Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation

Management of Fish Food Resources in Off-Channel Native Fish Habitats

2011–2013 Annual Report

July 2014

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Lower Colorado River
Multi-Species Conservation Program

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Prepared by:

Eric Loomis and Nathan Rudd, Fisheries Group

Lower Colorado River
Multi-Species Conservation Program
Bureau of Reclamation
Lower Colorado Region
Boulder City, Nevada
http://www.lcrmscp.gov

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Acronyms and Abbreviations

ANOVA	analysis of variance
CL	confidence limit
DF	degrees of freedom
DO	dissolved oxygen
kg	kilogram(s)
LN	natural logarithm
m	meter(s)
mL	milliliter(s)
mm	millimeter(s)
Reclamation	Bureau of Reclamation

Symbols

α	alpha
°C	degrees Celsius
$[NH_4]_3PO_4$	inorganic ammonium phosphate
%	percent

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INTRODUCTION

Lake Mohave is a Bureau of Reclamation (Reclamation) regulated reservoir that is home to the largest known remnant population of the endangered razorback sucker (*Xyrauchen texanus*). Lake Mohave elevations fluctuate annually within a 5-meter (m) vertical range for the purpose of downstream river operations (Mueller 1995). As a result, redistribution from sediment deposits form natural sandbars or berms that create a series of isolated lake-side backwaters during periods of high annual lake elevations. Backwaters typically fill to maximum pool by mid-May and become dry by October each year.

Since 1993, Reclamation and its partners in the Lake Mohave Native Fish Work Group have used these predator-free backwater ponds as grow-out areas for razorback sucker and bonytail (*Gila elegans*). The ponds are usually stocked in March with juvenile/subadult fish measuring in excess of 300 millimeters (mm) total length. Source fish are naturally spawned Lake Mohave larvae that are reared by the U.S. Fish and Wildlife Service at the Willow Beach National Fish Hatchery. Larvae are raised to target length for backwater stocking using standardized artificial feed regimens at the hatchery.

The backwater environments used for native fish grow-out are not supplemented with artificial feed. Fish must therefore rely on the natural food base available during a residence time of 2–7 months prior to repatriation into Lake Mohave. Little is known about the feeding habits of these fish in the backwaters. However, stomach content analyses of adult razorback suckers captured along the Lake Mohave shoreline have shown a cosmopolitan diet of mainly planktonic crustaceans, diatoms, and filamentous green algae (Marsh 1987).

Since 2005, Lake Mohave backwaters stocked with fish have been fertilized each spring under the Lower Colorado River Multi-Species Conservation Program. Annual fertilization regimes consist of a combination of inorganic ammonium phosphate ($[NH_4]_3PO_4$) and organic alfalfa pellets. Alfalfa meal has shown to be superior to other organic fertilizers in promoting reproduction and survival of large-bodied zooplankton, most notably cladocerans (Barkoh and Rabeni 1990). Combined fertilization regimes have been used to achieve high concentrations of zooplankton in ponds for the benefit of the target fish species (Geiger 1983a, 1983b; Geiger and Turner 1990). Despite efforts to enhance primary productivity, a detailed analysis of food resource dynamics in the backwaters has not been done. Because of limited knowledge of the food resource base in lake-side backwaters, the zooplankton community in selected grow-out ponds was characterized quarterly under the Lower Colorado River Multi-Species Conservation Program in 2009 and 2010 (Reclamation 2012). These preliminary plankton profiles were intended to produce baseline composition and relative abundance data prior to fertilization treatments. Beginning in 2011, zooplankton sampling was increased to monthly and continued through 2013. Monthly phytoplankton sampling was also added in 2011 to evaluate the effects of fertilization on primary productivity.

The purpose of this study is to attempt to quantitatively determine potential changes in food resource availability by using three experimental fertilization regimes in selected backwaters of Lake Mohave, Nevada. Known quantities of three types of fertilizer were distributed in ponds and manipulated from year to year with the goal of identifying fertilization regimes that can be used in future pond management to maximize the availability of natural food resources.

METHODS

Study Sites

Five lake-side ephemeral backwaters of Lake Mohave, Nevada, were selected for controlled fertilization in an attempt to increase primary productivity (figure 1). The ponds included North Nine Mile, Willow, Nevada Egg, Nevada Larvae, and a control (table 1). From 2005–2007, most of the backwaters received a treatment of 20 kilograms (kg) of $[NH_4]_3PO_4$ per 9 kg of alfalfa. Beginning in 2007, treatment efforts diminished, or were eliminated from all ponds until 2010, when Nevada Larvae and Willow received the aforementioned 20:9 treatment ratio. All sites were chosen based on close proximity and sharing the same Nevada shoreline.

Razorback Sucker Rearing

Annual razorback stockings occurred in either February or March of each year at Willow (n = 50) for grow-out before being released into Lake Mohave later in the season. Nevada Larvae received 50 fish in both 2011 and 2012. Consistently poor harvest results of razorback in this backwater prompted the Lake Mohave Native Fish Work Group to suspend stocking in 2013 and beyond. North Nine Mile, Nevada Egg, and the control ponds did not receive fish for the duration of this study.

Fertilization

Each backwater was treated experimentally with a combination of solid organic and inorganic fertilizers on March 3, 2011, February 14, 2012, and February 21, 2013 (table 2). Water quality profiles and plankton tows were collected immediately prior to each fertilization treatment. Fertilizers were broadcast manually from shore with a scoop and included either Grainland Select™ Rice Bran or Alfalfa Pellets combined with $[NH_4]_3PO_4$ (table 2). Wind speeds were non-existent or minimal during all treatments.

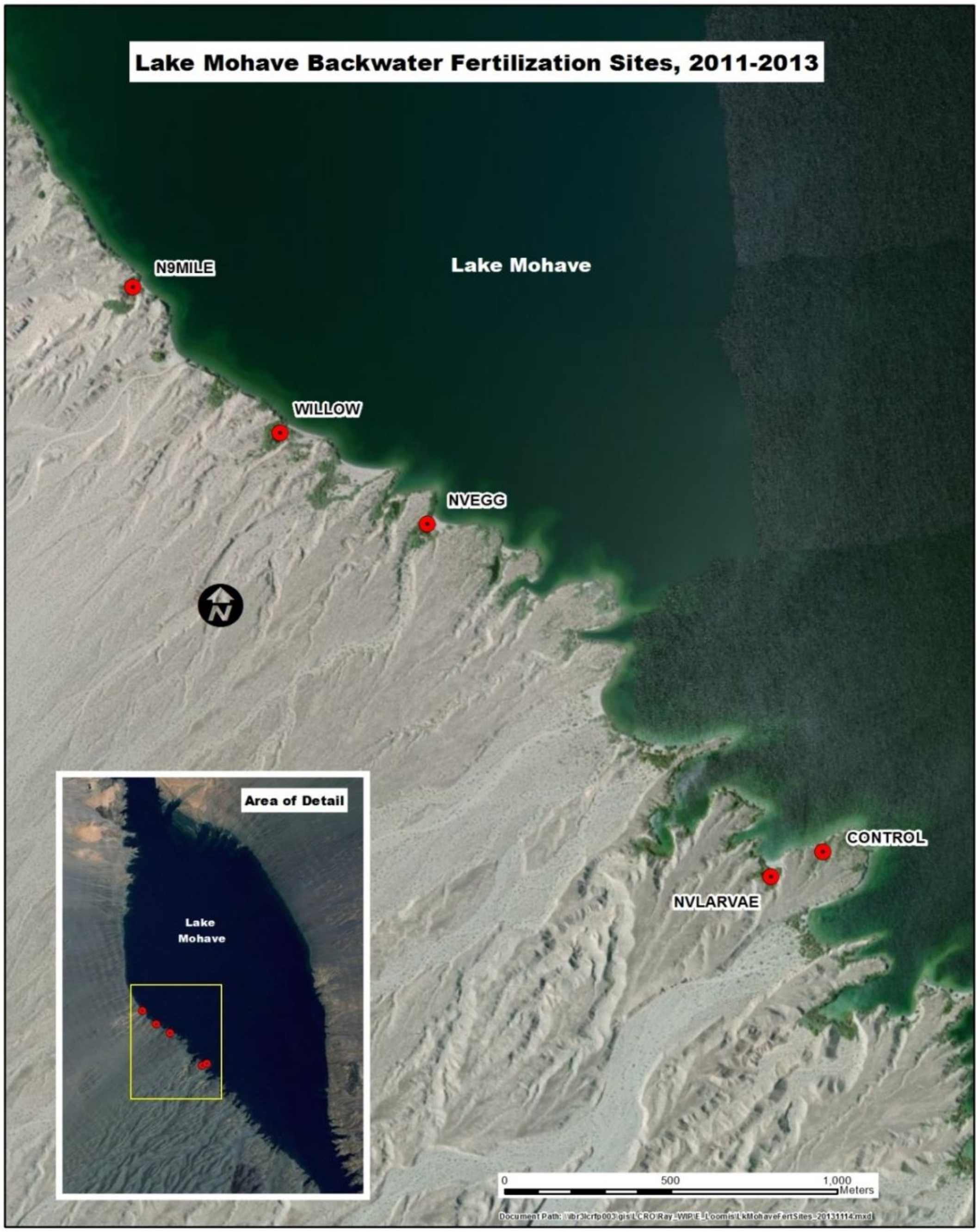


Figure 1.—Lake Mohave, Nevada, backwater fertilization sites, 2011–13.

Table 1.—Lake-side backwaters selected for fertilization experiments at Lake Mohave, Nevada, 2011–13

Backwater	Universal Transverse Mercator coordinates	Depth (m)[a]	Area (hectares)[b]
North Nine Mile	11 S 711117 3921603	1.3	0.33
Willow	11 S 711588 3921184	2.0	0.40
Nevada Egg	11 S 712036 3920917	1.3	0.18
Nevada Larvae	11 S 713051 3916488	2.3	0.22
Control	11 S 713209 3919889	0.9	0.07[c]

[a] Average maximum depth in meters.
[b] Surface area in hectares at maximum pool.
[c] Control backwater surface area is an approximation.

Table 2.—Experimental fertilization regimes for selected Lake Mohave backwater grow-out ponds on March 3, 2011, February 14, 2012, and February 21, 2013

Backwater	$[NH_4]_3PO_4$ (kg)	Alfalfa (kg)	Rice bran (kg)
North Nine Mile			
2011	19.8	0	36.5
2012	39.6	0	73.0
2013	59.1	0	109.5
Willow			
2011	22.7	45.4	0
2012	45.4	90.7	0
2013	67.8	135.9	0
Nevada Egg			
2011	10.9	22.7	0
2012	21.8	45.4	0
2013	32.4	67.8	0
Nevada Larvae			
2011	13.2	0	27.4
2012	26.3	0	54.9
2013	39.6	0	82.2

Fertilizer concentration loads were based on pond surface area (see table 1) in an attempt to increase large-bodied crustacean zooplankton production in experimental ponds of similar size (Barkoh et al. 2005; Barkoh and Rabeni 1990;

Ludwig and Tackett 1991). Organic components of rice bran and alfalfa were alternated based on similar-sized ponds. North Nine Mile and Willow were of similar surface area (see table 1); therefore, these ponds were paired with respect to the type and amount of organic fertilizer used (see table 2). Nevada Egg and Nevada Larvae were paired in the same manner. Fertilizer quantities were increased approximately threefold from the beginning of the study to see whether higher concentrations resulted in increased primary productivity or zooplankton biomass (see table 2).

Water Quality

Depth (m), temperature (degrees Celsius [°C]), dissolved oxygen (DO) in milligrams per liter, pH, and specific conductance (microsiemens per centimeter) were measured monthly (in conjunction with plankton sampling whenever possible) at each backwater using a YSI Pro-Series instrument. Water quality profiles were taken at the surface, every 0.5 m, and at the bottom of the backwater. Whenever possible, measurements were taken in the deepest portion of the pond, which was typically in the center, based on relatively uniform contours of each.

Zooplankton

Zooplankton samples were collected using 15-centimeter-diameter Wisconsin-style plankton net with 64-micrometer mesh. A single vertical tow was collected from the entire water column at each pond, and the contents were released into a 250-milliliter (mL) high-density polyethylene amber bottle. Each sample was preserved in Lugol's iodine solution at a rate of 1.0 mL solution per 100 mL of sample. Samples were collected from the deepest location in each backwater from one sampling event to the next to maintain consistency. Mean sample depth (m) was calculated for each pond and included in the results. Samples were shipped to BSA Environmental Services, Inc., Beachwood, Ohio, for analyses. Laboratory analyses of samples included enumeration of zooplankton by genus, species, and division. Biomass (micrograms per liter) calculations were performed based on tow length and volume.

Although baseline zooplankton samples were collected in 2009 and 2010, comparisons of temporal trends in biomass between pre- and post-fertilization years were not a reliable approach because baseline data were collected quarterly as opposed to monthly. Quarterly samples of 2009–10 may have failed to capture temporal variability in zooplankter distribution that was more evident in the monthly samples of 2011–13.

Phytoplankton

An integrated hose-style vertical sampler technique was used to collect phytoplankton in each backwater at the same location as the zooplankton sample. A modified standard pool hose measuring 3.5 centimeters in diameter was used to collect the composite sample. The hose was of a length suitable for maximum depth at each backwater and marked in 1-m increments. To prevent sediment from contaminating the sample, a length of the rope attached to a 2.2-kg lead weight at the end of the hose was slowly and evenly lowered to just above the maximum depth during sampling. The contents of the hose were then emptied into a bucket, and a composite sample was collected into a 250-mL amber sample bottle. The sample was preserved with Lugol's iodine solution at a rate of 1.0 mL solution per 100 mL of sample. The hose and collection bucket were thoroughly rinsed before and after collecting the sample at each backwater to prevent cross-contamination between sites. Samples were shipped to BSA Environmental Services, Inc., for analysis. Phytoplankton was tallied at the genus and division level. Total biovolume (cubic micrometers per milliliter) was calculated based on the composite sample volume.

The abundance of phytoplankton from data collected in 2011 was eliminated from this analysis based on an improper sampling technique. The integrated hose sampler was not implemented until 2012; therefore, no direct comparisons could be made between phytoplankton and other environmental variables for 2011.

Data Analysis

To ensure summary statistics were correctly calculated, 0-values for divisions without data for each sampling date were inserted prior to analysis. Trends in zooplankton and phytoplankton abundance from 2011–13 were graphed for each backwater both by and over plankton divisions in both untransformed and natural log (LN) transformed scales. The results are provided in this report only for total zooplankton, total phytoplankton, Cladocera and Copepoda (the most abundant zooplankton), and Cyanobacteria (blooms deplete DO). All graphs were generated using the statistical program R (version 3.01), and SAS (version 9.3) was used for statistical analyses.

Absolute and relative abundance of zooplankton were graphed by backwater to illustrate changes in composition at the division level over time. Annual means and standard error of zooplankton were graphed to examine potential differences in overall abundance among backwaters in conjunction with statistical analyses.

A multi-factor analysis of variance (ANOVA) was run using PROC MIXED to test for differences in zooplankton and phytoplankton abundance among backwaters and over years (2011–13 for zooplankton and 2012–13 for

phytoplankton). Separate analyses were done for Cladocera, Copepoda, and all zooplankton as well as all phytoplankton. The ANOVA model was: Y = backwater + year + month (year) + backwater * year, where the latter term represents an interaction between backwater and year. A significant backwater * year interaction could indicate a fertilization effect (i.e., zooplankton increasing differentially with higher or different types of fertilization but not in the control). This model assumes no interaction between month(year) and backwater due to a lack of replication within backwaters at each sample date. An analysis was done on LN transformed data (X + 1) to help meet assumptions of normally distributed residuals and homogeneous variances. Repeated measures of the same sites typically requires a mixed model analysis to allow for temporal autocorrelation, but a preliminary analysis indicated residuals were not highly correlated and roughly approximated the equal variance assumption. Pairwise comparisons (i.e., Fisher's Least Significant Difference) were run for significant backwater and year effects. Months within years were not compared because the focus of the analysis was on differences among years and backwaters; monthly variability was obviously large due to the seasonality of plankton blooms. Geometric means are reported for significant main effects; those followed by the same letter are not different at the
$\alpha = 0.1$ level. A more liberal alpha was used due to the relatively small number of backwaters, lack of spatial replication within backwaters, and large temporal variability in plankton abundance (i.e., low statistical power).

Trends of water quality parameters from 2011–13 were graphed using means for each sampling date from measurements at different depths (n = 2 to 7).

RESULTS

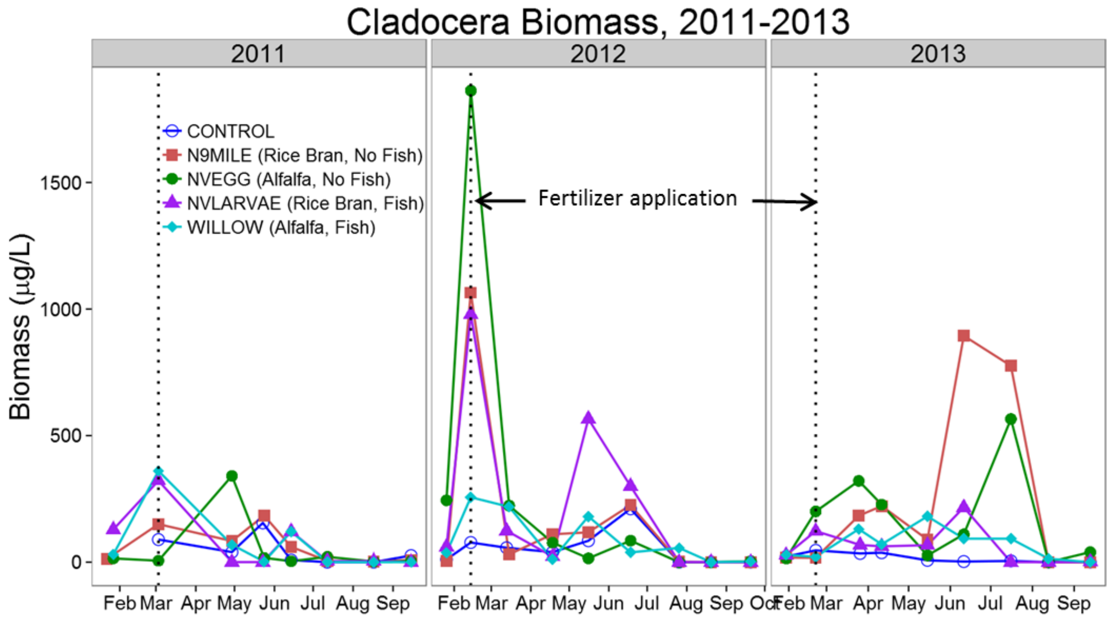
Zooplankton

An ANOVA on LN biomass of all zooplankton provided no clear evidence for response to fertilization (table 3). There was no indication of an interaction between backwater and year ($p = 0.2996$), and biomass did not vary significantly over years, but was highly variable within years. Thus, increased fertilization did not significantly increase annual mean total zooplankton biomass. The average zooplankton biomass over all years did vary significantly among backwaters, with Willow and Nevada Egg supporting approximately 2.5–4.5 times the biomass observed in the control based on pairwise comparisons (table 3) (i.e., the back-transformed difference between logarithms is a ratio). Ninety-five percent (%) confidence limits (CL) for differences from the control are: 1.4–5.0 times for Nevada Egg and 2.5–8.3 times for Willow.

Cladocera biomass varied significantly within and across years but not among backwaters (table 3). The highest peaks in Cladocera biomass occurred in 2012 before fertilizer was applied at North Nine Mile, Nevada Egg, and Nevada Larvae (figure 2). Higher zooplankton abundance at Willow was driven primarily by copepods (table 3, figure 3). Biomass averaged over time was highest at Willow, but it did not significantly vary among other backwaters. Copepod abundance remained similar at Willow across all three years despite increased levels of fertilization (figure 3).

Table 3.—Summary of ANOVA results for LN-transformed biomass of all zooplankton, Cladocera, and Copepoda
(Geometric means followed by the same letter are not different at α = 0.1. Error, degrees of freedom [DF] = 91.)

				Pairwise comparisons	
Effect	**DF**	**F**	**P**	**Backwater**	**Geometric mean**
All zooplankton					
Backwater	4	5.56	**0.0005**	Willow	225.0 a
Year	2	0.29	0.7476	Nevada Egg	133.3 a
Month (year)	23	4.38	< 0.0001	North Nine Mile	71.2 b
Backwater * year	8	1.21	0.2996	Nevada Larvae	67.1 b
				Control	48.9 b
Effect	**DF**	**F**	**P**	**Year**	**Geometric mean**
Cladocera					
Backwater	4	1.62	0.1766	2013	10.6 a
Year	2	2.49	**0.0885**	2012	9.7 a
Month (year)	23	7.94	< 0.0001	2011	5.6 b
Backwater * year	8	0.97	0.4679		
Effect	**DF**	**F**	**P**	**Backwater**	**Geometric mean**
Copepoda					
Backwater	4	8.26	**< 0.0001**	Willow	46.5 a
Year	2	2.18	0.119	Nevada Egg	14.0 b
Month (year)	23	2.27	0.0033	Control	10.4 b
Backwater * year	8	1.00	0.4406	Nevada Larvae	9.0 b
				North Nine Mile	5.9 b



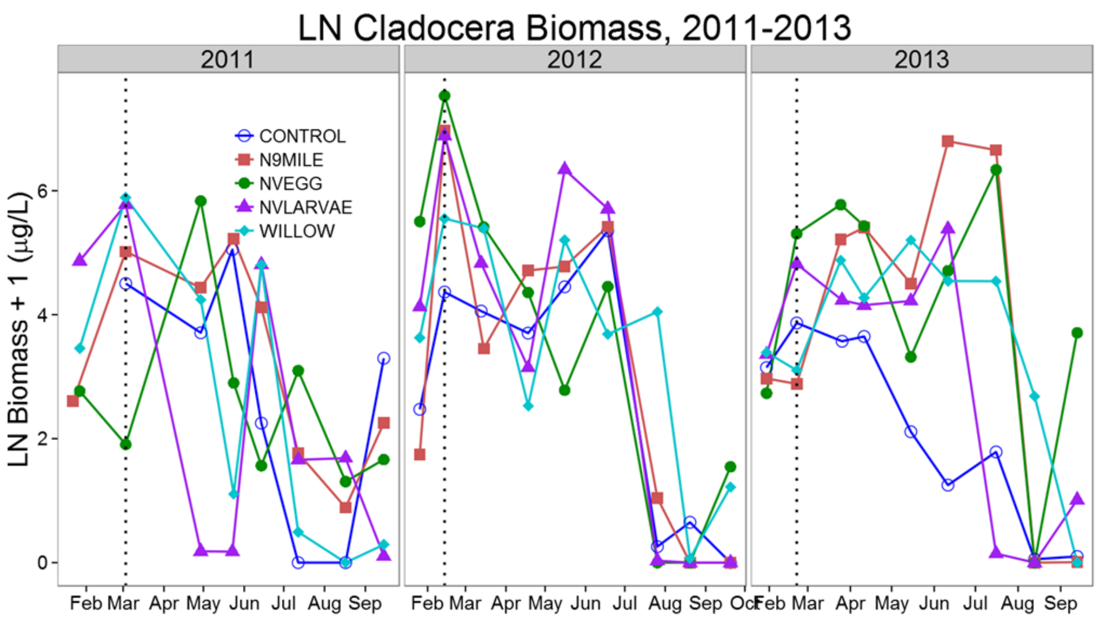
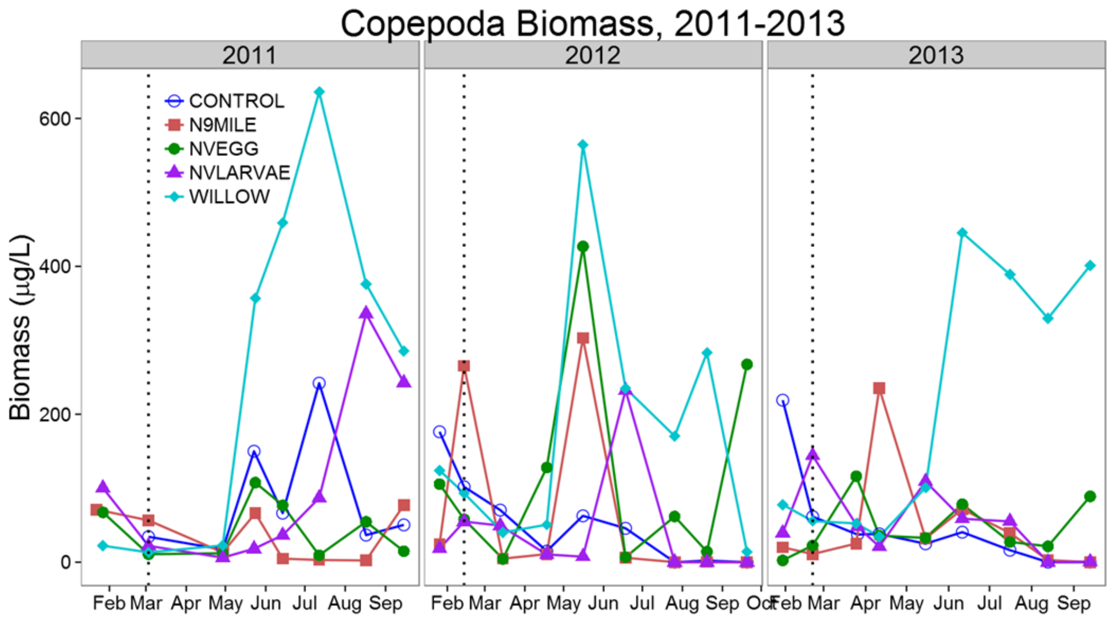


Figure 2.—Untransformed and LN-transformed monthly biomass of Cladocera at five backwaters, 2011–13.



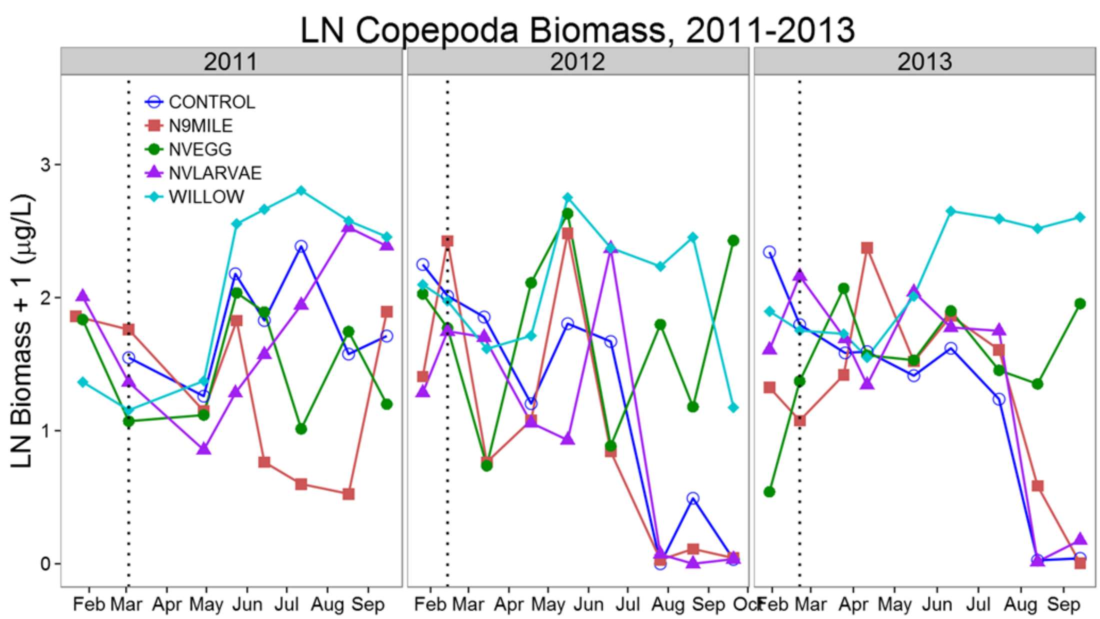


Figure 3.—Untransformed and LN-transformed monthly biomass of Copepoda at five backwaters, 2011–13.

Phytoplankton

Trends in LN-transformed phytoplankton biovolume were characterized by large increases in May or June of each year (figure 4). Cyanobacteria were most prevalent at North Nine Mile and Nevada Egg in May and June of each year and remained high throughout the summer at all backwaters (figure 5). Despite a higher prevalence of particular phytoplankton divisions during certain times at specific sites, temporal variation was quite large, with no significant differences over years. Total phytoplankton did vary among backwaters (p = 0.0066; table 4), with biovolume approximately 2–3.5 times greater at North Nine Mile than at the control, Nevada Larvae, or Willow (95% CL for differences: 1.1–3.9 times for control, 1.4–5.1 times for Nevada Larvae, and 1.9–6.9 times for Willow). Abundance was approximately 2.2–3.0 times higher at Nevada Egg than at Nevada Larvae and Willow (95% CL: 1.2 4.3 times for Nevada Larvae and 1.6–5.7 times for Willow).

Table 4.—Summary of ANOVA results for LN-transformed biovolume of all phytoplankton (Geometric means presented as cubic micrometers per microliter rather than per liter; geometric means followed by the same letter are not different at α = 0.1. Error, degrees of freedom [DF] = 64.)

Effect	DF	F	P	Pairwise comparisons: Backwater	Pairwise comparisons: Geometric mean (cubic micrometers)
All phytoplankton					
Backwater	4	3.92	**0.0066**	North Nine Mile	3,806.8 a
Year	2	1.62	0.2077	Nevada Egg	3,159.1 ab
Month (year)	16	4.48	< 0.0001	Control	1,864.1 bc
Backwater * year	8	1.04	0.3913	Nevada Larvae	1,408.7 c
				Willow	1,053.8 c

Water Quality

Mean water temperatures were predictably associated with increased ambient air temperatures throughout the summer months. Peaks typically occurred in July or August in all years (figure 6). As expected, there was a strong inverse relationship between water temperature and DO (figure 7; Spearman's r = -0.82, p < 0.0001, n = 90). Overall peaks in DO occurred in late April 2012–13 at

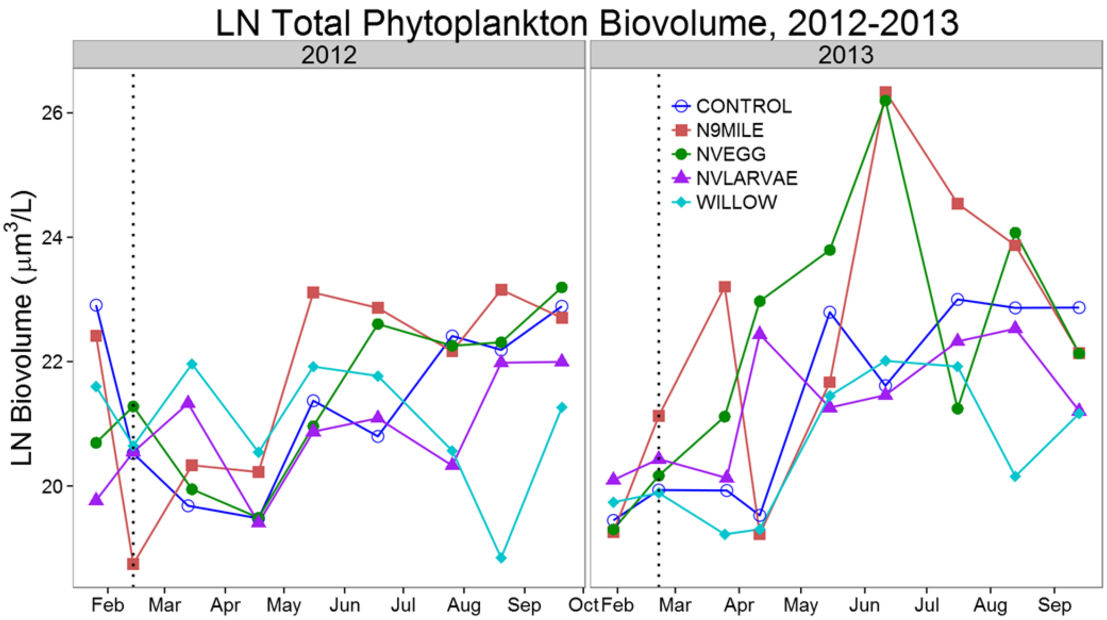


Figure 4.—Trends in LN phytoplankton biovolume (all divisions), 2012–13.

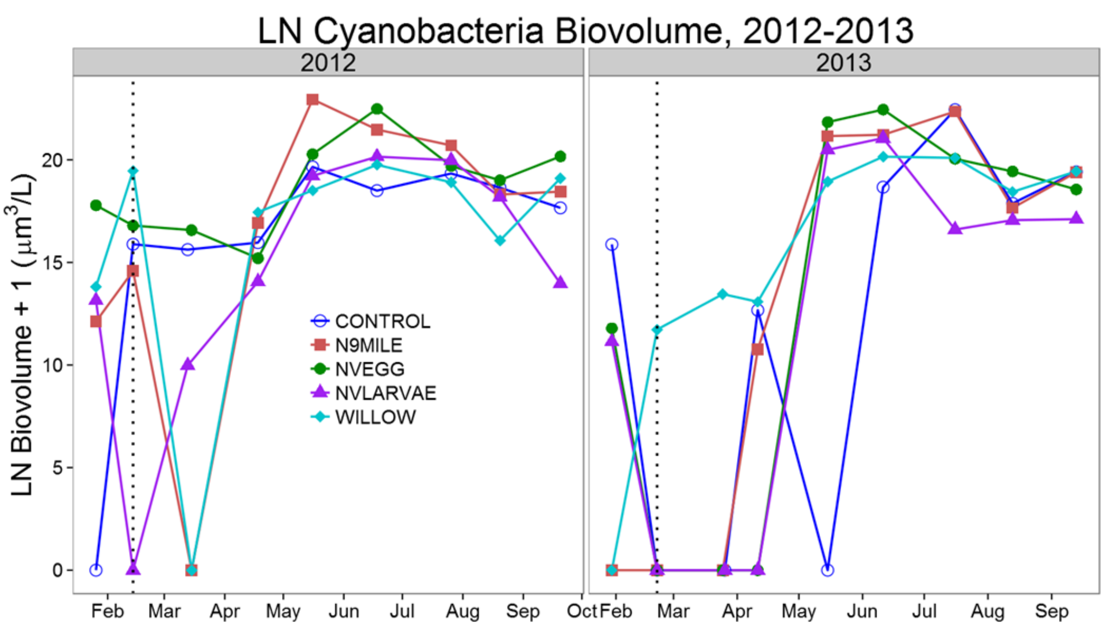


Figure 5.—Trends in LN Cyanobacteria biovolume, 2012–13.

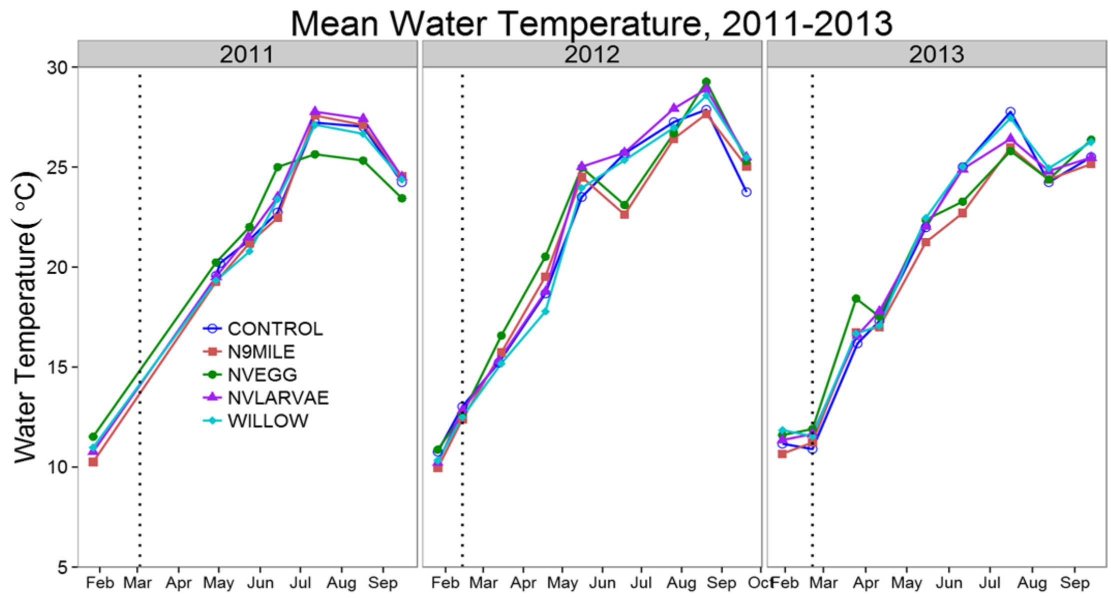


Figure 6.—Monthly water temperature at five Lake Mohave backwaters, 2011–13. Values are means over depth.

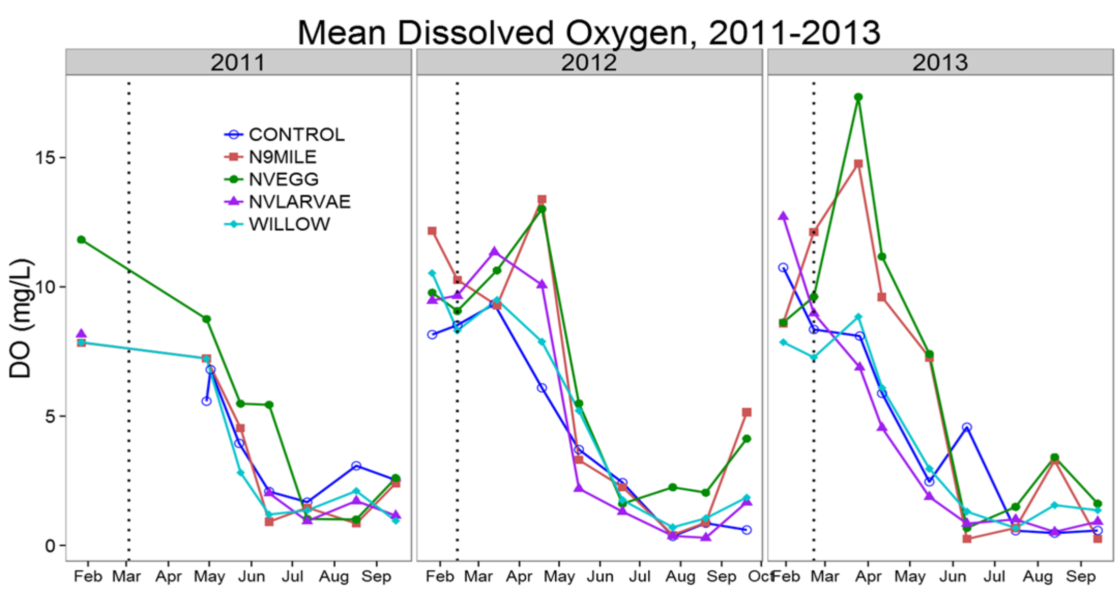


Figure 7.—Monthly DO at five Lake Mohave backwaters, 2011–13. Values are means over depth.

North Nine Mile and Nevada Egg, where it also remained highest during the summer months. In 2011, the control had the highest summer month DO concentrations.

DISCUSSION

Phytoplankton and zooplankton productions vary unpredictably among ponds and over time in the same pond (Boyd and Tucker 1998). The effects of fertilizers were considered on native fish resources in isolated ephemeral backwater ponds to evaluate primary productivity at different rates of fertilization. One fertilization application per year at each backwater pond and a single monthly sample over the duration of the 3-year study may have been inadequate for detecting an immediate, short-term plankton response. Additionally, the assumption that fertilizer treatments would enhance the quality of fish food was not supported by the data. Similar findings were reported by Mischke et al. (2003) of no significant difference in the nutritional value of zooplankton between fertilized and nonfertilized channel catfish (*Ictalurus punctatus*) nursery ponds.

Fertilizer treatments at two and three times the initial rate did not show tangible evidence of an increase in primary productivity with increasing concentrations of fertilizer. Conversely, increasing fertilizer treatments over time may result in unintended consequences. In a study of bass (*Morone* spp.) fingerling culture ponds, Ludwig (2002) found increased amounts of organic and inorganic fertilizer treatments over a 6-week period had increased desired zooplankton production but substantially decreased fingerling survival because the water quality had been adversely affected. There was no evidence of adverse effects on adult razorback suckers in Willow based on high variability in year-to-year harvest rates (2011: 44%, 2012: 94%, and 2013: 54%). Nevada Larvae yielded a diminished return on harvest rate (2011: 40%, 2012: 18%, and 2013: not stocked). However, it is unclear if an increase in nutrient load was responsible for this phenomenon or if consistently poor water quality over time has been the main contributing factor.

The peak reproductive period varies among different zooplankter groups (Geiger 1983b). Rotifer peak reproduction lasts only 3.5 days, owing to its short lifespan (Allan 1976). Copepods and cladocerans survive for about 50 days. However, peak reproductive periods differ. Copepods require 24 days to reach optimum reproductive success and cladocerans about 15 days (Allan 1976). Because of this variation, timing between when ponds fill to capacity and when fertilization treatments commence are important considerations for maximizing peak primary productivity.

Cladocerans constitute an important proportion of the razorback sucker diet (Marsh 1987). Overall adult cladoceran sizes vary considerably (0.47–2.17 mm; mean = 1.32 mm) (Culver et al. 1985). Mean annual surface water temperatures between 6 and 8 °C typically contain the largest individuals in middle latitudes (Pennak 2001). In Lake Mohave backwaters, water temperatures commonly reached annual means exceeding 20 °C (see figure 6). Large-bodied cladocerans like *Daphnia pulex* showed mean biomass peaks during winter and spring before declining in abundance as water temperatures increased (see figure 2). An exception occurred in 2013 at North Nine Mile and Nevada Egg due to an abundance of *Ceriodaphnia* spp. from these samples (see figure 2). It is not known why this increase occurred other than wide variation in distribution both spatially and temporally of the composition of plankton in the backwater environment. Overall, the observed decline in *Daphnia* is noteworthy because these large zooplankton species exhibit high nutritional value, and consistently maintaining high densities would provide increased forage for native fish species (Mischke et al. 2003).

High water temperatures associated with an extreme desert climate, smaller surface area, and shallow depth in the backwaters may result in smaller sizes and depauperate populations of cladocerans across all study ponds. Further, temperatures in excess of 20 °C have been associated with a reduction in some cladocerans, while others, such as *Diaphanosoma brachyurum* and exotic species like *Daphnia lumholtzi*, have shown increases (Work and Gophen 1999). However, this trend in the study ponds on Lake Mohave was not observed for these species. Mean species biomass of *D. brachyurum* was 5.83 micrograms per liter across all sampling sites for the 3-year study, with no observances of this organism after 2011. *D. lumholtzi* was not identified in any samples collected.

Conclusions and Future Recommendations

Natural food resource availability for native fishes in Lake Mohave backwaters during the grow-out phase has shown to be unpredictable. Despite concerted efforts to maximize primary productivity through experimental fertilization regimes, these techniques have yet to show positive results related to enhanced fish production.

A single monthly plankton sample and a single fertilizer application in each pond is likely inadequate for generating and/or detecting an immediate, short-term plankton response. If several samples can be taken both before and after fertilization, a before-after-control-impact analysis could be used to compare the average difference between control and treatment prior to fertilization to the average difference after fertilization.

Further investigations should include weekly samples for plankton and water quality. Similarly, spatial replicates within backwaters would provide more reliable abundance estimates for each sampling date and perhaps increase the ability of a repeated measures analysis to detect statistically significant differences among backwaters. Spatial replication could also allow for more accurate modeling of covariance/autocorrelation to avoid underestimates of standard error and inflated Type I (false-change) error rates. Unfortunately, sample schedules of this type may not be practical based on the amount of travel and labor necessary to reach multiple isolated backwaters on Lake Mohave. An alternative may be to design a fertilization study that focuses on a single backwater pond (with control) to alleviate labor and time constraints. However, limiting studies to smaller scales also runs the risk of inaccurate inferences to backwaters with different plankton composition and physical/chemical parameters.

Because of spatial variability in plankton communities, it is recommended that a more comprehensive strategy be implemented in future sampling technique. Instead of a single vertical tow, it is recommended that a longer horizontal tow over a larger area in each backwater be used to ensure a more representative sample.

Another consideration for increased survivability and fitness of fish in Lake Mohave backwaters is the exploration and implementation of an adaptive management model as it relates to stocking densities (Lorenzen 1995; Walters 1986, 2007). It is recommended that pond surface area dictate the total number of fish stocked each spring to maximize resource availability. Because razorback suckers are stocked during peak spawning season, physiological demands imposed on the fish become an added stressor in addition to overcrowding in limited-space backwater ponds and depleted DO levels.

An increased effort in monitoring the phytoplankton community, with an emphasis on DO content, is an essential component in maintaining the viability of these backwaters for annual success of the target species. Chlorophyll *a* is an indicator of the DO budget and, therefore, should be analyzed and compared with the phytoplankton standing crop (Boyd and Tucker 1998). Because some backwaters in this system experience high algal biomass, measurements of chlorophyll *a* would be a valuable determinant in the risk of seasonal fishkills due to depleted oxygen content.

Finally, proper use of mechanical aeration to maintain adequate DO levels during months of extreme temperature should be considered. Aeration has been implemented in various backwaters on Lake Mohave, but no investigations have been performed testing its efficiency in maintaining spatially optimal DO concentrations throughout the water column, especially during extreme summer climate in this region. Carefully scheduled aeration should be administered when DO levels drop below acceptable limits for optimal fish survival during summer months prior to harvest.

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