and taxa that are obligate to ephemeral ponds were significantly higher in natural playas and artificial waterbodies than in rock and salt pools (Table 1.3), these results are strongly influenced by differences in total densities and diversities. Natural playas and artificial waterbodies had more total invertebrates, and the other metrics simply followed this trend.

Physical Parameters

The correlation between diversity/number of organisms and ecosystem/habitat size is well known (Munroe 1948, MacArthur and Wilson 1963, Smith et al. 2005). Therefore, the lack of correlations between wetland area/depth and invertebrate metrics was unexpected. However, we propose that two factors may have caused the observed intermediate peaks found along the area and depth continua. The wetlands at the lower end of the area and depth continua are either very small (< 20 m^2) and/or shallow (< 10 cm deep). Since hydroperiod, the length of time the wetland has standing water, is controlled by wetland depth and shape (Garmendia and Pedrola-Monfort 2010), these shorter-lived habitats may be unable to support as diverse assemblages as the larger and deeper environments (King et al. 1996). Some species may be unable to complete their lifecycle within a smaller wetland with a shorter hydroperiod, their eggs may not survive longer periods of dryness, and vagile species may avoid these habitats when colonizing or laying eggs in favor of larger/deeper habitats with longer hydroperiods. On the opposite end of the area or depth continuum are the larger and deeper wetlands that may behave more like permanent wetlands. Most of the large branchiopod crustaceans, such as fairy shrimp (Anostraca), clam shrimp (Spinicaudata and Laevicaudata), and tadpole shrimp (Notostraca) may find these wetlands less suitable because their eggs are adapted to only respond to hatching cues after the wetland dries, so as to limit the number of times eggs hatch with insufficient hydroperiod remaining (Brendonck 1996). For non-crustacean members of the potential species pool, sources of immigrants would be smaller because permanent wetlands are rare and widely spaced in the semi-arid region we sampled.

Vegetation

Crustacean richness was the only invertebrate metric associated with vegetation cover. The large branchiopod crustaceans found in playas are largely adapted to planktonic habitats. Extremely dense vegetation often holds habitat for insect predators and/or could impede their swimming, thereby limiting access to food and mates (Brendonck et al. 2008, Rogers 2009). However, mixed habitat, where there is much open water, plus some vegetation stabilizing the sediments and providing additional substrate on leaves and stems for periphyton growth could allow more niches for more crustacean species. Additionally, water completely absent of vegetation could increase susceptibility to predation by ducks and wading shorebirds. Therefore, playas with only sparse or low levels of vegetation may provide a suitable refuge from both types of predators and create additional feeding conditions for higher crustacean richness.

Nutrients and Chemical Parameters

The negative correlations of alkalinity with species richness and insect richness, and pH with species richness and number of insects may simply be a matter of the chemical tolerances of organisms to salt levels, because saline playas had the highest alkalinity and pH. Some colonists may be approaching or surpassing their physiological tolerances and thus eliminated from the saline environments rather than being limited by alkalinity or pH levels.

Anthropogenic Impacts

Artificial Habitats

The artificial waterbodies were mainly long and narrow roadside ditches and stock ponds which have distinct differences in their aquatic habitat compared to nearby natural ephemeral wetlands (Fig. 1.4). Yet analysis of individual invertebrate metrics and multivariate techniques showed that their communities were indistinguishable from natural playas. Several factors could have caused these similarities. One reason could be that all natural playas in these areas are degraded to some degree. However, the Pawnee and Sheyenne National Grasslands, while not pristine, have some of the best habitat available for this type of study. Another reason could be that the sampling scheme may not have been as responsive to differences due to overlapping group membership that were responding to various disturbances. Nevertheless, some form of artificial waterbodies may be a more practical alternative to conserving species normally found in natural wetlands (Elphick 2000, Huner et al. 2002, Longoni 2010, Li et al. 2013). Ephemeral wetland communities are made up of members that are extremely tolerant generalists, in the sense that they have high tolerances for the fluctuations found in ephemeral habitats. They may however have little tolerance for certain aspects of permanent wetlands, such as fish predation. These fauna may be flexible enough to overcome the challenges of artificially created habitats. Many large branchiopod crustaceans, the hallmark taxa of playa habitats (Brendonck et al. 2002), can thrive in many types of field and laboratory habitats as long as predation is not intense and physicochemical hatching requirements (temperature, salinity, etc.) are met. For example, resting eggs of commercially available “sea monkeys” (often *Artemia* fairy shrimp) or *Triops* (tadpole shrimp) are successfully raised in glass jars, the most artificial of habitats, as long as proper food is available.

Figure 1.4 - Top – Naturally formed playa. Bottom – Artificially formed waterbody in roadside ditch

Native Vegetation Buffer

While the MRPP showed a difference (A = 0.00846) in the communities between buffered and unbuffered wetlands, the effect size was much less than A = 0.01, which is considered to be very small (McCune and Grace 2002). If an artificial habitat such as a roadside ditch is acceptable habitat, the presence of several hundred meters of native vegetation may not significantly improve the habitat for the majority of invertebrates. However, this study only examined invertebrate communities, and we would expect to see differences in other aspects if the ephemeral wetland were surrounded by roads on one or more sides, such as: (a) sedimentation problems if next to a tilled field (Evans 2010); (b) differences in the surrounding plant community; and (c) abundance and diversity of birds and other vertebrates.

Grazing

The community differences between grazed and ungrazed wetlands were striking. Grazing can impact properties of a landscape (Bremer et al. 2001, Gerlanc and Kaufman 2003), including habitat characteristics of wetland/riparian areas (Fleischner 1994, Robertson and Rowling 2000). The higher nitrite and nitrate concentrations in ungrazed wetlands were unexpected given the obvious signs of defecation and urination within the grazed wetlands. Grazing may be stimulating rates of primary production so that nutrients may be used up faster in grazed wetlands.

There is a historical perception that grazing is detrimental to biodiversity (Pyke and Marty 2005, Sayre et al. 2012), yet recent research has shown that grazing may be beneficial and actually maintain some aspects of ephemeral wetland fauna (Marty 2005, Pyke and Marty 2005). The ungrazed playas had higher numbers of opportunistic species, which mostly consist of dipterans, including mosquitos and other taxa considered less-desirable in relation to human health concerns. Indeed, one playa we sampled in 2011 was divided by a fence with grazing on one side only. The grazed side was dominated by crustaceans and taxa obligate to ephemeral wetlands, whereas the ungrazed side was dominated by opportunistic species, especially mosquitos and other diptera. Communities are responding to grazing, even in the same playa. While this single data point is anecdotal at best, it fits in nicely into the overall pattern, and is an area for future research. Additionally, interaction tests showed that species richness and crustacean richness were found to be unaffected by tilling in ungrazed playas. Tilling had an overwhelming negative effect when analyzed separately, and these results of the interaction test point to an even higher importance of grazing in these systems.

The invertebrates found in these habitats are resilient and may be adapted to respond positively to the presence of large mammals and the impacts of cattle grazing. It is important to note that the type of open range cattle grazing in and around these wetlands does not have the same impact as confined feeding operations. At the grazing densities we observed, the cattle appear to be keeping the water column more open by reducing wetland vegetation density (Rogers 1998), may be transporting eggs as other mammals do (Vanschoenwinkel et al. 2008, Vanschoenwinkel et al. 2011), creating microhabitats with their hoofprints (Johnson and Rogers 2003), and mixing the egg bank (Rogers 2014). As long as densities remain low, cattle grazing increases spatial heterogeneity and enhances species diversity of prairies (Collins 1987, Hartnett et al. 1996); and while cattle may not be the best substitute, they may functionally replace native grazers (Knapp et al. 1999) such as bison (*Bison bison*), mule deer (*Odocoileus hemionus*), and pronghorn (*Antilocarpa americana*).

Tilling

Tilling from row crop agriculture had a consistently negative effect on invertebrate communities, as evidenced by our results of lower crustacean richness, a depleted general invertebrate fauna, fewer species obligate to ephemeral wetlands, and higher proportions of insects and diptera. Ephemeral wetlands are often the most vulnerable to impact and slowest to recover from agricultural disturbances (Bartzen et al. 2010). Tilling and the resulting monoculture crop severely change the landscape in and around ephemeral wetlands, and over half of the world's wetlands have been lost, primarily to agriculture (Zedler and Kercher 2005). Tilling can result in increased sedimentation (Hernandez et al. 2006, Evans 2010), which is one of the most widespread and detrimental impacts to ephemeral wetlands (Luo 1997, Haukos and Smith 2003). Pesticides, herbicides, and fertilizers may be changing the chemical environment of wetlands (Thurman et al. 2000), and the repeated physical turnover of soils may select for different communities (Devictor et al. 2007). It appears that ephemeral wetland communities are not resistant or resilient enough to overcome the disturbance from tilling for row crop agriculture.

Conclusions

These invertebrate communities live in habitats that undergo extreme environmental fluctuations. Consequently, these communities have great flexibility, which seems to be translating to resilience under some anthropogenic forces (e.g., roads, artificial impoundments). The invertebrate communities have high enough tolerances to live in artificially created or unbuffered playas with little to no effect on the community. These organisms are adapted to the disturbances caused by large mammal grazing, and actually do better in grazed playas.

However, these communities show consistent negative responses to tilling and row crop agriculture. The value of playa wetlands should not be overlooked, yet, the United States Government currently does not protect them (Haukos and Smith 2003). Wherever found, natural playas should be protected, but the value of artificial waterbodies should not be discounted. While many countries require a "no net loss of wetlands", and wetland mitigation banks have been created for many years, the scientific community often shuns artificial habitats as inferior habitats that deserve little recognition. Closer research on these issues will provide a sound scientific basis for the conservation and management of ephemeral wetlands in the future.

Acknowledgements

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Chapter 2

Untangling food web structure in an ephemeral ecosystem

Abstract

Scientists know relatively little about the ecology of ephemeral aquatic habitats beyond the importance of hydroperiod and vertebrate predators, especially regarding trophic structure. We used playa wetlands, common ephemeral habitats common in arid to semi-arid regions, as our study ecosystem. We predicted that more species-rich, larger playas would have food webs with larger dimensions (longer food chain and more energy sources used), more variation (more niches filled), and more redundancy (niche overlap). We used geometric properties of stable C and N isotope bi-plots to explore: (a) what regulates trophic structure in ephemeral wetlands; (b) the role of anthropogenic forces in altering factors controlling trophic structure; and (c) how trophic structure in ephemeral systems differs from that typical of permanent systems.

We sampled aquatic animal food webs of 21 playa wetlands located mostly in the short-grass prairies of the Pawnee National Grassland in the U.S. state of Colorado. Playas with higher insect diversity had more complex trophic structures than those dominated by large branchiopods (tadpole, clam, and fairy shrimps). Insect diversity seemed dependent on length of, and position (time since filling) within the playa's hydroperiod, both of which in turn are determined by playa depth. The key to understanding trophic structure in playas is an interaction between hydroperiod and the traits, lifespan, and trophic niche of the species present.

The trophic structure of naturally created playas was marginally more complex than artificial playas, and playas buffered by native vegetation had a higher number of food webs that included unique trophic strategies. Food webs of playas influenced by row crop agriculture had a broader selection of food sources. Playas grazed by cattle had food web structures comparable

to those in ungrazed playas, suggesting that playa biota may be adapted to large mammal disturbances. The trophic structure of ephemeral aquatic habitats may not respond to disturbance in the same way as permanent systems. Indeed food chain length in a playa can actually increase as ephemeral ecosystem approaches the end of its hydroperiod. Measures of ecosystem size need to include more than the two dimensions of surface area when being applied to general ecological theory. Food webs increase in complexity with time, and many of the same factors that control trophic structure in ephemeral habitats could be important at least at some time in the life of a relatively permanent ecosystem.

Introduction

Scientists know relatively little about the ecology of ephemeral wetlands beyond the importance of hydroperiod (Spencer et al. 1999, Bilton et al. 2001, Gascon et al. 2005, Waterkeyn et al. 2008, Sim et al. 2013) and vertebrate predators, such as salamanders and anuran larvae, which have been shown to affect ephemeral communities (Dickman 1968, Burton and Likens 1975, Seale 1980, Wilbur 1997, Ghioca-Robrecht and Smith 2008, Ghioca-Robrecht and Smith 2011). Ecological research has primarily focused on permanent ecosystems, and studies lack integration among diverse ephemeral habitats (cf. Williams 2006), despite some ecological commonalities. An important difference between permanent and ephemeral systems is that many groups of organisms common to permanent ecosystems are markedly absent from ephemeral systems (Williams 2006) due to the extreme fluctuations in environmental conditions. Permanent residents of these habitats require a suite of specialized attributes (Williams 2006), which can include resting eggs (Wiggins et al. 1980), ability to enter diapause (Brendonck 1996), short generation times (Shepherd and Brantley 2005), rapid growth rates (Zedler 2003), and high vagility of propagules (Vanschoenwinkel et al. 2008a) or adults for dispersal.

Beyond these broad generalizations, many questions remain. What regulates trophic structure in ephemeral systems? How do anthropogenic forces affect factors controlling trophic structure? How does trophic structure in ephemeral systems differ from that more typical of permanent systems?

Many important aspects of trophic structure can be explored to answer these questions. For example, size of the web, or the number of species interacting in the web, is an important characteristic that can correlate with other properties of trophic structure. Webs with more species have been shown to have a more complex trophic structure (Wiggins *et* al. 1980, Wellborn 1996, Urban 2004). The dimensions of the web, which refers to the number of trophic levels and/or the breadth of energy sources used in a web, may offer insights into community structure, ecosystem processes, and contaminant concentrations (Post 2002). The variation of the web, or the number, types, and spread of niches exploited, can be used to examine trophic diversity of a food web (Layman et al. 2007) and to explore the trophic specificity/generality of individuals (Bearhop et al. 2004) and whole communities (Layman et al. 2007). Finally, the redundancy of the web, or the extent to which species fulfill similar trophic niches, may have implications on competition (Layman et al. 2007), the stability of a food web (Borrvall, Ebenman and Jonsson 2000, McCann 2000), and trophic functioning (Fridley 2001). All these aspects of trophic structure can be analyzed using a set of metrics developed by Layman *et al.* (2007). These metrics extract data from the geometric properties of taxa based on their position on an isotope bi-plot (most commonly using C and N isotopes). This approach is founded on the concept of an isotopic niche (Layman et al. 2012), which is a proxy for the Hutchinsonian n-dimensional hypervolume (Hutchinson 1957) and also likely correlated with a trophic niche (Jackson et al. 2011). With careful interpretation and sampling, these metrics can be useful (Layman and Post 2008), and they have been successfully employed to describe trophic structure in many different ecosystems (Darimont, Paquet and Reimchen 2009, Martinez del Rio et al. 2009, Olsson et al. 2009, Quevedo, Svanbäck and Eklöv 2009, Golléty et al. 2010, Turner *et al*. 2010, Zambrano *et al*. 2010). However, all of these studies and most studies that use stable isotopes to determine food chain length or trophic structure are from permanent ecosystems.

We used playa wetlands as our study ecosystem. Playa wetlands, common, ephemeral aquatic features of many arid to semi-arid climates, are often roughly circular (Luo et al. 1997), clay-lined, shallow depressions (Evans 2010) which can arise from a combination of geomorphic, hydrochemical, and biologic processes (see Smith (2003), Gurdak and Roe (2009), and Evans (2010)). These highly homogenous environments often have little vegetation or habitat structure. The southern High Plains of the United States has the highest concentration of natural playas in the world (Evans 2010). However, similar habitats can be found in many arid to semi-arid areas on other continents and are known by many names, such as the pans of Australia and South Africa, and the takyr, sabkha, and kavir of Central Asia, Saudi Arabia, and Iran, respectively. A playa may be dry for decades during droughts or may fill and dry several times in a given year depending on evaporation and precipitation.

The large branchiopods (clam, fairy, and tadpole shrimps) are a group well adapted to surviving in playa wetlands due to their highly resistant resting eggs, and they are often the dominant group of organisms early in the hydroperiod (Jocque, Vanschoenwinkel and Brendonck 2010). Insects also frequent these habitats (Haukos and Smith 2003) but usually disperse from adjacent aquatic habitats. Amphibians are common in these habitats when inundated (Williams 2006), and waterfowl often use wet playas as resting, breeding, and feeding sites (Haukos and Smith 1992). Towards the end of the hydroperiod, playas become dominated by predatory invertebrates (Boix et al. *2*006). Beyond this, very little is known about the basic ecology of playas (Haukos and Smith 2003); and to our knowledge, our study is the first of its kind to employ stable isotopes to analyze food web structure in playa wetlands.

Playa wetlands are excellent systems for testing factors potentially controlling the trophic structure of food webs. We expected size of the web (species richness) and physical ecosystem size to have positive effects on trophic structure. We predicted that larger ecosystems would have more species, which would then allow the food web to have larger dimensions (longer food chain, more energy sources exploited), more variation (more niches filled), and more redundancy (more species would create a higher probability that any two species would have the same niche). Conversely, we initially expected anthropogenic impacts, such as cattle grazing, plowing for row crops, and habitat modification, to alter the control of the system, with artificially created habitats and these agriculturally impacted systems having more simple trophic structures. We predicted that food webs in impacted systems would be simpler, with smaller dimensions (short food chains, few energy sources exploited), reduced variation (fewer niches), and higher redundancy (more generalists).

In addition to testing these hypotheses, we highlight what ephemeral systems can tell us, in general, about the trophic structure of food webs. We explore hydroperiod and disturbance and how they interact to control trophic structure in playas and many ephemeral systems, and we emphasize some differences in food web structure between permanent and ephemeral systems.

Methods

Study site

We sampled 21 playa wetlands throughout northeastern Colorado, with most located in the Pawnee National Grassland in Weld County (Fig. 2.1). This national grassland is a "checkerboard" of short-grass prairie and agricultural land which is owned by a variety of governmental and private entities. Playas ranged in size from 12–88,000 m^2, with a median of $2000m^2$. The hydrologic cycles of these playas are driven by sporadic thunderstorm events and often are wet for a month or two per year, depending on overall precipitation trends for the year (2011 - ~1 month of hydroperiod in June and July 2012 – Dry entire year 2013 – most playas were wet for 3 weeks in May, and 1.5 months in September and October). Some had been grazed or plowed, whereas others were more natural with a buffer zone of native short-grass prairie. Four of the playas were artificially created wetlands (roadside ditches and stock ponds). We chose most sites from a database from two organizations: the Playa Lakes Joint Venture and the Rocky Mountain Bird Observatory. Several other unmarked playas were fortuitously discovered and sampled while traveling through the national grassland.

Figure 2.1 – Map of sampled playa sites.

Field sampling

We visited each playa once in the summer of 2011, and recorded relevant physical parameters (size, depth, and presence and density of vegetation). We also noted evidence of grazing, plowing, presence and length of short-grass prairie buffer, and/or whether the playa was a natural depression or artificially created. We considered a playa grazed if it had cattle prints or visible manure within and around the playa. Evidence of plowing consisted of plow marks or crop vegetation within or directly adjacent to the playa border. We considered a playa buffered if the area surrounding the playa on all sides was short-grass prairie, the prevailing native vegetation of the area. If any border of the playa touched a road or crop field it was considered unbuffered. Artificial playas were those water-filled, man-made depressions found in roadside ditches or in stock tanks. We collected separate invertebrate samples for characterization of community composition and for stable isotope analysis. For aquatic community composition, we quantitatively sampled the middle of the playas with a 1 m net sweep (25cm x 18cm; 400μm mesh), which resulted in a total of 45L of water sampled. If the playa was shallower than the 18 cm height of the net, we extended the sweep to fulfill the 45L requirement (e.g. water 9cm deep = 2m sweep). We then retrieved a qualitative sample to collect the less abundant species and for use in stable isotope analyses. Qualitative sampling continued until all of the following conditions were met: 1) all areas of the playas were repeatedly sampled; 2) repeated net sweeps failed to collect new taxa; and 3) each taxa had sufficient numbers for isotope sample replication. In several of the smaller playas, only single individuals of the larger predatory insects (Hydrophilidae, Dytiscidae, Anisoptera) were found; but for most taxa, we collected at least several individuals. This generally allowed for 3–5 stable isotope replicates for each taxon. Invertebrate samples were immediately preserved in 75% EtOH, and later identified in the laboratory. We collected five tadpoles (Plains spadefoot toad, *Spea bombifrons*) from each playa, euthanized them in MS-222, and preserved them in 75% EtOH. Sediment, plant detritus, and any visible algae were sampled and preserved in 75% EtOH, and any live plants found within the playa were sampled, put on ice, and frozen once back at the lab.

Species identifications and stable isotope analyses

We counted all collected metazoan invertebrates and identified the invertebrates using Thorp and Covich (2010) and Merritt, Cummins and Berg (2008). Crustaceans and amphibians were identified to species, most insects to genus, and dipterans and annelids to higher taxa. For stable isotope analysis, we dried the samples at 60°C for 48 hr, ground them into a fine, homogenized powder using a Wig-L-Bug® Mixer/Amalgamator, and held them in desiccators until analyzed. Samples were placed in tin capsules, weighed (2–3 mg dry mass), and analyzed at the University of Kansas W.M. Keck Paleoenvironmental and Environmental Stable Isotope Laboratory using a ThermoFinnigan MAT 253 continuous flow system mass spectrometer. Data collected included total N and C, and $\delta^{13}C$ and $\delta^{15}N$ values. The $\delta^{13}C$ and $\delta^{15}N$ values were determined by the relative difference in isotopic ratio between the samples and known standards as represented by the following equation:

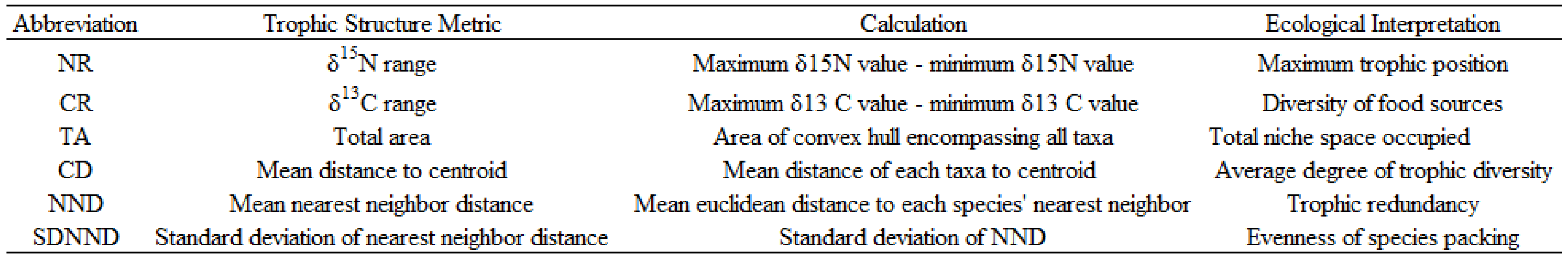
$$\delta X = ((R_{sample} / R_{standard}) - 1) \times 1000$$

where X is either ^{13}C or ^{15}N and the corresponding ratio is either $R = {^{13}C}/{^{12}C}$ or $R = {^{15}N}/{^{14}N}$. Vienna Pee Dee Belemnite and atmospheric nitrogen were used as the C and N standards, respectively.

Statistics and data analyses

The trophic structures of playa invertebrate communities were quantified using metrics (Table 2.1) developed by Layman et al. (2007), and included: (a) $\delta^{15}N$ range [NR], (b) $\delta^{13}C$ range [CR], (c) total area [TA], (d) mean distance to centroid [CD], (e) mean nearest neighbor distance [NND], and (f) standard deviation of nearest neighbor distance [SDNND]. We chose the original metrics of Layman et al. (2007) in lieu of newer Bayesian approaches (Jackson et al. 2011) that are more appropriate for the niche of a single taxon. All trophic structure metrics were calculated using the centroids of each animal taxon. Therefore, if a playa had seven taxa, the trophic structure metrics were derived from seven points, those seven points being the centroids of each taxon. NR and CR are simple calculations of the lowest $\delta^{15}N$ and $\delta^{13}C$ value subtracted from the highest. TA was calculated by drawing convex hulls around points of the C-N isotope bi-plot and calculating the area in ArcMap v. 10 (ESRI, Redlands, CA, USA). For CD, the centroid of each bi-plot was calculated in Excel (Microsoft Inc., Redmond, WA, USA), and then distances were calculated from each taxon point to the centroid and averaged. NND was calculated using a distance matrix (Euclidean distance) created in PCORD v. 6 (MJM Software Design, Geneden, OR, USA) and averaging the shortest distances between each taxon and its nearest taxon. SDNND used the same distance matrix and was computed by taking the standard deviation of the nearest neighbor distances. See Layman *et al*. (2007) for more detailed instructions and alternate ways to calculate the trophic structure metrics. All statistical tests were completed using Minitab v. 16 (Minitab Inc., State College, PA, USA). Two-way interaction effects for the various explanatory variables were explored using interaction plots and the general linear model function in Minitab.

Table 2.1 – Trophic structures metrics as developed from Layman et al. (2007). Columns indicate abbreviation, full name, method of calculation, and ecological interpretation.



Abbreviation	Trophic Structure Metric	Calculation	Ecological Interpretation
NR	δ^{15}N range	Maximum δ15N value - minimum δ15N value	Maximum trophic position
CR	δ^{13}C range	Maximum δ13 C value - minimum δ13 C value	Diversity of food sources
TA	Total area	Area of convex hull encompassing all taxa	Total niche space occupied
CD	Mean distance to centroid	Mean distance of each taxa to centroid	Average degree of trophic diversity
NND	Mean nearest neighbor distance	Mean euclidean distance to each species' nearest neighbor	Trophic redundancy
SDNND	Standard deviation of nearest neighbor distance	Standard deviation of NND	Evenness of species packing

Results

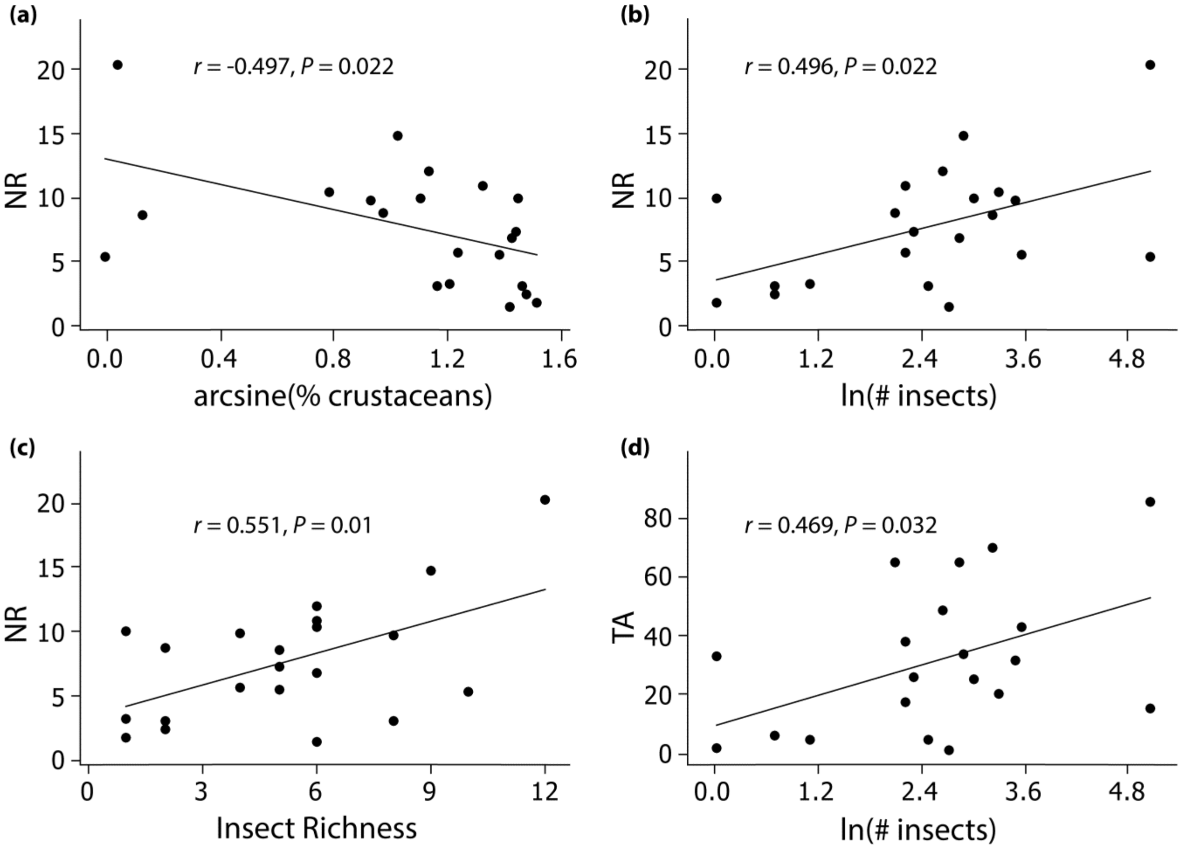
General results

Playas were dominated by a mixture of crustaceans which were mostly large branchiopods: tadpole shrimp (Notostraca) – *Triops longicaudatus;* fairy shrimp (Anostraca) species in *Thamnocephalus, Branchinecta,* and *Streptochephalus*; and clam shrimp (Spinicaudata and Laevicaudata) species in *Lynceus, Cyzicus,* and *Eulimnadia;* among others crustaceans. We also collected insects (Hemiptera – Corixidae and Notonectidae; Coleoptera – Dytiscidae and Hydrophilidae; Diptera – Culicidae, Chironomidae, Ephydridae, and Syrphidae; among others), and amphibians (*Spea bombifrons*). Densities of organisms varied greatly, averaging 12.74 invertebrates/L and ranging from 2.2 to over 46 invertebrates/L. For additional information on the community dynamics of these playa wetlands, contact the senior author or see O'Neill, Thorp, and Rogers (in prep).

Trophic structure results

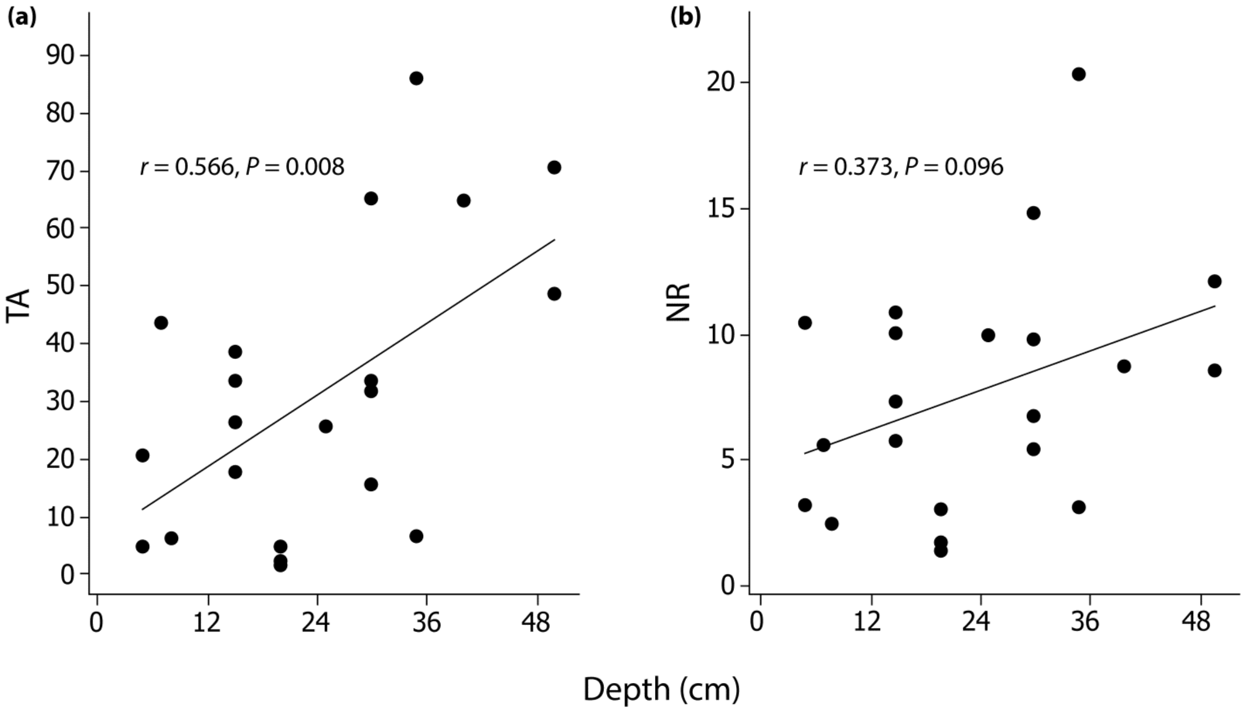
Playas held an average of 12.2 taxa, ranging from 6 to 18 taxa. No trophic structure metrics (NR, CR, TA, CD, NND, or SDNND, as described in Table 2.1) were correlated with species richness or any related metrics of diversity such as Shannon-Weiner Diversity Index, Evenness, or Simpson's Diversity Index. However, several trophic structure metrics were correlated with various community metrics (Fig. 2.2). NR was negatively correlated with arcsine(% crustaceans) (r = -0.497, P = 0.022), positively correlated with ln(# insects) (r = 0.496, P = 0.022), and positively correlated with Insect Richness (r = 0.551, P = 0.01). Additionally TA was positively correlated with ln (# insects) (r = 0.469, P = 0.032). CR, TA, CD, NND, and SDNND were not correlated with these community metrics.

Figure 2.2 – Selected trophic structure metrics (NR = Nitrogen Range, TA = Total Area) regressed on selected community metrics. R = Pearson Correlation Coefficient.



Playas ranged in area from 12 m^2 to 88,000 m^2, with a median of 2,000 m^2. NR, CR, TA, CD, NND, or SDNND were not correlated with playa area. Playa depth ranged from 5 cm to 50 cm and averaged 23.8 cm. Playa depth was not correlated with CR, CD, NND, or SDNND, however, it was correlated with TA ($r = 0.566$, $P = 0.008$) (Fig. 2.3). Additionally, NR was marginally, positively correlated with playa depth ($r = 0.373$, $P = 0.096$) (Fig. 2.3).

Figure 2.3 – TA and NR regressed on depth of playa. *r* = Pearson Correlation Coefficient.



While not statistically significant, natural playas (n=17) showed some differences in trophic structure metrics compared to artificially created playas (n=4). TA (t_8 = 2.09, P = 0.07) and CD (t_7 = 2.01, P = 0.085) were marginally lower in artificial playas (Fig. 2.4). All other trophic structure metrics showed no difference. All correlations and statistical tests were repeated excluding artificial playas in case the possible hydrological and morphological differences were affecting the results. All of the tests showed the same results as those where artificial playas were included, thus the tests excluding artificial playas are not reported herein. Of the 21 playas we sampled, 6 showed evidence of plowing either within or directly next to the playa. Plowed playas had higher CR and CD values (t_7 = 2.71, P = 0.03; t10 = 2.64, P = 0.025, respectively; Fig. 2.5) compared to unplowed playas. All other trophic structure metrics were not significantly different in plowed vs. unplowed playas. Furthermore, no trophic structure metrics were found to be statistically different in the 13 grazed vs. 8 ungrazed playas. NR, CR, TA, CD, or NND were not significantly different between playas buffered with native vegetation vs. those without a buffer. Of the 21 playas, we sampled 11 buffered by native vegetation. However, all but one of the unbuffered playas had low values of SDNND, while playas with a buffer had a greater range of SDNND values (Fig. 2.6). While this association is not statistically significant (high degree of overlap between both groups), many buffered playas had much higher values of SDNND than the unbuffered playas. Interaction plots and the general linear model function of Minitab v.16 showed that there were no significant two-way interaction effects between the explanatory variables of playa type, buffering, grazing, and plowing.

Figure 2.4 – TA (Total area of convex hull) and CD (mean distance to centroid) in natural and artificial playas.

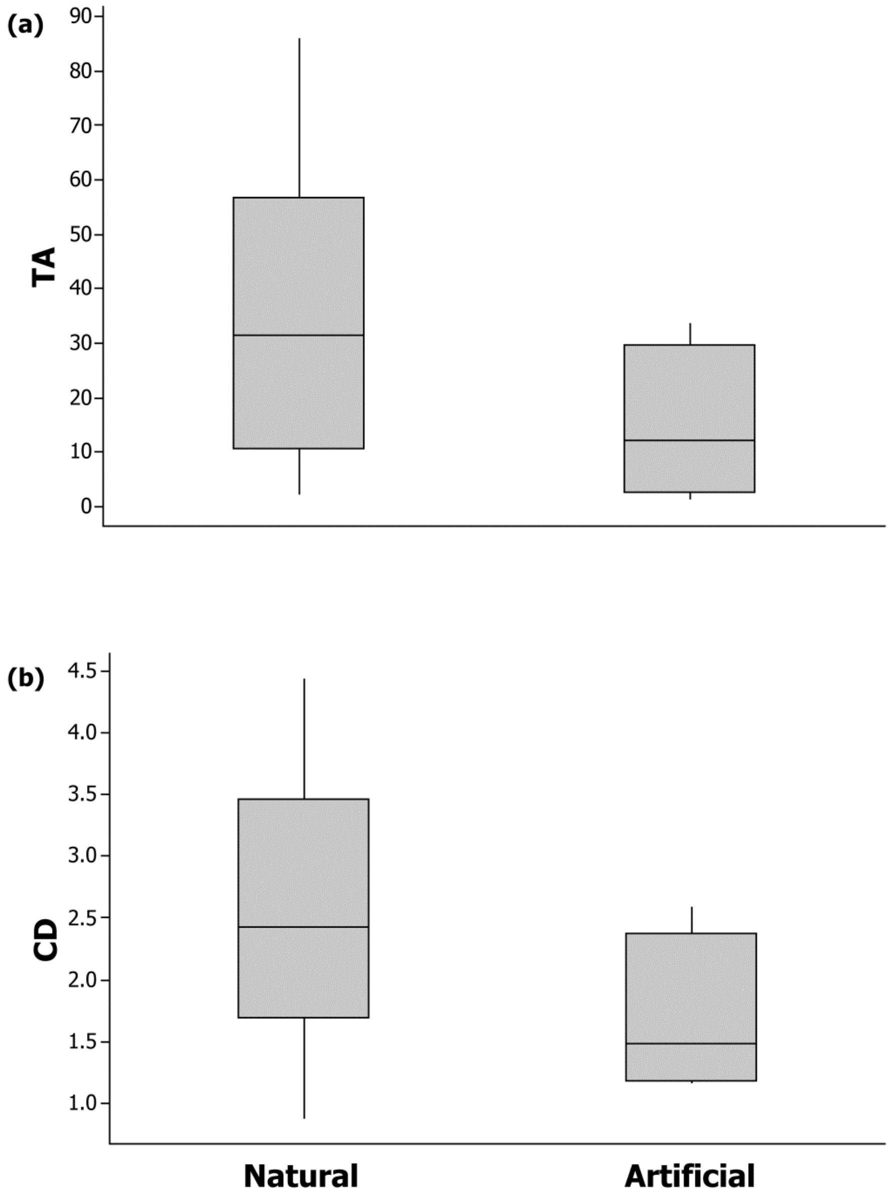


Figure 2.5 – CR (δ^{13}C range) and CD (mean distance to centroid) in plowed and unplowed playas.

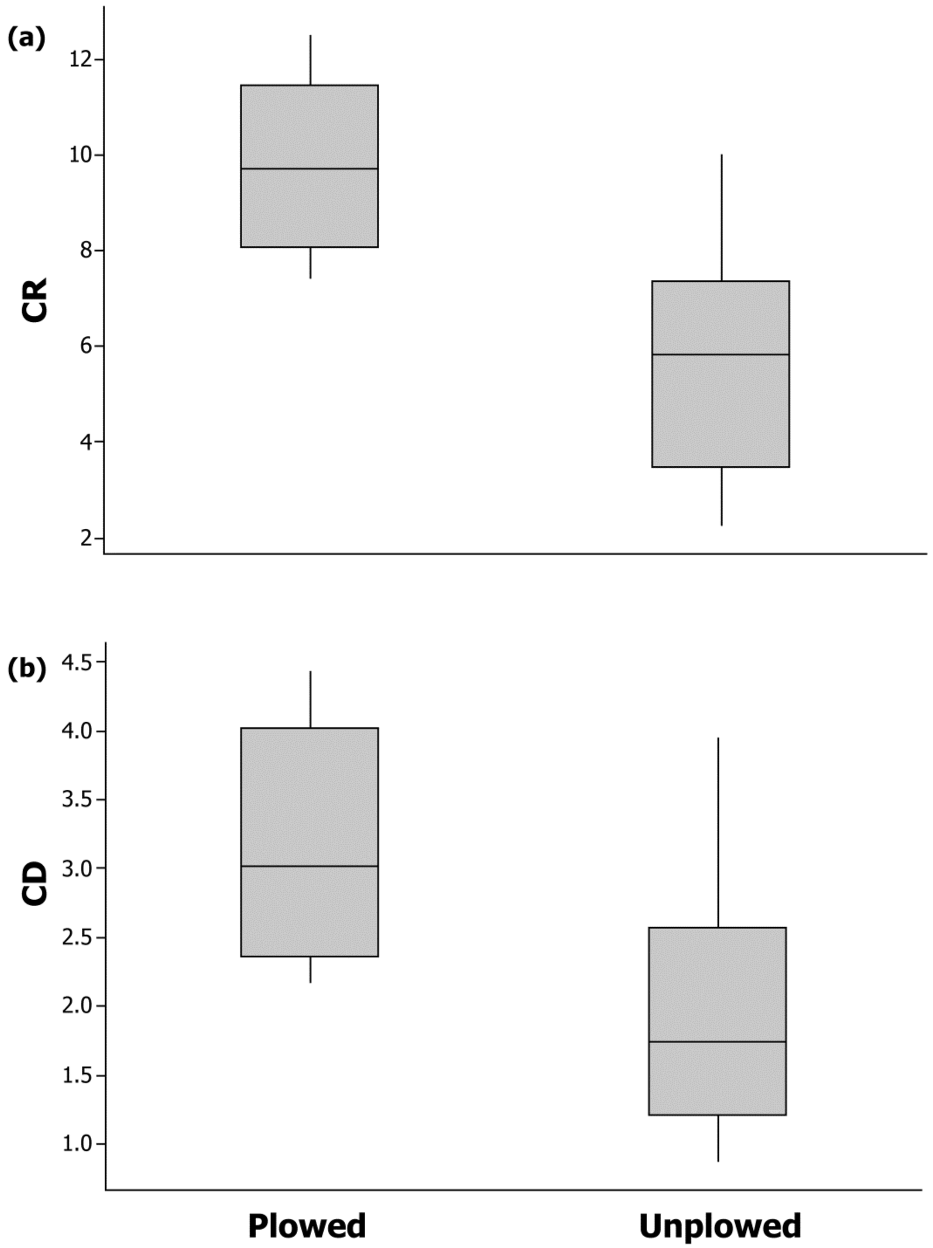
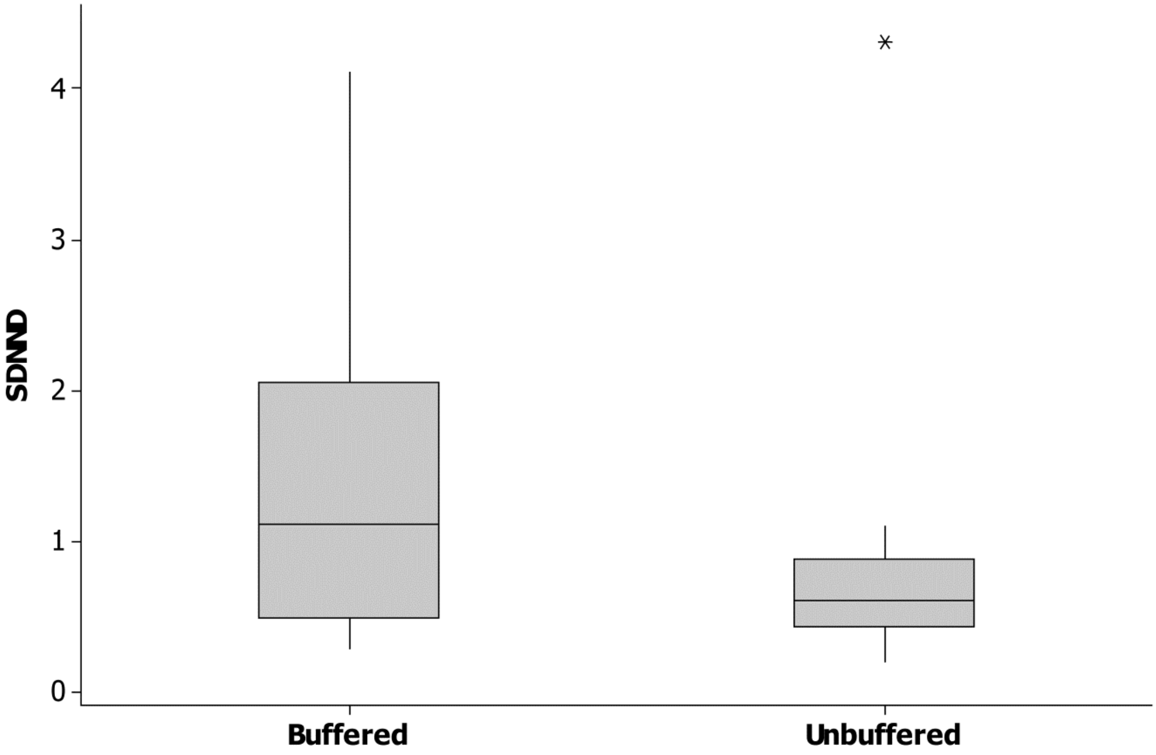


Figure 2.6 – Boxplot of SDNND (standard deviation of nearest neighbor distance) grouped by buffered and unbuffered playas. Bottom and top whiskers show 1st and 4th quartile, respectively. Shaded box shows interquartile range (IQR) with horizontal line as the median. * = outlier (> Q3 +1.5(IQR)).



Discussion

What controls the complexity of trophic structure in playas?

Species diversity is intimately linked to aspects of ecosystem structure and functioning, such as food web properties (Paine 1966), material and energy processing (Cardinale, Palmer and Collins 2002), and stability (Rooney and McCann 2012). Although increases in diversity are often associated with greater trophic complexity in many ecosystems (Wiggins *et* al. 1980, Wellborn 1996, Urban 2004), our study did not show this trend. However, the negative correlation with crustacean dominance (Fig. 2.2a) and positive correlations with insect diversity and numbers (Fig. 2.2b-d) show that trophic structure complexity is not simply a matter of species numbers, but may rely on species-specific traits. In particular, most of the abundant crustaceans inhabiting playas are large filter-feeding branchiopods (fairy, clam, and tadpole shrimp). They can consume phytoplankton and bacteria (Brendonck 1993a, Brendonck 1993b, Dierckens et al. 1997), rotifers (Starkweather 2005), nematodes (Jocque et al. 2010), small invertebrates (Mertens et al. 1990), and even glass spheres (Brendonck 1993a). Thus, most large branchiopods are using the same food sources in the same way (filter feeding), making them trophically redundant. Consequently, playas could have many species of large branchiopods, but still have simple trophic structures. It is insects that introduce novel trophic strategies and lengthen the food chain in playas.

Insects and branchiopods have different life strategies. Branchiopods hatch from resting eggs 1–5 days after a playa fills (Brendonck 1996, Lahr et al. 1999) and can mature in 3–4 days (Brendonck et al. 1998). Playas gain insect species throughout their hydroperiod (Jocque et al. 2010), and predator diversity increases (Schneider and Frost 1996). Consequently, the farther along in its hydroperiod, the more complex a playa’s trophic structure will tend to be. The playa wetlands sampled in this study were all relatively close together (15 of the 21 playas were within ~70 km of one another, with a maximum of 280 km). While we do not expect these playas to have filled with water on the exact same day, the antecedent weather conditions were relatively uniform over the study area, and we expect our sampling coincided at relatively the same time within the playas' hydroperiods. Factors that determine hydroperiod are the amount of water and evaporation rate. However, among playas that are reasonably close together, evaporation would be equal per unit of surface area, leaving the hydroperiod mainly determined by water depth (Garmendia and Pedrola-Monfort 2010). Therefore, it is not surprising that significant correlations exist between depth and TA, a measure of total niche space occupied (Fig. 2.3a), and between depth and NR, which measures food chain length (Fig. 2.3b). Deeper playas have food webs with more complex trophic structures either because the longer hydroperiod allows for more niches, or colonizing and ovipositing insects choose deeper playas.

These two main results – identity of species present and playa depth – provide insight into a hypothetical hydroperiod (Fig. 2.7). Shortly after a playa fills, large branchiopods dominate the system (Jocque et al. 2010, O'Neill and Thorp, unpublished data). Food chain length and trophic complexity is low at this time due to high trophic redundancy. Later, insects colonize the ecosystem, thereby bringing new trophic species that can increase food chain length and trophic structural complexity (Wiggins et al. 1980, Wellborn 1996, Urban 2004, O'Neill and Thorp, unpublished data). Branchiopod populations are quickly ravaged by insect predators (Dumont and Schorreels 1990, Wissinger et al. *1*999, Brendonck, et al. 2002, De Roeck, Artois and Brendonck 2005) and almost disappear. As the playa dries, some species are eliminated, decreasing trophic complexity (O'Neill and Thorp, unpublished data). As the time since first inundation (filling) lengthens, the community becomes increasingly dominated by interspecific

(Boix et al. 2006) and cannibalistic predators, thereby increasing food chain length. Eventually the food web collapses as the playa completely dries. Overall, the key to understanding trophic structure in these ephemeral wetlands is an interaction between hydroperiod, which is mediated by playa depth (rather than simple area), and the species' traits, lifespan, and trophic niche.

How do anthropogenic influences alter control of trophic structure in playas?

While we expect many of the same forces to act on the trophic structure of anthropogenically influenced systems, the relative importance of various control factors may be altered. In the Pawnee National Grassland, roadside ditches collect water and contain many of the same invertebrates as naturally formed playas. These ecosystems may be functioning as ephemeral wetlands, and at the very least it is evident that large branchiopods were successfully reproducing. We only sampled 4 artificial playas and the statistical power was low, yet we found some interesting differences. Natural systems had marginally higher values of TA and CD (Fig. 2.4), yet these differences were not statistically significant (for TA $P = 0.07$, for CD $P = 0.085$). The possibility of higher TA values in natural systems could mean that more trophic niches are filled, and higher CD could mean that trophic diversity of natural food webs is higher. Overall, the trophic structure of artificial playas needs to be more conclusively studied to determine the conservation value of these common habitats.

While SDNND was not significantly different between buffered or unbuffered playas, most unbuffered playas had low SDNND (Fig. 2.6). SDNND measures evenness of species packing. Thus, our unbuffered playas were distributed evenly on the C-N bi-plot, whereas our buffered playas had bi-plots with taxa that were spread widely and unevenly. This could mean that those widely spread taxa have very different and unique trophic strategies. Therefore, to have a diverse trophic structure with different trophic strategies and niches, a playa must be buffered by native vegetation.

The above anthropogenic perturbations could be negatively impacting trophic structure, and we expected agriculture to simplify trophic structure. However, playas with row crops adjacent or directly in the playa had higher CR and CD values (Fig. 2.5). CR measures diversity of food sources, and CD measures trophic diversity. Corn, the most common crop near our playas, was expected to increase CR, because vegetative parts of the C4 corn plant is more ^{13}C enriched than the native C3 plants around playas. This could spread the corn-consuming taxa along the x-axis of the bi-plot, which could simultaneously increase CR and CD. The higher values of these metrics suggest that this new food source is being exploited by some playa organisms. By adding corn and potentially other food sources to the playa food web, more complex trophic structures could result (cf. Gagic *et al*. 2012). Plowing may also affect the hydroperiod and other aspects of playa habitat, but this should have minimal impact on the range of carbon signatures found within a food web.

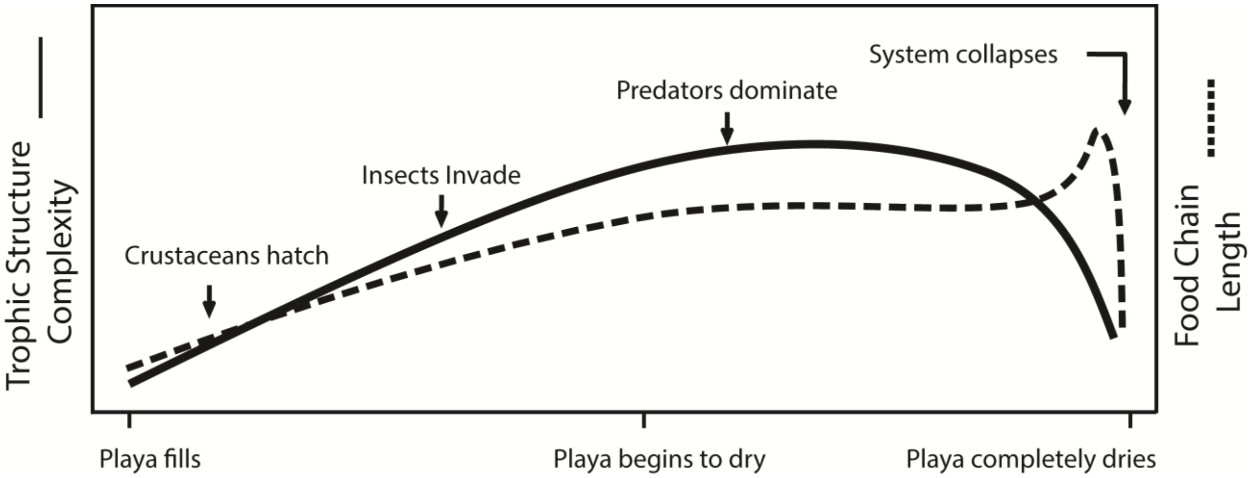
Historically, playas had natural grazers, such as bison (*Bison bison*). They continue to be used by pronghorn (*Antilocarpa americana*) and mule deer (*Odocoileus hemionus*) – if not for forage, then as a source of drinking water. Differences in historical and modern intensities of grazing are uncertain, but cattle and bison are known to affect aquatic ecosystems in different ways (Steuter and Hidinger 1999). Ranchers use Pawnee National Grassland as pasture; and when wet, the playas provide convenient water sources. While the plant (terrestrial and aquatic) communities did not noticeably differ between grazed and ungrazed playas, grazed playas often contained cattle feces and were covered in hoof prints. We expected this would simplify trophic structure, yet none of the metrics showed significant differences between grazed and ungrazed playas. Playa organisms may be adapted to grazing. In fact, Marty (2005) found invertebrate richness was 28% lower in ungrazed California vernal pools than in grazed pools. Hoof prints could create unique microhabitats (Johnson and Rogers 2003) and expose buried eggs. Cattle may be transporting propagules from different playas, as wild boar (Vanschoenwinkel et al. *2*008b) and African mammals do (Vanschoenwinkel et al. *2*011), all of which could potentially increase species diversity and maintain a healthy egg bank.

What do ephemeral systems have to add to the knowledge of trophic structure?

In many ecosystems, it logically follows that disturbances may shorten food chain length or simplify trophic structure. However, meta-analyses do not always support this supposition (slight negative effect – Sabo et al., (2009), no effect - Takimoto and Post (2012)). Organisms in ephemeral wetlands experience large changes in abiotic conditions over the hydroperiod as the system shrinks, including temperature, solute concentrations, and habitat size. While these forces are in one sense disturbances, organisms have adapted to them via resting eggs (Wiggins et al. 1980), diapause (Brendonck 1996), and escape to other habitats. Predators often dominate at the end of the hydroperiod (Schneider and Frost 1996, Boix et al. *2*006), and our Figure 2.7 shows how food chain length may increase as playas dry. A counter-argument is that food chain length is shorter because there are only one or two trophic levels of predators. However, food chain length cannot be reliably evaluated at a single moment in time. In this case, a true measure of food chain length should include basal organisms that have already been eliminated but which have enabled the present community composition to exist. The absence of the base of a food web at a given time may not be as apparent in permanent systems. Notable exceptions include highly seasonable food webs where organisms simply wait for the base of the food web to return, such as streams with yearly energy inputs from salmon corpses (Polis, Anderson and Holt 1997).

We contend that aspects of time, be it hydroperiod, seasonal changes, or ontogeny, are vitally important to how we evaluate trophic structure, especially in ephemeral systems.

Figure 2.7 – Hypothetical playa hydroperiod showing the expected behavior of trophic structure complexity and food chain length. Trophic structure complexity increases with time as insects invade and eventually decreases as playa is dominated by predators. Food chain length increases as insects invade and further increases at the end of the hydroperiod due to elevated predation and cannibalism.



In both disturbed and pristine systems, ecosystem size in one form or another seems to control trophic structure (McCann, Rasmussen and Umbanhowar 2005, Sabo et al. 2009, Takimoto and Post 2012). Ecosystem size has been repeatedly associated with species diversity (Munroe 1948, MacArthur and Wilson 1963, Smith et al. 2005) and can be a substitute for habitat complexity (larger systems often have more habitats (Lomolino, Riddle and Brown 2006)) or total energy of a system (Post, Pace and Hairston 2000). Most studies of permanent aquatic ecosystems use surface area to measure ecosystem size. However, playas are simple clay-lined bowls, often with no vegetation, making the entire habitat very homogenous, regardless of size. We need to recognize that the term “ecosystem size” is more than simply two dimensions of surface area. In playas, the hydroperiod is mediated by the third dimension, depth of the playa (Garmendia and Pedrola-Monfort 2010), which translates to the fourth dimension, time (deeper playas last longer). When determining the biodiversity of ephemeral systems, surface area has been shown to be relatively unimportant compared to durational patterns (Garcia-Valdecasas, Lop and Camacho 1984, Aubin and Leblanc 1986, Ebert and Balko 1987, Schneider and Frost 1996). Hydroperiod length is central to many aspects of the food web (Spencer et al. 1999, Bilton et al. 2001, Gascon et al. 2005, Waterkeyn et al. 2008). While hydroperiod may not be applicable to permanent ecosystems, “time since habitat creation” certainly would apply (e.g., in comparing biodiversity in post-glacial vs. ancient lakes). Food webs tend to increase in complexity and diversity with time and are more resistant to external perturbations (Emmerson and Yearsley 2004, Moore et al. 2004, Rooney and McCann 2012). During the early stages of any ecosystem, many of the same factors that control trophic structure in ephemeral habitats would be important. We believe that to create a general theory of what controls trophic structure, more holistic metrics that apply to all ecosystems should be considered.

Acknowledgements

We thank Christopher Rogers for help with sample collection and project development, Andrea Romero with sample preparation, and both the Playa Lakes Joint Venture and the Rocky Mountain Bird Observatory for assistance in finding playa sites. Our study was funded by the Region 8 office in Denver of the Environmental Protection Agency (CD968115-01), but conclusions of this study do not necessarily reflect the policy of EPA. All vertebrates were treated according to the University of Kansas Institutional Animal Care and Use Committee Protocol# 193-04.

Chapter 3

Birth, life, and dormancy of an ephemeral food web

Abstract

The study of how communities assemble provides insights into interactions among trophic levels through space and time. However, community assembly studies typically do not span sufficient time periods to answer their questions or only focus on extremely small ecosystems or habitats. Herein we use playa wetlands, ephemeral aquatic features common to arid and semi-arid ecoregions, to explore whether traditional community assembly rules apply to ephemeral ecosystems.

We tracked diversity and abundance within the invertebrate food web through multiple hydroperiods in six playa wetlands in northeastern Colorado. We also used stable isotope techniques to investigate several key aspects of trophic structure, including the size, variation, and redundancy of playa food webs throughout the observed hydroperiods.

Playa wetland communities generally increased in diversity and abundance through the early phase of the hydroperiod and then declined as the playa dried. Playa communities were more organized in time rather than by space. However, food web structure was not associated with time. Food chain length and food web complexity were statistically associated with aspects of community membership, such as overall taxon, insect, and predator richness. Food web redundancy was negatively associated with total invertebrate density and biomass.

Community assembly in playa wetlands can proceed differently depending upon which components of the species pool are active at the time of the hydroperiod. While community density and diversity did increase early, the community decline could be due to a combination of many factors including predation, competition, and life history constraints. Food web structure

is more closely tied to community membership than stage of the hydroperiod. Specific traits and behaviors of individual organisms that are immigrating and emigrating the playas, such as trophic level, would have larger impacts on trophic structure. The unstable nature of ephemeral ecosystems means that many traditional community assembly rules need to be reconsidered to incorporate the disconnection of temporal scales or the ideas of intermittency common in these systems.

Introduction

The study of how communities assemble provides insights into interactions among trophic levels through space and time. Community assembly is often studied profitably using model simulations, but these methods require assumptions that simplify real ecosystems (Levin 1992) or suffer intractability. Additionally, comprehensive empirical studies of community assembly and food-web structure are relatively rare (Baiser et al. 2012). Studies typically do not span sufficient time periods (Schrama et al. 2013) or only focus on extremely small ecosystems or habitats, such as phytotelmata (e.g. Baiser et al. 2012) or animal dung (e.g. Valiela 1974, Sladecek et al. 2013). Finding sufficient replication of newly created ecosystems is difficult, if not impossible, for larger ecosystems. One way around these problems is to use ephemeral wetlands. Communities in ephemeral wetlands must assemble *de novo* when the habitat becomes suitable for life. These make excellent study sites which allow for sufficient replication to answer the questions, enable incorporation of realistic ecosystem processes into the study, limit the intractability (common to models with many species) due to their relatively low species diversity, and can be studied for the entirety of the ecosystem’s existence.

Community assembly models are often initiated from an empty community and carried through equilibrium (Capitán et al. 2011), but disturbance and other factors can prevent natural systems from reaching equilibrium (Pickett and White 1985). For example, ephemeral ecosystems may never reach equilibrium because of time constraints or continually changing internal conditions (Williams 2006). This raises the questions, do traditional assembly rules (cf. Belyea and Lancaster 1999) apply to ephemeral ecosystems and do patterns hold across the range of system permanence?

In our study we use playa wetlands to explore these questions. Playa wetlands are common, ephemeral aquatic features of many arid to semi-arid ecoregions, including the U.S. Great Plains. They are often roughly circular (Luo et al. 1997), clay-lined, shallow depressions (Evans 2010), which can arise from a combination of geomorphic, hydrochemical, or biologic processes (e.g., Smith, 2003, Gurdak and Roe 2009, Evans 2010). These highly homogenous environments often have little vegetation or habitat structure (Fennessy and Craft 2011). The southern High Plains of the United States has the highest concentration of natural playas in the world (Evans 2010), yet similar ecosystems are found on other continents, such as the pans of Australia and South Africa, and the takyr, sabkha, and kavir of Central Asia, Saudi Arabia, and Iran, respectively. A playa may be dry for decades or may fill and dry several times per year depending on climate. Playa wetlands are endangered ecosystems. By the 1980s, at least 90% of playas in the United States Southern High Plains region had been impacted by humans (Haukos and Smith 2003). The importance of playas and other ephemeral habitats has often been overlooked (Bratton 1990, Williams 1985) by researchers and laypeople alike.

Knowledge of playa wetlands is largely descriptive and often geologic in nature (Merickel and Wangberg 1981). Several ecological studies have highlighted the importance of playas to regional biodiversity (Haukos and Smith 1994, Baar et al. 2008, Smith et al. 2011), stressed hydroperiod as the overriding driver of playa and ephemeral wetland ecology (Spencer et al. 1999, Bilton et al. 2001, Gascón et al. 2005, Waterkeyn et al. 2008, Sim et al. 2013), showed that anthropogenic modification of playas negatively affected amphibian growth and health (Gray and Smith 2005, McMurry et al. 2009), and documented how natural and anthropogenic factors influence playa trophic structure (O'Neill and Thorp 2014). Few studies have looked deeply into the ecological patterns and processes of playa wetland communities (Merickel and Wangberg 1981).

In the current study, we explored whether traditional community assembly rules apply to playa wetlands by following the aquatic invertebrate communities of several playa wetlands through multiple hydroperiods. To do so, we tracked taxa diversity and abundance and used stable isotope techniques to investigate aspects of trophic structure, such as the size, variation, and redundancy of playa food webs. We predicted that community diversity and density would increase immediately after playas fill, and then decrease as playas dry. We also predicted that communities would be organized in time rather than space. In other words, playa communities would be more similar within dates across playas, compared to within playas across dates. We anticipated predator richness and abundance to start out low and increase through time. Similarly, we expected trophic structure metrics (food-web size, variation, and redundancy) to increase through time and eventually decrease as playa communities collapse at the end of the hydroperiod. Overall, we analyze and discuss some ecological patterns and processes that can only be seen in ephemeral ecosystems.

Methods

Study site

We sampled 6 playa wetlands within the Pawnee National Grassland in Weld County, Colorado, USA (Table 3.1). The Pawnee National Grassland is a “checkerboard” of short-grass prairie and agricultural land that is owned by a variety of governmental and private entities. We sampled playas on land with varying amounts of grazing pressure and anthropogenic impact. Playas 3 and 4 had active grazing during our sampling season, but all playas had some contact with cattle grazing in the past. Native short-grass prairie vegetation buffered all playas from roads by 700, 60, 60, 430, 130, and 130 m for playas 1-6, respectively. No sampled watersheds had tilled areas for row-crops. Playa 3 had nearby tilling (~70m), but the watershed was disconnected from it by a gravel road and subsequent ditches.

Table 3.1 – Latitude and longitude of study sites.

Playa	Latitude	Longitude
1	N 40.9171	W 103.8325
2	N 40.9173	W 103.8603
3	N 40.96218	W 103.8986
4	N 40.99653	W 103.8693
5	N 40.7537	W 104.6394
6	N 40.7965	W 104.5031

Field sampling

We sampled throughout two separate hydroperiods in 2013: three times in the spring (May 3, 12, and 24; hereafter spring-dates 1–3, respectively) and five times in late fall (September 14, 22, October 1, 10, and 18; hereafter fall-dates 1–5, respectively). The first sampling date of both hydroperiods occurred within ~3 days after playa filling for all but one playa. The spring hydroperiod was the result of snowmelt from the previous 3–7 days. The fall hydroperiod started from region-wide rainfall on September 9–12. We suspect that Playa 5 filled during a rain event around August 23 because we found 22-mm long fairy shrimp (Anostraca: *Thamnocephalus platyurus*) and tadpole shrimp (Notostraca: *Triops longicaudatus*) with carapace lengths up to 10.8mm in playa 5 (important for later data interpretation). This strongly suggests that the playa filled ~3 wk prior to our first sampling because these taxa cannot colonize as adults, and based on laboratory growth rates of 0.46mm carapace length/day (Obregón-Barboza et al. 2001), need at least 21.7 days to reach this size. Some playas could only be qualitatively sampled on three dates due to low water levels. Playa 1 was dry throughout the spring hydroperiod, and other playas had various times to complete desiccation in both hydroperiods (playas 1–5 dry by spring-date 3, playa 3 dry by fall-date 4 and 5). Cattle incursion prevented sampling playa 4 on fall-date 5. Our sampling captured the entirety of the hydroperiods because all playas were dry by our last sampling dates or had only 2–3 cm of water remaining.

During each sampling, we recorded playa size and depth and measured turbidity in NTUs using an Aquafluor ® turbidimeter. We took quantitative and qualitative samples of the invertebrate and amphibian communities, following procedures described in O’Neill and Thorp (2014). Quantitative samples consisted of three haphazardly located 1-m net sweeps (25 cm x 18 cm; 400-µm mesh; 45 L of water sampled). If the playa was shallower than the 18 cm net height, we extended the sweep to fulfill the 45-L sampling requirement (e.g. 9 cm deep water = 2 m sweep). Qualitative samples consisted of continuous sampling until three conditions were met: (i) all areas of the playa were repeatedly sampled; (ii) repeated net sweeps failed to collect new taxa; and (iii) each taxon had sufficient numbers for isotope sample replication. In some playas, on various dates, only single individuals of certain taxa could be collected, but most taxa had enough individuals for three stable isotope replicates per taxon. Samples were immediately preserved in 75% EtOH and identified later in the laboratory. Amphibians (Plains Spadefoot Toads, *Spea bombifrons*, and Western Chorus Frog, *Pseudacris triseriata*) were euthanized by immersion in MS-222 and preserved in 75% EtOH.

Species identifications and stable isotope analyses

We counted all collected metazoan invertebrates and identified them using various sources (Thorp and Covich 2010, Merritt, Cummins and Berg 2008, McCafferty 1983, Collins 1974). Amphibians were identified to species, most insects and crustaceans to genus, and dipterans and annelids to higher taxa. For stable isotope analyses, we dried the samples for 48 h at 60 °C, ground them into a homogenized powder using a Wig-L-Bug® Mixer/Amalgamator, and stored them in desiccators until analyzed. Samples were put in tin capsules, weighed (1–1.5 mg dry mass) and analyzed at the University of Kansas W.M. Keck Paleoenvironmental and Environmental Stable Isotope Laboratory using a ThermoFinnigan MAT 253 continuous flow system mass spectrometer. Data collected included $\delta^{15}N$ and $\delta^{13}C$ and total N and C. $\delta^{15}N$ and $\delta^{13}C$ were determined as the relative difference in isotopic ratio among the samples and known standards (Vienna Pee Dee Belemnite and atmospheric nitrogen for C and N, respectively) as the following equation: $\delta X = ((R_{sample}/R_{standard}) - 1) \times 1000$ where X is either ^{13}C or ^{15}N, and the corresponding ratio is either $R = {}^{13}C/{}^{12}C$ or $R = {}^{15}N/{}^{14}N$.

Statistics and data analyses

Nonmetric Multidimensional Scaling (NMS) and Multi-Response Permutation Procedure (MRPP) were completed using PCORD v. 6 (MJM Software Design, Gleneden, OR, U.S.A.) following the recommendations of McCune and Grace (2002). The NMS ordination used the Sorensen (Bray-Curtis) distance measure, starting with six dimensions stepping down to one, 50 runs with real data, and 15 iterations to evaluate stability with a stability criterion of 0.00001. A supplied seed integer of 4483 was used for the starting configuration. The possibility of a better-than-random solution was evaluated using a Monte Carlo test with 10,000 iterations. A scree plot and final stress was used to determine the number of dimensions to use in the final test. Stability of each ordination was evaluated by using the plot of stress vs. iteration number and by the final instability. MRPP used the Sorensen (Bray-Curtis) distance measure. Univariate statistics were completed using Minitab v. 16 (Minitab Inc., State College, PA, U.S.A.).

The trophic structures of playa food webs were quantified using metrics (Table 2.1) developed by Layman et al. (2007): $\delta^{15}N$ range (NR), $\delta^{13}C$ range (CR), total area (TA), mean distance to centroid (CD), mean nearest neighbor distance (NND), and standard deviation of nearest neighbor distance (SDNND). These metrics were chosen instead of Bayesian approaches (Jackson et al. 2011) which are more appropriate for evaluating the niche of a single species. All trophic structure metrics were calculated using the centroids of each taxon. Thus, if a food web had eight taxa, the six metrics were determined from eight points (the eight taxa centroids). NR and CR were calculated by subtracting the lowest $\delta^{15}N$ and $\delta^{13}C$ value from the highest. TA was measured by creating a convex hull around points of the C-N isotope biplot and determining the area in ArcMap v.10 (ESRI, Redlands, CA, U.S.A.). CD was computed by finding the centroid of each biplot in Excel (Microsoft Inc., Redmond, WA, U.S.A.), and averaging the distances from each taxon point to the centroid. NND was calculated using a distance matrix (Euclidean distance) from PCORD v. 6 and averaging the shortest distances among each taxon and its nearest taxon. SDNND was determined from the same distance matrix by taking the standard deviation of the nearest neighbor distances. See Layman et al. (2007) for more detailed instructions and alternate methods to calculate these metrics. To eliminate discrepancies in baseline nitrogen between residents and new colonists, only taxa incapable of emigrating from the playa were used in the trophic structure analyses (e.g. crustaceans, insect larvae, and tadpoles).

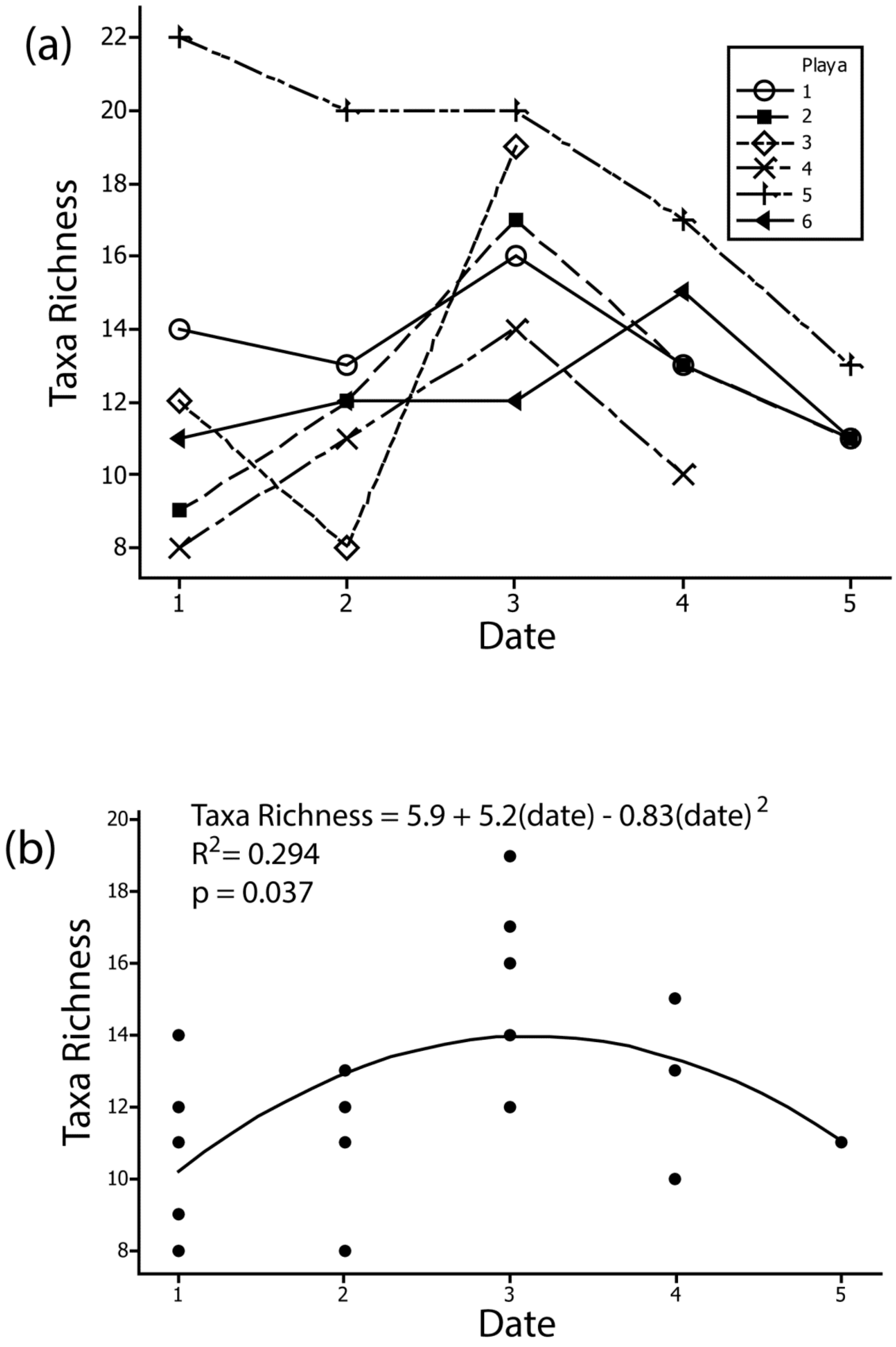
To further explore food web relationships, we explored the isotope signatures of various species alone. Herein we only show the analysis of the tadpole shrimp *Triops*. The δ^{15}N values of *Triops* were standardized by the lowest δ^{15}N value found in their respective playa to eliminate problems with differences in baseline nitrogen values.

Results

Community patterns through time

Taxa richness varied within and among playas during the fall hydroperiod, with a minimum of eight taxa and a maximum of 22 taxa per playa. In most playas, taxa richness increased in the beginning, peaked at sampling date three or four, and then decreased. However, total taxa richness continuously decreased throughout the study for playa 5 (Fig. 3.1A), the playa we believe filled much earlier than all the others. Richness data, omitting playa 5, showed a significant quadratic regression on time (taxa richness = 5.854 +5.191 x date – 0.8299 x date^2, R^2 = 0.294, $p = 0.037$) (Fig. 3.1B).

Figure 3.1 – A-Total taxa richness values for the fall hydroperiod through time. B – Fitted line plot of taxa richness values through time, with Playa 5 omitted.



Invertebrate numbers, total invertebrate biomass, and predator biomass followed a similar parabolic pattern. Invertebrate numbers for all playas combined, omitting playa 5, averaged 1.22/L on the first fall sampling date and reached a maximum of 24.21/L on the second sampling date. Thereafter, invertebrate numbers decreased until the end of the hydroperiod (Fig. 3.2A). Invertebrate numbers showed a significant quadratic regression on time: ln(invertebrate numbers) = 1.071 + 3.769 x date – 0.6749 x date^2, with $R^2 = 0.375$, $p = 0.005$ (Fig. 3.2B). Total invertebrate biomass per playa sample ranged from 4.7 mg invertebrate biomass to 5041.7 mg. For the fall hydroperiod, invertebrate biomass also followed a parabolic pattern (Fig 3.3A), showing a significant quadratic regression on time: ln(total biomass) = 0.707 + 3.738 x date – 0.6424 x date^2, with $R^2 = 0.364$, $p = 0.006$ (Fig. 3.3B). Predator biomass per playa sample ranged from 0.9 mg to 264.6mg. For the fall hydroperiod, predator biomass again followed a parabolic pattern (Fig. 3.4A), showing a significant quadratic regression on time: ln(predator biomass) = 0.7159 + 2.383 x date – 0.4055 date^2, with $R^2 = 0.317$, $p = 0.012$ (Fig. 3.4B).

Figure 3.2 – A – Average number of invertebrates for the fall hydroperiod through time. Values shown are the natural logarithm of average number of invertebrates. B – Fitted line plot of average number of invertebrates through time.

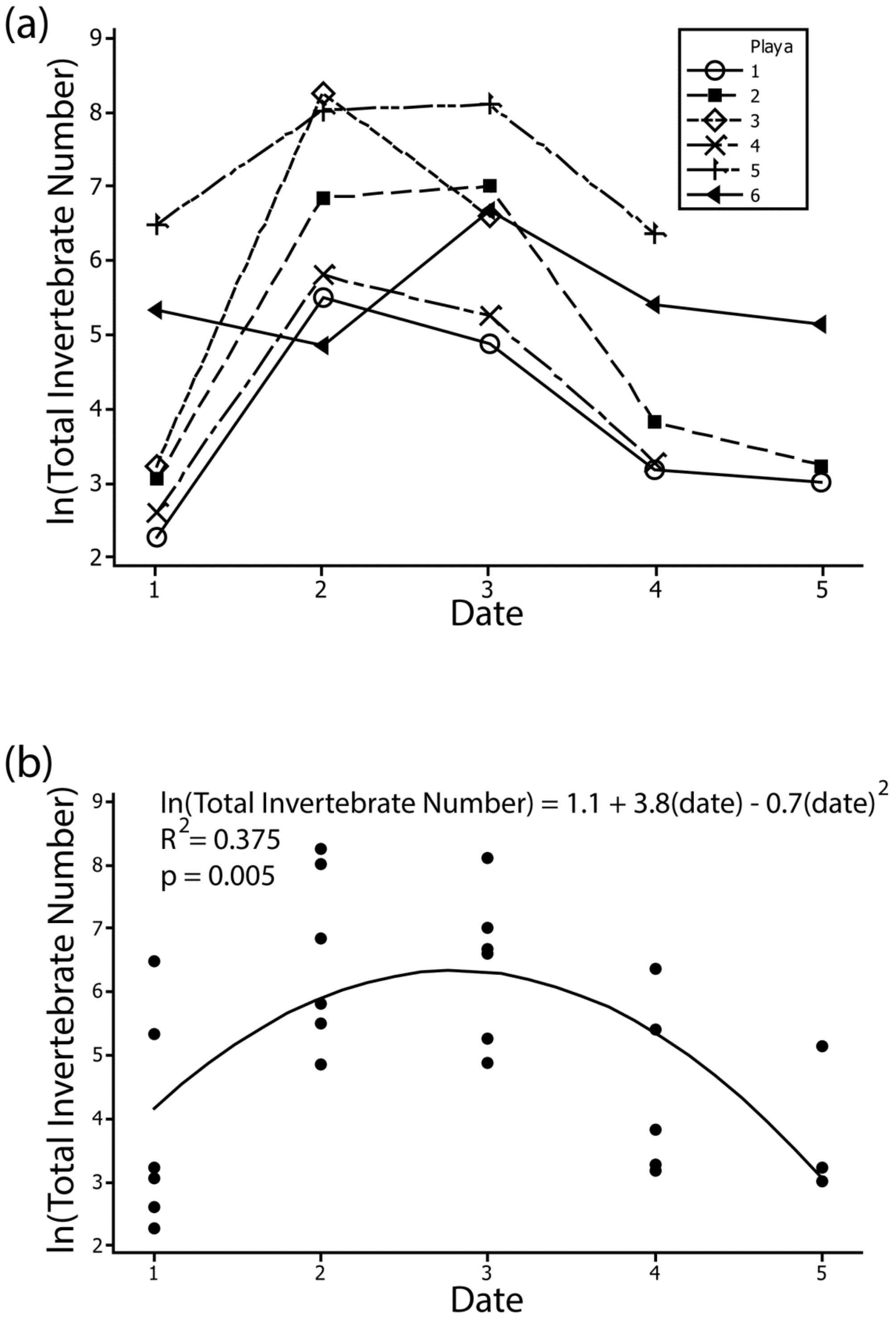


Figure 3.3 – A – Total biomass of invertebrates for the fall hydroperiod through time. Values shown are the natural logarithm of total biomass. B – Fitted line plot of total biomass through time.

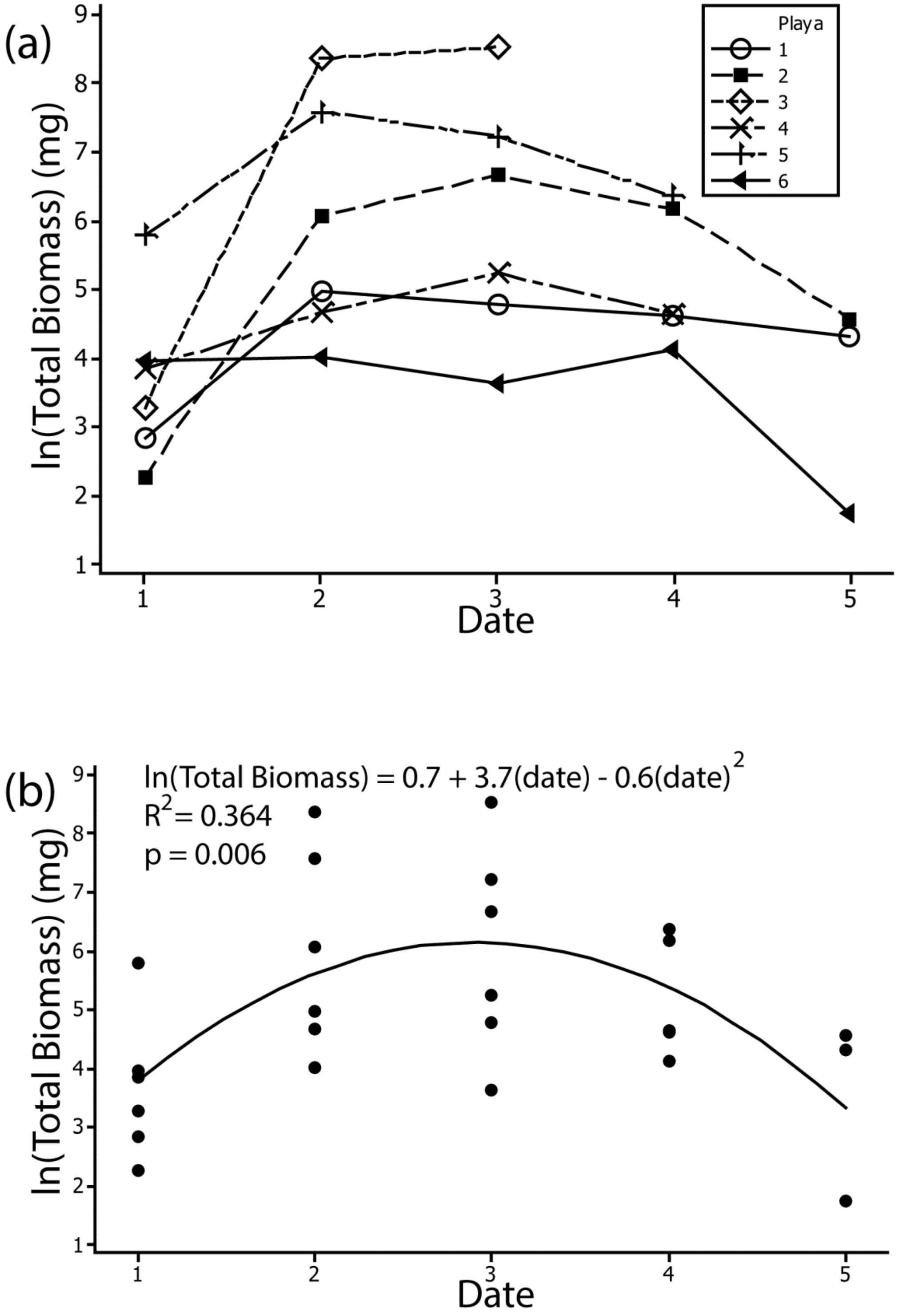
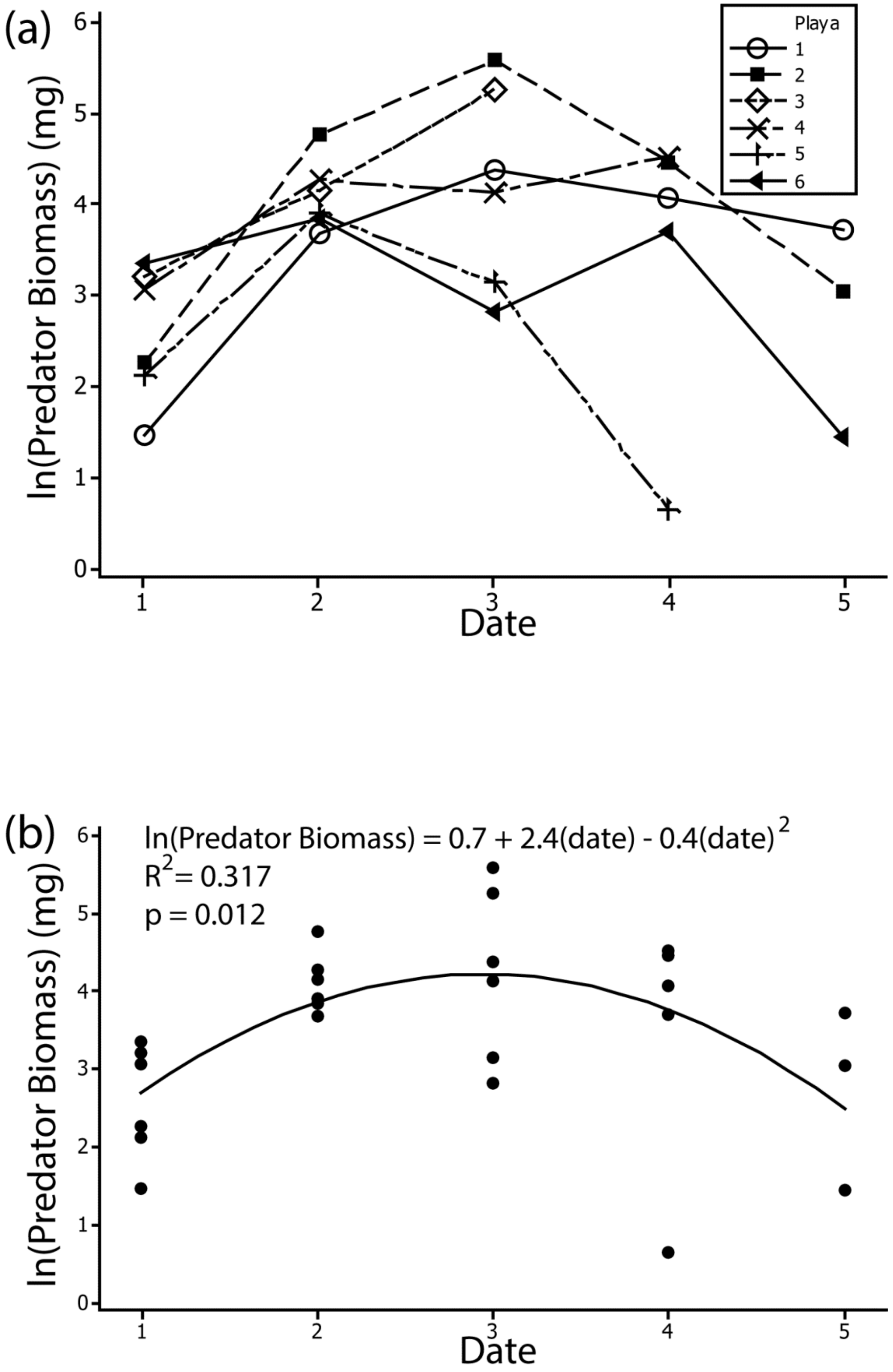
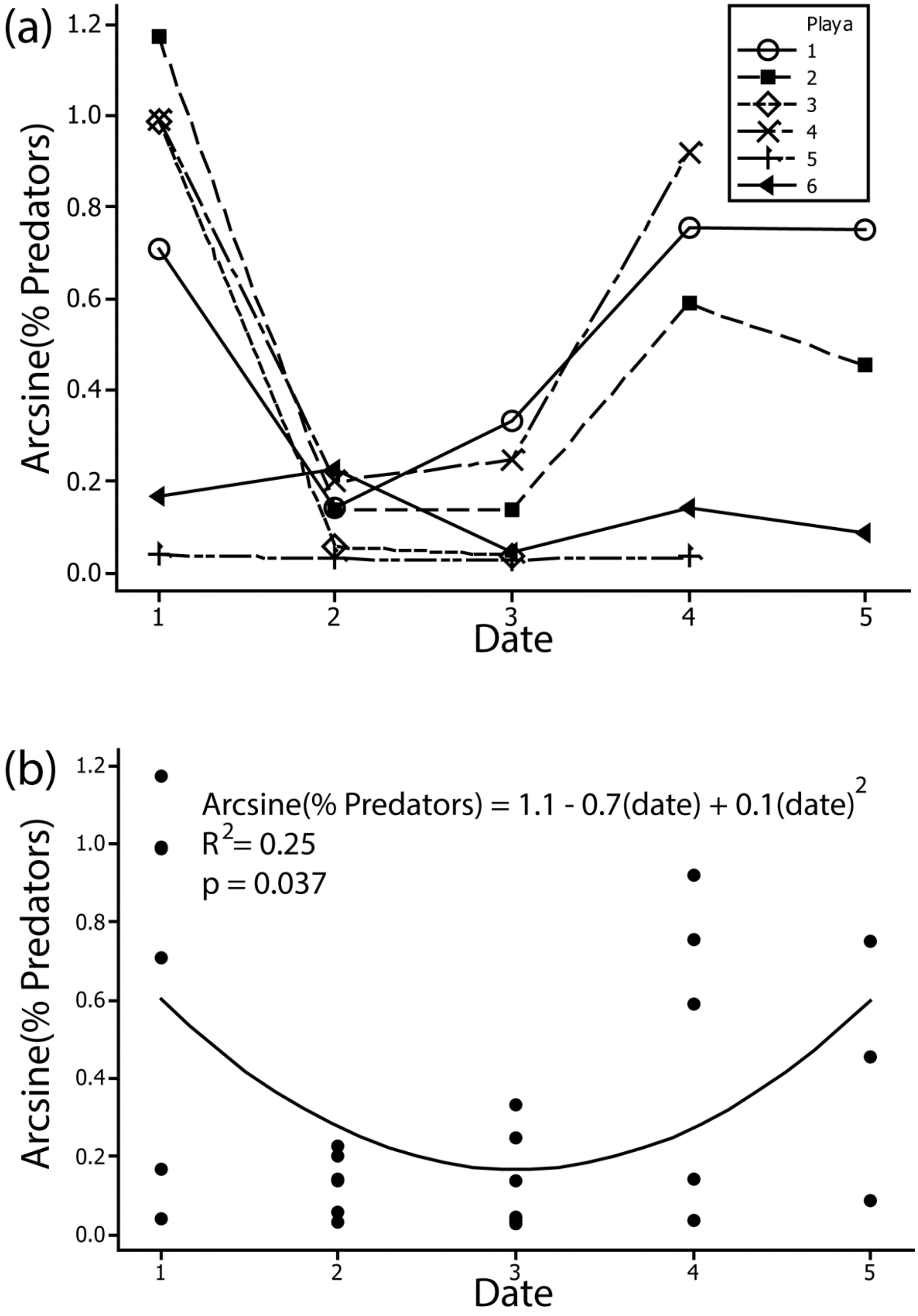


Figure 3.4 – A - Predator biomass for the fall hydroperiod through time. Values shown are the natural logarithm of predator biomass. B – Fitted line plot of predator biomass through time.



Predator dominance, as defined by the percentage of individuals in the sample that were predators, followed an opposite pattern in the fall hydroperiod (Fig. 3.5A). Predator dominance for all playas averaged 29.7%, 2.2%, 3.1%, 28.6%, and 22.2% of sample dates 1–5, respectively. These data show a significant quadratic regression on time (arcsine(% predators) = 1.149 -0.6555 x date + 0.1091 x date^2, $R^2 = 0.25$, $p=0.037$) (Fig. 3.5B).

Figure 3.5 – A - Predator dominance for the fall hydroperiod through time. Values shown are the arcsine of predator dominance. B – Fitted line plot of predator dominance through time.



General patterns of the fall hydroperiod invertebrate communities contrasted strongly with the spring. We can only give qualitative/anecdotal evidence describing the spring communities and food webs (May 3–24) due to the shorter hydroperiods and our inability to sample quantitatively during those low water periods. Generally, spring playa communities started with mostly depauperate communities of newly-hatched branchiopods, such as fairy shrimp (Anostroca), tadpole shrimp (Notostraca), and clam shrimp (Spinicaudata and Laevicaudata). At this time, insect species were absent or rare. Taxa richness increased during the next sampling date and playa communities had much higher densities of branchiopods and insects, including predator species. The last spring sampling date included only playa 6, because the others were dry. At this time in playa 6, large ostracods, insect predators, and small tadpoles were concentrated in water-filled, cattle hoofprints.

Nonmetric multidimensional scaling (NMS) showed that playa communities were more similar to each other on a given date (Fig. 3.6A). A single playa community was not very similar to itself through time (Fig. 3.6B); that is, playa communities were organized more by time than space. The NMS solution had a final stress of 12.48 in two dimensions (Kruskal's rule of thumb states stress of ~10 is satisfactory for analyzing (McCune and Grace 2002)). The final instability was 0.00017 after 209 iterations. Multi-response permutation procedure (MRPP) showed the same trend. While both MRPP analyses were significant (Date – A = 0.1239, p = 0.0005; Playa – A = 0.1306, p = 0.0008), pairwise comparisons showed large differences between the two analyses. When organized by date, pairwise comparisons showed that most playa communities were significantly different from communities of non-adjacent dates (Table 3.1A). However, when analyzed by playa, little statistical significance was seen. Only playa 5 had a significantly distinct community, and playa 6 was significantly distinct from all but playa 4 (Table 3.1B).

Figure 3.6 – Nonmetric multidimensional scaling plot of community data. Each point is a playa assemblage on a single date. A – Convex hulls bound all playas from each sampling date. B – Convex hulls bound all sampling dates from each playa.

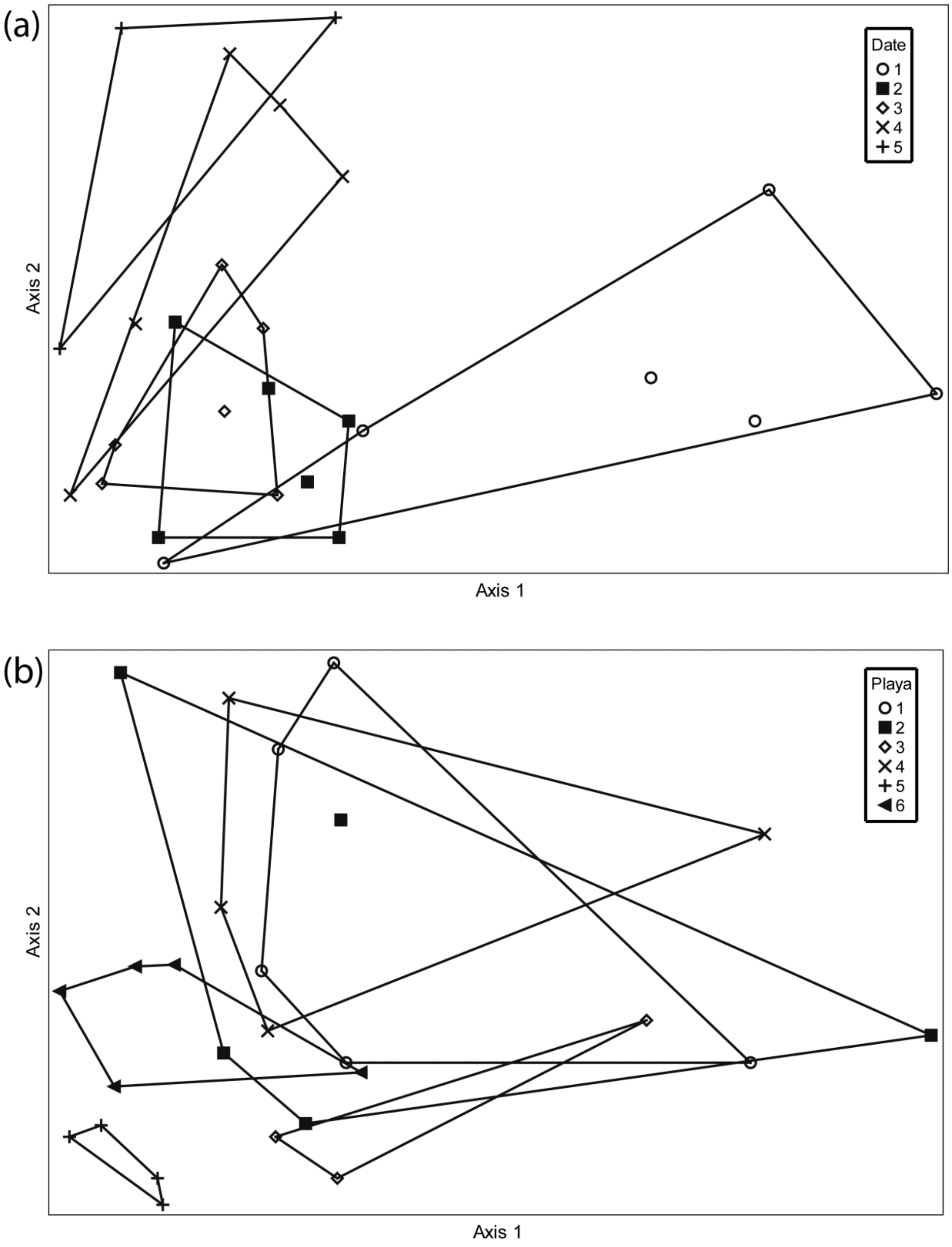


Table 3.2- Multi-response permutation procedure pairwise comparisons. Values in table include A (chance-corrected within-group agreement) and associated p values. A – Pairwise comparisons grouped by sampling date. B – Pairwise comparisons grouped by playa.

(A)

Date	2	3	4	5
1	A=0.117 p=0.019	A=0.147 p=0.009	A=0.131 p=0.01	A=0.089 p=0.053
2		A=-0.002 p=0.472	A=0.106 p=0.01	A=0.087 p=0.017
3			A=0.043 p=0.14	A=0.05 p=0.119
4				A=-0.049 p=0.783

(B)

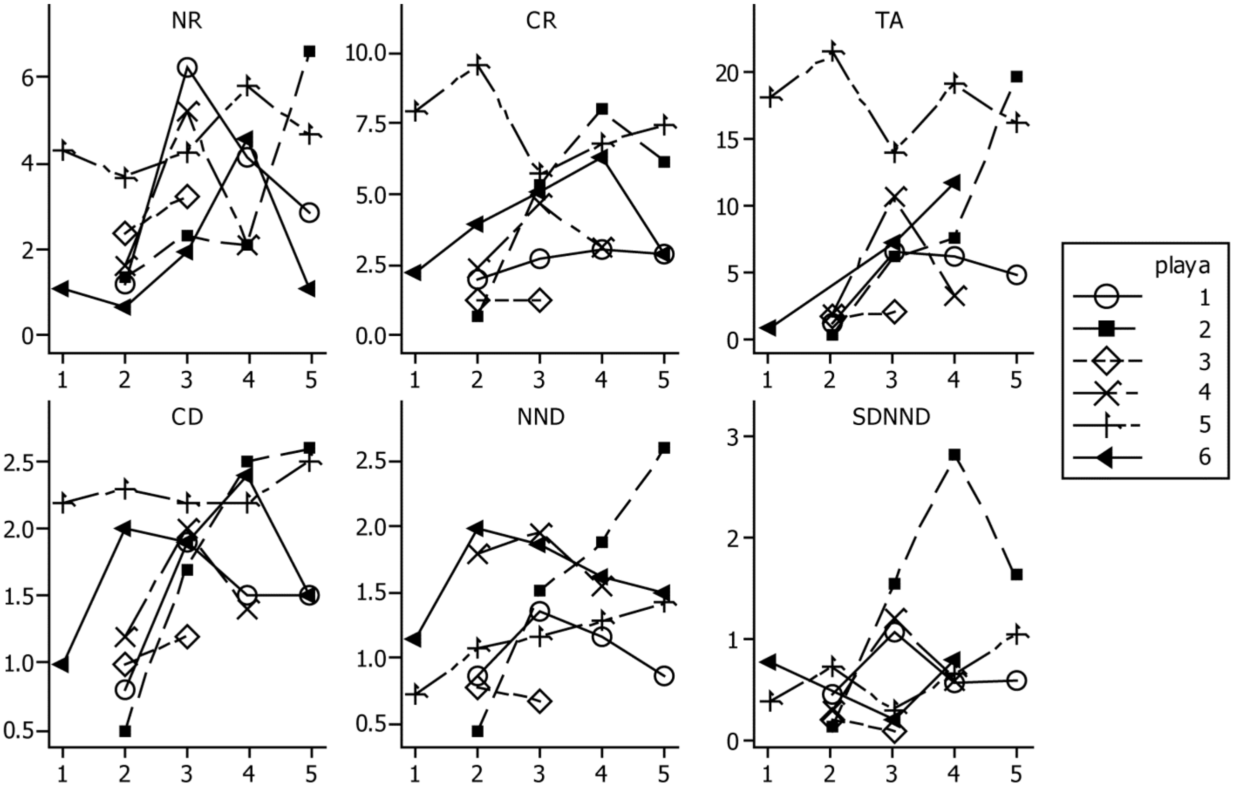
Playa	2	3	4	5	6
1	A=0.016 p=0.331	A=0.056 p=0.181	A=-0.003 p=0.484	A=0.224 p=0.003	A=0.089 p=0.0197
2		A=-0.007 p=0.5	A=-0.027 p=0.685	A=0.174 p=0.003	A=0.086 p=0.007
3			A=0.054 p=0.189	A=0.208 p=0.009	A=0.107 p=0.029
4				A=0.245 p=0.005	A=0.04 p=0.087
5					A=0.181 p=0.0037

Food-web patterns

Taxa richness

No metrics of trophic structure, including δ^{15}N range (NR), δ^{13}C range (CR), total area (TA), mean distance to centroid (CD), mean nearest neighbor distance (NND), nor standard deviation of nearest neighbor distance (SDNND) were correlated with sampling date nor with any measure of time (Fig 3.7). However, taxa richness was positively correlated with NR ($R = 0.421$, $p = 0.046$), CR ($R = 0.52$, $p = 0.011$), and TA ($R = 0.538$, $p = 0.012$). Crustacean richness was positively correlated with NR ($R = 0.646$, $p = 0.001$), and insect richness was positively correlated with CR ($R=0.535$, $p = 0.009$) and TA ($R = 0.537$, $p = 0.012$). Predator richness was positively correlated with NR ($R = 0.414$, $p = 0.049$) and CR ($R = 0.432$, $p = 0.04$).

Figure 3.7 – Scatterplots of δ^{15}N range (NR), δ^{13}C range (CR), total area (TA), mean distance to centroid (CD), mean nearest neighbor distance (NND), and standard deviation of nearest neighbor distance (SDNND) through time.



Invertebrate numbers and biomass

TA was negatively correlated with predator number (R = -0.522, p = 0.018), the natural logarithm of predator biomass (R = -0.603, p = 0.005), and the arcsine of % predator biomass (R = -0.481, p = 0.032). NND was negatively correlated with the natural logarithm of invertebrate density (R = -0.423, p = 0.05) and the natural logarithm of invertebrate biomass (R= -0.447, p = 0.037). Zooplankton density (*Daphnia, Moina*, other cladocera, and copepods) was negatively correlated with SDNND (R = -0.475, p = 0.034).

Taxa specific isotopic patterns

δ^{15}N signatures of tadpole shrimp (*Triops*) consistently increased through time (standardized $\delta^{15}N = 0.104 + 0.503 \times \text{date}$, $R^2 = 0.445$, $p < 0.001$) and stepwise linear regression eliminated animal size, as measured by carapace length, from the regression model.

Discussion

Community assembly

Playa food webs are excellent systems for studying aspects of community succession and food-web assembly through time. While the long timescales of many terrestrial habitats make succession difficult to study (Boit and Gaedke 2014), playa wetlands can fill and dry completely multiple times per year, providing repeated observations of complete assembly dynamics. However, assembly may not always follow the same path in all playas at all times (Michalski and Arditi 1995, Morton and Law 1997), especially with playa hydroperiods scattered through different seasons, resulting in colonization from different subsets of the species pool. In the spring, insects did not colonize the playas in any great numbers until the second sampling date, whereas the fall hydroperiod saw the immediate colonization of insect predators (Notonectids, Corixids, and others). Playas can also fill midwinter, where very few insects are available for colonization. Additionally, varying hatching requirements by species cause the crustacean community to differ seasonally (Vanderkerkhove 2005). In essence, the species pool from which colonization occurs varies temporally. Consequently, the sequence of change in food-web trophic structure also varies seasonally.

The nature of ephemeral systems is rarely incorporated within "textbook" ecological theory. Simulated models of food webs often necessitate that prey items colonize before predators (e.g. Piechnik et al. 2008, Liu et al. 2012). However, our playas had adult predatory insects appearing first, as immigrants from other aquatic habitats. These immigrating predators need wait only a short time to exploit the extremely fast growth rates of the soon-to-hatch, large branchiopod prey items; this behavior prevents these predators from only being allochthonous organisms (cf., Ingimarsdóttir et al. 2013). The short time scales of ephemeral ecosystems mean that many traditional assembly rules can be "broken" or are at least less appropriate. For example, the inherently unstable nature of ephemeral systems often precludes multi-species coexistence stability (cf., Pawar 2009). However, on a larger temporal scale, a stable metacommunity that is organized temporally can be created by persistence of dormant propagules (Hanski 1987). We suggest that it would be valuable to revise many food-web assembly rules to incorporate ideas about intermittency or the disconnection of temporal scales and processes in ephemeral ecosystems.

Food-web assembly theory dictates that taxa richness and organism density should increase through ecologically relevant time scales (Post and Pimm 1983, Drake 1990, Law and Morton 1996). Our playa communities somewhat followed this pattern (except for the anomalous playa 5). In the fall, taxa richness and invertebrate density and biomass generally increased through the first three sampling dates, but declined thereafter (Fig. 3.1). These declines may be due to several natural processes. For instance, branchiopods hatch and dominate early on (Jocque et al. 2010) but usually decline afterward because they are constrained by having only a single generation per hydroperiod. Eggs of most species must undergo diapause and changing environmental conditions before hatching (Brendonck 1996). In addition, insects born in the playa, such as dytiscid and hydrophilid beetles, mayflies, and Odonata, emerge and leave the aquatic habitat. Another factor likely driving diversity declines is the increase of predation by shorebirds and waterfowl in playas. As invertebrate densities rise, playa habitats finally have sufficient invertebrate biomass to support the presence of transient waterfowl. Playa invertebrates can be an important food source for waterfowl (Anderson et al. 2000), and optimal foraging theory suggests that these highly mobile birds should concentrate their foraging effort on later-stage playas, with more invertebrate food. Adult branchiopods are especially vulnerable to predation because of their mostly pelagic behavior, which makes them easy targets for bird, amphibian (salamanders and spadefoot tadpoles), or invertebrate predators (Jocque et al. 2010). The increase in invertebrate density will concomitantly increase encounter rates among the aquatic residents, which can amplify competition and predation rates (Boix et al. 2006). Large invertebrate predators (e.g., Hydrophilidae, Dytiscidae, and Anisoptera nymphs) eventually reach a size where they can have a large impact on the community. While predation levels are changing, the aquatic habitat gradually harshens throughout the hydroperiod. In these arid to semi-arid regions, filling events are relatively rare, so salinity, temperature, and temperature fluctuations tend to increase as water levels decline, which combined could lower invertebrate density and eliminate many species. Ultimately, taxa richness collapses to zero as the aquatic habitat disappears, or is at least replaced with dormant stages and terrestrial inhabitants of dry playas.

With the combination of the above processes, it is not surprising that our multivariate analyses showed playa communities to be more similar on the same date across different playas, compared to communities in the same playa across different dates. Most taxa found in the study were cosmopolitan, and only a few taxa were unique to any single playa. The playas were also not that far from each other (maximum 70 km), and with the exception of playa 5, were exposed to relatively similar weather. We expect that the hydroperiod length was the overriding factor determining playa community state through time, given that: (a) all our playas were relatively homogenous in structure within and among playas (Haukos and Smith 1994); (b) immigration by adults and colonization by resistant eggs would have been roughly comparable in these spatially clustered habitats; and (c) all playas were close enough to experience similar weather conditions. Indeed, most research has stressed that hydroperiod is the most important factor in ephemeral wetland communities (Spencer et al. 1999, Bilton et al. 2001, Gascon et al. 2005, Waterkeyn et al. 2008, Sim et al. 2013).

Food-web assembly

While hydroperiod/temporal patterns seem to be important to the above aspects of the invertebrate community, we did not find the same patterns to be associated with trophic structural metrics (Fig. 3.7). Richness and other standard community metrics are constrained by design to treat all species equally. The addition or deletion of one species typically has no great statistical effect on total richness or a multivariate analysis. However, depending on the species, it can have great impact on the trophic structure of a food web. Therefore, it is not surprising that we found more important effects of assemblage membership, such as crustacean, insect, and predator richness, on trophic structure, rather than temporal effects. It is a taxon's feeding characteristics that influence trophic structure in playas. For example, predator richness was correlated with NR, a measure of food chain length, and FCL generally increased with a rise in number of predator species. The addition or deletion of a trophically redundant species would not be expected to change trophic structure, unless they are very abundant, such as total invertebrate biomass increasing food web redundancy (as measured by NND), and zooplankton density influencing species packing (SDNND). O'Neill and Thorp (2014) predicted that food-web complexity would decrease at the end of a playa hydroperiod for various reasons including high predation. The current study shows that when there were high predator numbers, biomass, and dominance, the total trophic complexity of the food web, as measured by TA, was low. This could be evidence that high predation is consuming large portions of the community and simplifying the food web.

Time, however, may be important to many taxa within playa food webs. The $\delta^{15}N$ signatures of the tadpole shrimp (*Triops*) were found to increase through time, irrespective of body size. Such $\delta^{15}N$ increases are usually indications of an increase in trophic level. *Triops* undergo an ontogenetic diet shift from planktivore, to detritivore, to predator, which is often linked to body size (Pont and Vaquer 1986). Our finding suggests that temporal changes in the trophic status in *Triops* may be linked to factors other than simply body size. Certain branchiopods can optimize resource allocation between growth and reproduction depending on hydroperiod length (Huang et al. 2010). As a *Triops* ages, it may sense in some way that the playa is starting to dry and shift resources to reproduction, no matter the animal's size. At this time it may require more dietary protein for egg production, and the resulting increase in predatory behavior could cause the $\delta^{15}N$ increase. However, the number of potential prey was declining towards the end of the hydroperiod in our playas. Given that starvation can inflate $\delta^{15}N$ signatures (Bowes et al. 2014) and that *Triops* can be food limited during the drying phase (Boix et al. 2006), the increase in apparent FCL may have been misleading.

Conclusions

While ephemeral ecosystems may not always “follow the rules” when it comes to community assembly, they do organize themselves in discernible patterns. How well these patterns resemble those in permanent systems depends on several factors, including which taxa colonize, how they interact with current residents, and the length of the hydroperiod. The study of ecosystem processes in ephemeral ecosystems, such as community and food-web assembly, has much to add to our current knowledge of how communities and food webs are assembled. Inclusion of the processes common in ephemeral ecosystems, such as the interruption of temporal mechanisms, will only enrich our understanding of community assembly and food-web processes.

Acknowledgements

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Chapter 4

Habitat structure, hydroperiod, and trophic complexity of playa wetlands: a mesocosm experiment

Abstract

Ephemeral ecosystems undergo many changes throughout their hydroperiod which can subsequently change community diversity and food web structure. Previous food web studies on one type of ephemeral wetlands – the playas of the arid to semi-arid U.S. Great Plains –were purely correlative and unable to definitively determine what drives the food webs. Direct experimentation in mesocosms allowed us to explore how habitat structure, the hydroperiod, and community assemblages all interact to create the playa food web.

Mesocosms at the University of Kansas Field Station were “seeded” with playa propagules from sediment collected in dry Colorado playas and allowed to colonize throughout subsequent hydroperiods. Our mesocosm experiment consisted of two treatments: (1) a control group with sediment and water only; and (2) and an enhanced habitat complexity group with added habitat structure group (plastic plants). Invertebrate community diversity and aspects of the trophic structure (size, redundancy, and variability) were tracked through the hydroperiod. Trophic structure metrics were measured using geometric properties of stable isotope (C^{13} and N^{15}) biplots.

Mesocosms generally gained more species through time. The mesocosms with added habitat structure showed consistently more diversity, but were not significantly different. Trophic structure was more closely associated with insect diversity rather than crustacean diversity. Trophic structure was also more complex in mesocosms with more total, insect, and predator diversity.

Many playa wetland invertebrates ignore habitat structure created by vegetation, but structure that increases the hydroperiod, such as irregularities or deeper depressions in a playa floor, may impact playa invertebrates. Food web complexity is not simply associated with species richness numbers, but rather the characteristics of the individual taxa within the ecosystem.

Introduction

Playa wetlands are common, ephemeral aquatic features of many arid to semi-arid ecoregions, including the U.S. Great Plains. They are often roughly circular (Luo et al. 1997), clay-lined, shallow depressions (Evans 2010), which can arise from a combination of geomorphic, hydrochemical, or biologic processes (e.g., Smith, 2003, Gurdak and Roe 2009, Evans 2010). These highly homogenous environments often have little vegetation or habitat structure (Fennessy and Craft 2011). The southern High Plains of the United States has the highest concentration of natural playas in the world (Evans 2010), yet similar ecosystems are found on other continents, such as the pans of Australia and South Africa, and the takyr, sabkha, and kavir of Central Asia, Saudi Arabia, and Iran, respectively. A playa may be dry for decades or may fill and dry several times per year depending on climate.

Hydroperiod has long been recognized as the main ecological determinant in playas and other ephemeral wetlands and waterbodies (Spencer et al. 1999, Bilton et al. 2001, Gascón et al. 2005, Waterkeyn et al. 2008, Sim et al. 2013). Through the hydroperiod, water conditions change (Williams 2006), habitat often shrinks, and species richness fluctuates, with initial increases due to emergence from resting stages and colonization from other aquatic or terrestrial habitats. These factors can alter food web structure through changes in community composition, behavior of individuals and species (Kondoh 2003), and impacts of outside abiotic forces (Schmitz and Booth 1997).

Single-visit sampling designs, which characterized most previous playa studies including our earlier research (Chapters 1-2; O'Neill and Thorp 2014), hinder the ability of scientists to positively identify the main factors controlling food webs of these hydrologically fluctuating, ephemeral wetlands. Although large sample sizes of playas (as in Chapter 1) and multiple sampling events in the same playa (as in Chapter 3) provide valuable insights to food web control mechanisms, there are still site-specific differences in playa wetlands that cannot be explored without experimental manipulation, especially those related to vegetation cover. For example, the playas we studied previously (Chapters 1-3; O'Neill and Thorp 2014) differed in habitat size, vegetation density and thus habitat structure, hydroperiod length, and community composition. To adequately tease apart the effect and strength of these factors, we need equal water chemistry, habitat size, hydroperiods, seasonality, and equivalent species pools on which to draw (same colonists, same propagules). Direct experimentation in mesocosms allows us to fulfill these requirements by following communities and food webs in a controlled manner.

To resolve these problems, we manipulated habitat structure in mesocosms to track diversity and abundance of the invertebrate community and employed stable isotope techniques to examine aspects of trophic structure, such as size, variation, and redundancy of the mesocosm food web (cf. Chapter 2 and O'Neill and Thorp 2014). These experiments allowed for more conclusive answers to the following three questions: What are the effects of adding habitat structure on artificial playa wetland communities? When communities start with the same propagule pool, how do they change through time? How do differences in community assemblage change the food web? We hypothesized that: (1) added habitat structure increases community diversity, and that insects respond more strongly than crustaceans to increased habitat complexity; (2) trophic structure responds positively to increases in diversity, including total diversity and predator diversity; (3) trophic structure responds more strongly to insect diversity than crustacean diversity; and (4) slight differences in communities can make large differences in trophic structure, depending upon which species are present.

Methods

Experimental design and sampling

Mesocosms consisted of circular pools (95 cm inside diameter, 20 cm deep) that held ~140 L of water when full (Fig. 4.1). We added ~3 cm of playa substrate to "seed" the mesocosms with playa propagules and two pieces of concrete (generally 10 x 10 x 3 cm) for added weight. We collected substrate for the mesocosm experiments from two playa wetlands in northeastern Colorado (Playa 1 - Latitude: N 40.917, Longitude: W 103.832; Playa 2 - N 39.55, W 103.075) in May 2012. These playas had no standing water, and the sediment was dry at time of substrate collection. The top 1–2 cm of sediment were collected from all areas of the playa floor to ensure as many types of dormant eggs as possible. Sediments were stored in sealed containers in a climate controlled building until use in the mesocosms. Before "seeding", we mixed equal volumes of sediment from each playa to homogenize the potential propagules.

Figure 4.1 – Photograph of a mesocosm pool. Pools measure 95 cm in diameter and 20 cm in depth.

The experiment took place at the University of Kansas Field Station over two summers (2012 and 2013) and consisted of six mesocosms each year. Ten plastic aquarium plants were added to three pools (randomly chosen) to simulate plant density commonly found in natural playas. Plastic plants were a variety of shapes and sizes but generally mimicked *Ceratophyllum*, *Myriophyllum*, and *Vallisneria*. We filled mesocosms with groundwater one week before the first invertebrate sampling, and refilled as needed (approximately every 7 days) to keep the water level above ~6 cm. Before the second year, mesocosms were cleaned and fresh substrate was added to eliminate interference from the previous year's experiment.

We sampled the mesocosms three times in 2012 (May 29, June 5, and June 14), and four times in 2013 (July 5, 12, 27, and August 3). Sampling in all but the last date consisted of qualitative samples and visual inspections of mesocosm biota. This qualitative sample consisted of collecting at least one of each taxon found within each mesocosm by net (400 μm mesh) for isotope sampling. We collected all macroinvertebrates in the mesocosm on the last sampling date. For this last sample, we swept the net through the entire water column, including the top ~1 cm of substrate, multiple times. The sediment was then allowed to settle and was visually inspected for more invertebrates. We then repeated net sweeps in each mesocosm. Invertebrates were then taken back to the lab, preserved in 75% EtOH, and identified using a variety of sources (Arnett et al. 2001, 2002; Darsie and Ward 2005; Merrit et al. 2008; Thorp and Covich 2010).

For stable isotope analyses, we dried the samples for 48 h at 60 °C, ground them into a homogenized powder using a Wig-L-Bug® Mixer/Amalgamator, and stored them in desiccators until analyzed. Samples were put in tin capsules, weighed (1–1.5 mg dry mass) and analyzed at the University of Kanas W.M. Keck Paleoenvironmental and Environmental Stable Isotope

Laboratory using a ThermoFinnigan MAT 253 continuous flow system mass spectrometer. Data collected included δ^{15}N and δ^{13}C and total N and C. δ^{15}N and δ^{13}C were determined as the relative difference in isotopic ratio among the samples and known standards (Vienna Pee Dee Belemnite and atmospheric nitrogen for C and N, respectively) as the following equation:

$$\delta X = ((R_{sample}/R_{standard}) - 1) \times 1000$$

where X is either ^{13}C or ^{15}N, and the corresponding ratio is either R = ^{13}C/^{12}C or R = ^{15}N/^{14}N.

Statistics and trophic structure metrics

We conducted statistics using Minitab v. 16 (Minitab Inc., State College, PA, USA), which mainly consisted of ANOVAs, two-sample t-tests, ANCOVAs, and correlations. The trophic structures of playa food webs were quantified using metrics (Table 2.1) developed by Layman et al. (2007): δ^{15}N range (NR), δ^{13}C range (CR), total area (TA), mean distance to centroid (CD), mean nearest neighbor distance (NND), and standard deviation of nearest neighbor distance (SDNND). These metrics were chosen instead of Bayesian approaches (Jackson *et al*. 2011) which are more appropriate for the niche of a single species. All trophic structure metrics were calculated using the centroids of each taxon. Thus, if a food web had eight taxa, the metrics were determined from eight points (the eight taxa centroids). NR and CR were calculated by subtracting the lowest δ^{15}N and δ^{13}C value from the highest. TA was measured by creating a convex hull around points of the C-N isotope biplot and determining the area in ArcMap v.10 (ESRI, Redlands, CA, U.S.A.). CD was computed by finding the centroid of each biplot in Excel (Microsoft Inc., Redmond, WA, U.S.A.), and averaging the distances from each taxon point to the centroid. NND was calculated using a distance matrix (Euclidean distance) from PCORD v. 6 and averaging the shortest distances among each taxon and its nearest taxon. SDNND was determined from the same distance matrix by taking the standard deviation of the nearest neighbor distances. See Layman et al. (2007) for more detailed instructions and alternate methods to calculate these metrics.

Results

Invertebrate taxa richness increased through time (Fig. 4.2), but may have also been affected by treatment (Fig. 4.3). An ANCOVA showed that taxa richness was significantly different among dates but not among treatments (F_{date} = 9.82, p = 0.005; $F_{treatment}$ = 1.52, p = 0.232). The slopes of the two groups appear to be slightly different (Fig. 4.3), but not significantly so (F = 0.68, p = 0.42). Correlation of taxon richness with sampling date showed that for all data pooled, taxa richness was positively correlated with sampling date (R = 0.584, p = 0.004). However, when split by treatment, taxa richness in the added structure treatment was significantly correlated with sampling date (R = 0.649, p = 0.022), whereas the control group was not (R = 0.491, p = 0.149). Additionally, δ^{15}N range (NR) was positively correlated with sampling date in only the control group ($R_{control}$ = 0.637, p = 0.048; $R_{added\ structure}$ = 0.134, p = 0.732; $R_{all\ data}$ = 0.408, p = 0.083).

Figure 4.2 – Taxon richness vs. sampling date. Data grouped by mesocosm. Structure refers to the mesocosms with plastic plants added. Points offset slightly for better readability.

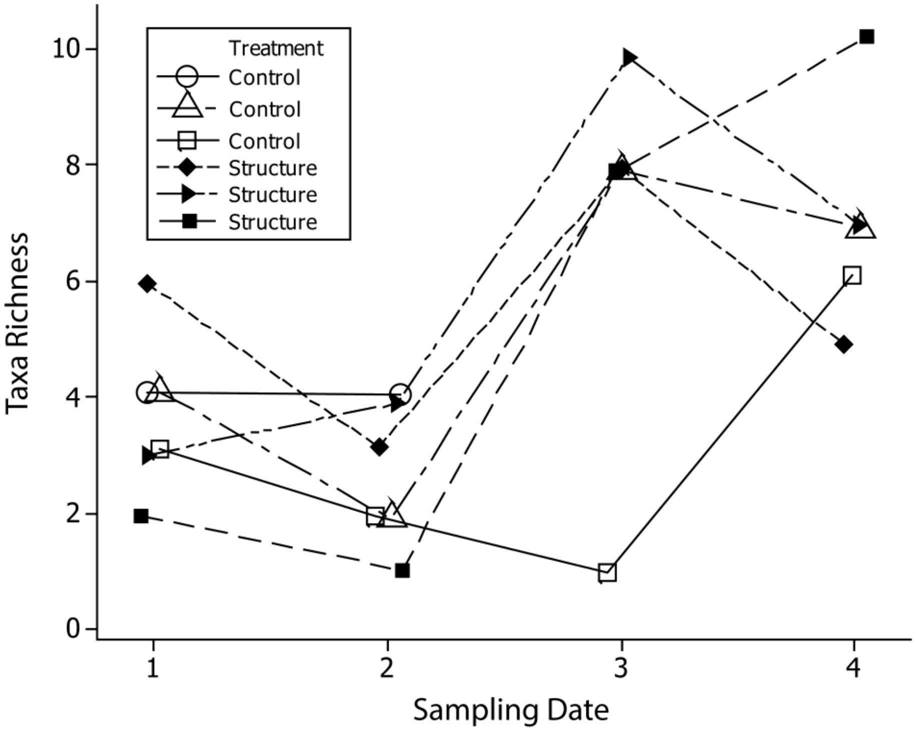
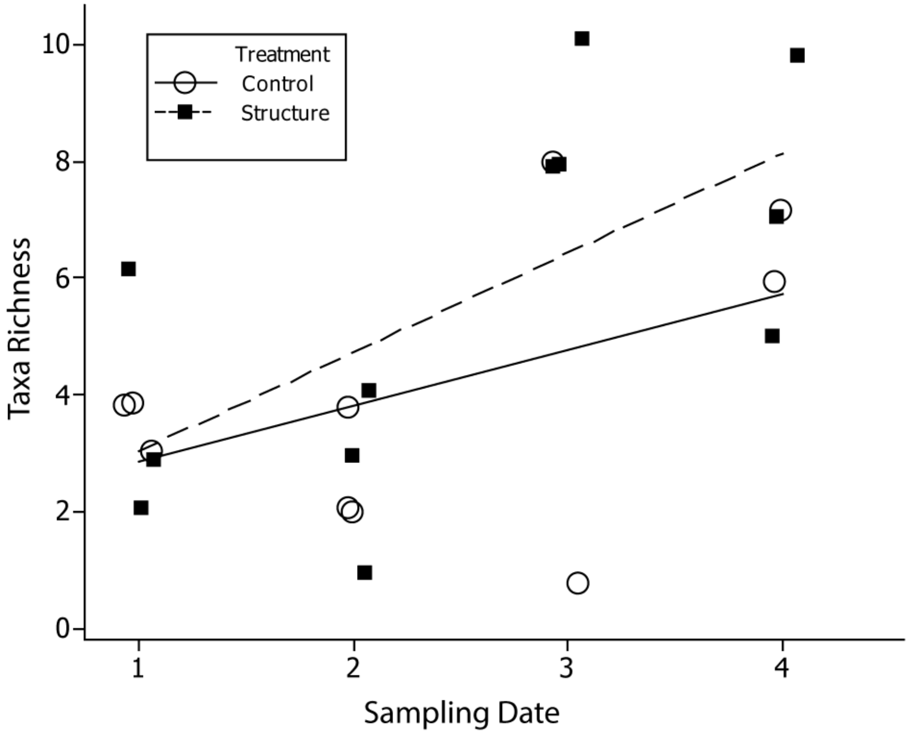


Figure 4.3 – Scatterplot of taxa richness vs. sampling date. Data are separated by treatment. Points offset slightly for better readability. Lines represent linear regressions.



Taxa richness was also associated with trophic structure metrics. An ANCOVA showed that δ^{15}N range (NR) was positively correlated with taxa richness, but not necessarily treatment ($F_{richness} = 4.8$, $p = 0.044$; $F_{treatment} = 3.19$, $p = 0.093$). δ^{13}C range (CR) was associated with taxa richness and treatment (ANCOVA $F_{richness} = 10.58$, $p = 0.005$; $F_{treatment} = 5.01$, $p = 0.04$), with the control group having higher CR. Mean centroid distance (CD) was significantly correlated with taxa richness ($R = 0.654$, $p = 0.04$) in only the control group. Total area (TA) was higher with increases in taxa richness (ANCOVA $F_{richness} = 7.66$, $p = 0.017$; $F_{treatment} = 2.79$, $p = 0.121$).

Taxa group specific results

Crustaceans and insects responded differently throughout the experiment. There were no differences among the control and added structure treatment for the two taxa, yet, crustacean richness generally decreased through time (correlation $R = -0.694$, $p < 0.001$), while insect richness generally increased (correlation $R = 0.754$, $p < 0.001$) (Fig. 4.4). NR was significantly lower when the mesocosms were dominated by crustaceans (ANCOVA $F_{\%crustaceans} = 11.33$, $p = 0.004$; $F_{treatment} = 3.62$, $p = 0.075$). Insects generally had a positive effect on trophic structure metrics (Fig. 4.5). NR, CR, CD, and TA increased with higher insect richness (NR ANCOVA $F_{insect\ richness} = 8.44$, $p = 0.01$, $F_{treatment} = 4.31$, $p = 0.054$; CR ANCOVA $F_{insect\ richness} = 16.42$, $p = 0.001$, $F_{treatment} = 6.32$, $p = 0.023$; CD ANCOVA $F_{insect\ richness} = 6.04$, $p = 0.026$, $F_{treatment} = 2.8$, $p = 0.113$; TA ANCOVA $F_{insect\ richness} = 8.41$, $p = 0.013$, $p = 0.172$, $F_{treatment} = 2.11$, $p = 0.172$).

Figure 4.4 – Crustacean and insect richness through time.

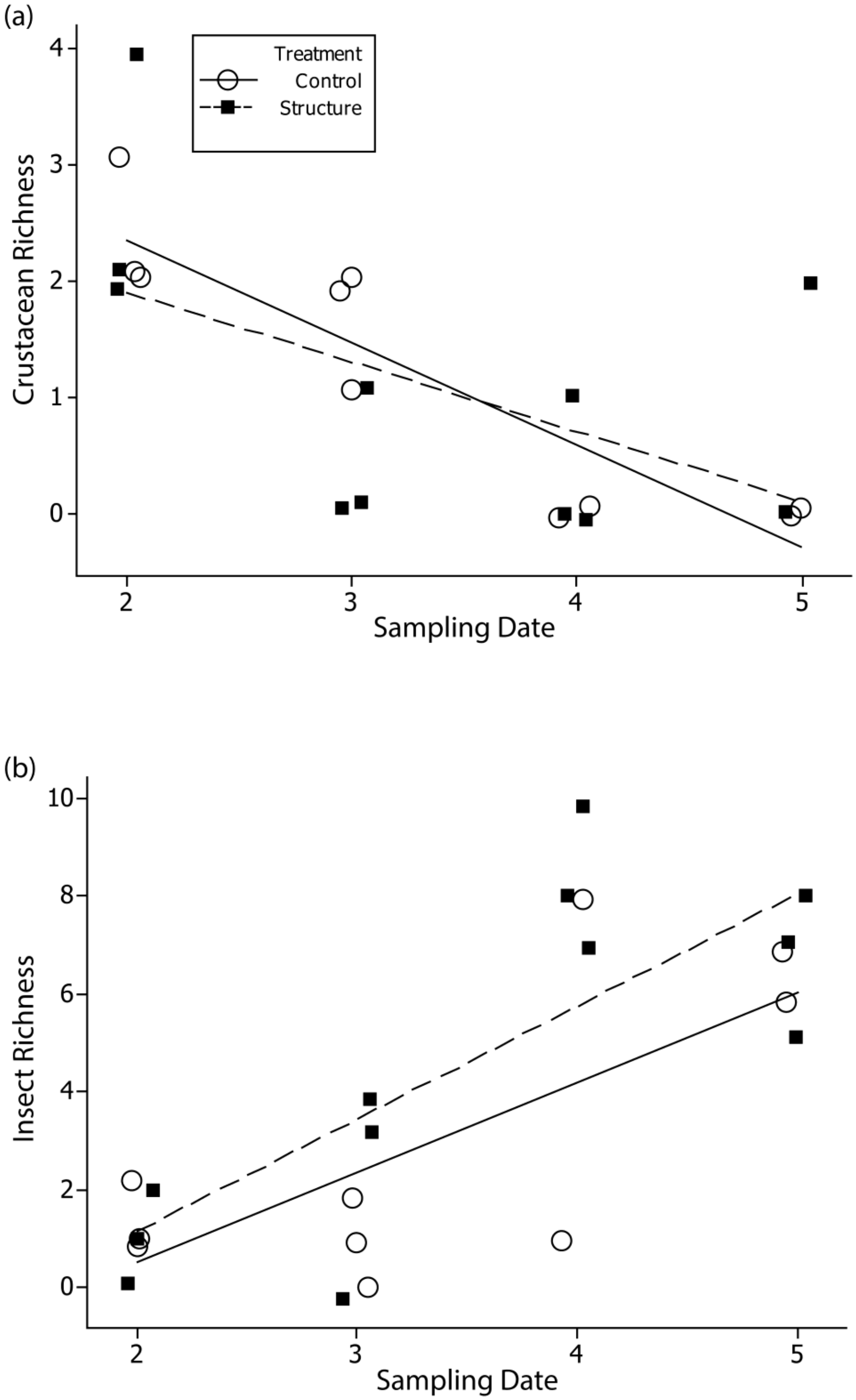
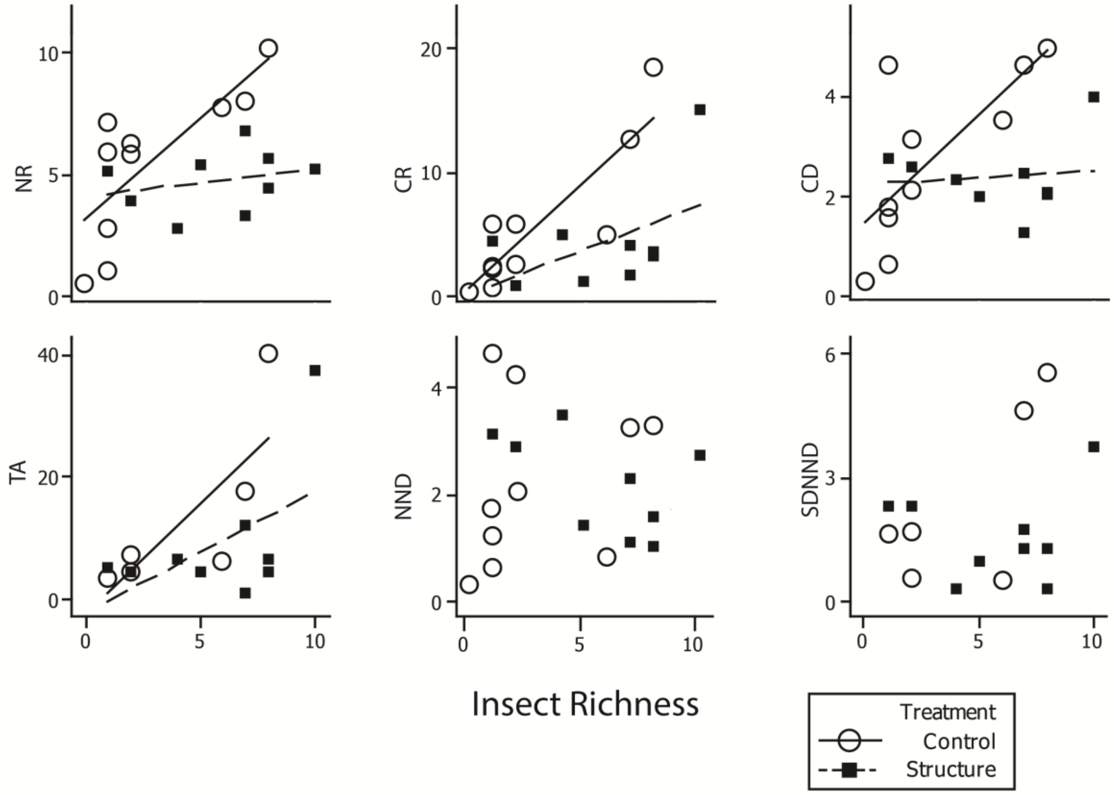
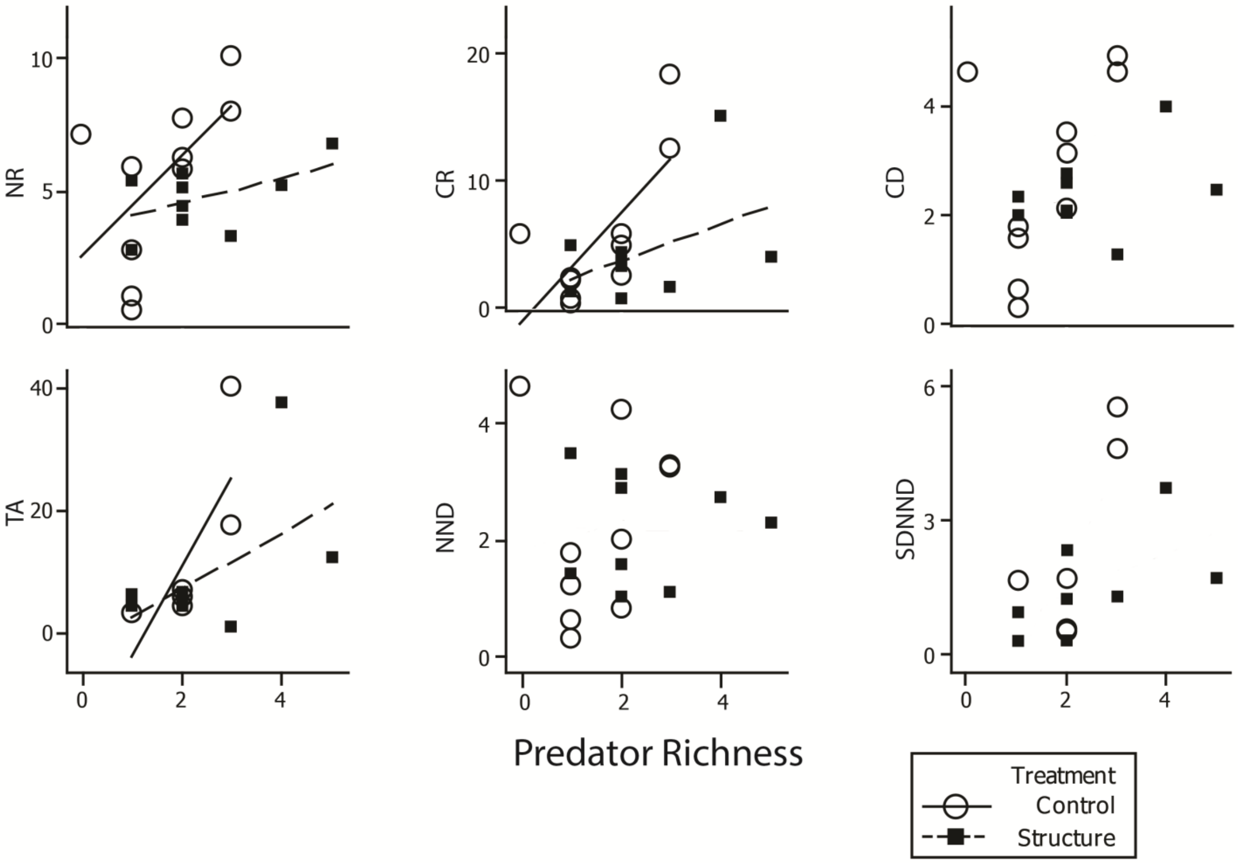


Figure 4.5 – Trophic structure metrics vs. insect richness. Data grouped by treatment. Significant correlations denoted by lines.



Predator richness also had a generally positive effect on trophic structure metrics (Fig 4.6). NR increased with higher predator richness (ANCOVA $F_{\text{predator richness}} = 4.61$, $p = 0.047$; $F_{\text{treatment}} = 2.23$, $p = 0.155$), along with CR (ANCOVA $F_{\text{predator richness}} = 7.39$, $p = 0.015$; $F_{\text{treatment}} = 2.46$, $p = 0.136$), and TA (correlation $R = 0.546$, $p = 0.035$). The only trophic structure metric associated with nonpredators was CR; higher CR values increased with non-predator richness (ANCOVA $F_{\text{nonpredator richness}} = 4.72$, $p = 0.045$; $F_{\text{treatment}} = 2.42$, $p = 0.139$)

Figure 4.6 – Trophic structure metrics vs. predator richness. Data grouped by treatment. Significant correlations denoted by lines.



Discussion

Researchers have long recognized the overwhelming importance of the hydroperiod in ephemeral wetlands and waterbodies (Spencer et al. 1999, Bilton et al. 2001, Gascón et al. 2005, Waterkeyn et al. 2008, Sim et al. 2013). Boix et al. (2004) even classified ephemeral wetland communities into different groups depending on the stage of the hydroperiod. Previous work (Chapter 3) showed that many aspects of playa communities were determined by hydroperiod stage. Our results with mesocosm experiments were similar to patterns observed in natural playa wetlands (Chapter 3). The species diversity increase through time, and gradual replacement of crustacean species with insect species, occurred in the mesocosms as well as the playa wetlands. These previous field empirical results showed these strong changes, but were limited because they were purely correlative. The playas were in the same type of grassland, relatively close to each other, and similar in size and morphology. Nonetheless, they could have varied in important ways that could have obscured the results. For example, some of the playas dried out more quickly than others, with local geomorphology perhaps causing slightly different water, temperature, and wind patterns. The playas may have also started with different propagule pools. While the branchiopod fauna of the playas were similar and most species overlapped, several species were only found in a single playa. This mesocosm experiment allowed us to expose our sampling units to more uniform conditions. Since we found similar patterns and results in our mesocosms compared to our field studies, we can say more definitively that the stage of the hydroperiod is the primary driver in playa communities, rather than aspects of individual playas.

Playa wetlands are extremely homogenous habitats, which do not vary significantly in habitat complexity, irrespective of that created by vegetation, within or among playas, nor with increases in playa size (Luo et al. 1997). Most playas have very little vegetation, so even a small increase in plant density is expected to greatly augment habitat structure or complexity, which can increase species diversity (e.g. Cummings and Lauf 1969, Minshall and Minshall 1977, Tolkamp 1982). Our mesocosm communities may have shown some differences with the increased habitat structure treatment, albeit weakly. Trophic structure metrics appeared higher in the added structure treatment (Fig. 4.5), but sample size was insufficient to statistically separate the effects of treatment from the more strongly associated variables, such as taxa richness and hydroperiod. However, invertebrates primarily associated with playas are less likely to respond to increases in habitat structure.

The resting eggs of branchiopods, the hallmark organisms of playas (Brendonck et al. 2002), mainly respond to temperature, salinity, and water depth cues for hatching (Vandekerkhove et al. 2005). The addition of plant structure, and especially our plastic plants in the mesocosms, may not affect any of the hatching cues upon which branchiopods rely. Playa invertebrates do not need to use macrophytes as refuges from fish predation; therefore, most live a planktonic lifestyle. Branchiopods are poorly adapted to grasp or use plant stems or structures in any way. However, being wholly planktonic in shallow habitats can increase predation risk (Jocque et al. 2010) by invertebrate predators, wading shorebirds, and waterfowl. Bird predation could have significantly impacted our mesocosms. We observed Killdeer (*Charadruis vociferous*) actively hunting in our mesocosms. This may be one reason why crustaceans disappeared towards the end of the experiment. In natural settings, water can concentrate in cattle hoofprints, and be viable habitats for smaller playa inhabitants (Chapter 3), effectively lengthening the hydroperiod for a short time. This type of habitat structure may have a larger effect on playa communities, rather than the structure created by vegetation. Future research should be conducted into how playa communities react to different types of habitat structure.

Food-web complexity, or complexity of trophic structure, has been shown to increase with species diversity (e.g. Paine 1966). Some food-web studies have even used species richness as a measure of food-web complexity (e.g., Williams et al. 2002). As food webs develop, higher species numbers allow for more total interactions. Yet, data show that food-web connectance, the proportion of actual links to possible links, is stable across food-webs with different numbers of species (Martinez 1992). Beyond some threshold, all species cannot interact with every other species in a food web, due to diet constraints and foraging behavior (Beckerman et al. 2006). Our previous work in playa wetlands (Chapters 2-3; O'Neill and Thorp 2014) showed that at the diversity level common to playa wetlands, trophic structure is strongly tied to community membership (identities of the species present) rather than species richness or any diversity metric. These food webs may be at the stable connectance point, where any further increases in diversity may not affect trophic structure complexity. However, this was hard to definitively prove in our real ecosystems. Our mesocosms allowed us to eliminate many of the differences between playas.

While the total number of all invertebrate species was not tied to trophic structure, the numbers of insect species were. The insects of playa wetlands have varied lifestyles and feeding strategies. In a single playa there can be large separation of niches among insects, unlike the branchiopod crustaceans. Diverse feeding strategies may be of importance for trophic functioning (Fridley 2001). A playa could have water striders (Gerridae) predating on the water's surface, backswimmers (Notonectidae) and beetle larvae (Dytiscidae and Hydrophilidae) predating below, mosquitos (Culicidae) filtering in the water column, mayflies (Baetidae) gathering food particles on the benthos, and other omnivorous beetle adults. Our mesocosms responded in kind with higher insect richness increasing trophic structure complexity. Conversely, crustaceans either had no effect or a negative effect on trophic structure. They do not have the varied feeding strategies that insects do. Most of the crustacean branchiopods common to playas filter-feed organic particles from the water column, making them trophically redundant (cf. Blondel 2003).

Predator richness also increased trophic structure complexity. Only a few of the branchiopods found in playa wetlands act as predators, such as the tadpole shrimp *Triops*, and several less common fairy shrimp (*Branchinecta gigas*, *Branchinecta raptor*). Therefore, the overwhelming majority of increases to food-chain length (as measured by NR) and other trophic structure properties come from the colonizing insects. Overall, simply using species numbers is a poor way to measure trophic complexity. The addition of particular traits of individual species makes more of a difference to food webs than overall numbers.

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Conclusion

Playa wetlands appear, from an outside perspective, to be unpredictable habitats with no discernible patterns to their communities, food webs, or ecology. The assemblages of playa communities can change with large scale geographic patterns and even in the same playa within and between hydroperiods. Due to their high tolerances to extreme conditions, playa communities seem to be unaffected by factors such as habitat size, depth, and within-playa habitat structure, all of which typically affect communities in permanent ecosystems. However, the fauna follow predictable patterns and react consistently to certain environmental cues. Tilling for row crops negatively impacts playa communities and simplifies food webs. Yet, not all anthropogenic forces are detrimental to these ecosystems. Cattle grazing in playas, at least at the levels seen in our study sites, appears to positively impact community diversity and may maintain playa communities. This may reflect prior effects of native grazers, such as bison and pronghorns. Artificially-created waterbodies often support invertebrate communities of equal diversity and abundance to natural playas. However, simply looking at overall diversity or species richness tells very little about food web complexity. It is the community membership, or identity of the species found and their corresponding characteristics, that influences how food webs function. Playa communities are more organized by time ~~rather~~ than site specific characteristics, and they follow predictable patterns of colonization through the hydroperiod. Toward the beginning, food webs are relatively simple, and food chains are short when branchiopods dominate the system. Shortly into the hydroperiod, insects begin to colonize and greatly increase food-web complexity and food-chain length. As long as playas have sufficient water or a hydroperiod of sufficient length, they will show patterns of increasing taxa richness, food-web complexity, and food-chain length until the playa begins to dry. As the playa dries, many species are eliminated due to increased competition and predation. During this stage, food-web complexity declines, but apparent food-chain length can actually increase. Finally, as the water completely evaporates, the invertebrates either flee to other aquatic habitats or deposit resting eggs that will hatch when water returns.

Playa wetlands are unique habitats with important functions for regional biodiversity. They hold some of the only drinking water sources for many large mammals in many prairie regions and contain invertebrate and plant food important to migrating waterfowl and shorebirds. Moreover, they hold invertebrate communities that are important and interesting in themselves, especially large branchiopods such as fairy and tadpole shrimp. Playa wetlands, and many ephemeral waterbodies worldwide, are highly endangered. Ephemeral habitats are often disregarded and destroyed without thought to the organisms that use and reside in them. This body of work has shown that playa wetlands, while highly resistant and resilient to natural and human-induced disturbances, cannot survive everything. Playas need to be protected where they still are in their natural state, and conserved and wisely managed wherever possible. Ephemeral wetlands have much to inform us about how food webs assemble and how they are maintained in the face of large environmental fluctuations.