Rapid recovery of a fish assemblage following an ecosystem disruptive algal bloom

Richard M. Zamor[1,2,4], Nathan R. Franssen[1,5], Clayton Porter[3,6], Tim M. Patton[3,7], and K. David Hambright[1,2,8]

[1]Program in Ecology and Evolutionary Biology, Department of Biology, University of Oklahoma, Norman, Oklahoma 73019 USA
[2]Plankton Ecology and Limnology Laboratory, Department of Biology, University of Oklahoma, Norman, Oklahoma 73019 USA
[3]Department of Biological Sciences, Southeastern Oklahoma State University, Durant, Oklahoma 74701 USA

Abstract: Disturbance of freshwater ecosystems through cultural eutrophication has resulted in an increased global occurrence of harmful algal blooms (HABs). Ecosystem disrupting algal blooms (EDABs) are a subset of HABs that produce extensive disturbances across entire ecosystems. *Prymnesium parvum* is an EDAB species that has invaded freshwater systems worldwide, causing massive fish kills and other negative effects. Fish kills frequently occur during HABs and EDABs, but few studies exist of the long-term implications of these fish kills and the resilience and recovery of fish assemblages following kills. We sampled fish near- and offshore over an annual cycle encompassing a *P. parvum* EDAB in 2 coves (i.e., a bloom site and a reference site) of a southern Great Plains reservoir, Lake Texoma, Oklahoma–Texas (USA). Our objective was to document the extirpation and recovery of a fish assemblage in response to the disturbance of an EDAB event. *Prymnesium parvum* bloomed in 1 cove from mid-December 2008 until May 2009 and eliminated all fish during this period. Fish toxicity bioassays indicated no substantial differences in susceptibility among fish species to *P. parvum* toxins. Fish recolonized the bloom site rapidly in May 2009 after the bloom diminished. Fish assemblages were resilient to the *P. parvum* EDAB, and recovered to previous abundance, richness, and composition within 6 mo. Our results suggest that the reservoir-wide fish meta-assemblage enabled a rapid recovery of local fish assemblages after a spatially heterogeneous EDAB.

Key words: ecosystem disruptive algal bloom, EDAB, harmful algae, fish kill, *Prymnesium parvum*, Lake Texoma

Ecological communities can be influenced profoundly by ecosystem disturbances and environmental perturbations (Hutchinson 1953, Connell 1978, White and Pickett 1985). Natural and anthropogenic disturbances can shape community structure by changing resource availability and by creating opportunities that can be used by newly arriving species or invasive species within the disturbed community (White and Pickett 1985, Davis et al. 2000, Lockwood et al. 2007). Freshwater ecosystems have experienced extensive anthropogenic disturbances associated with increased nutrient loading (Smith 2003, Smith and Schindler 2009), which has caused an increased incidence of harmful algal blooms (HABs) worldwide (Hallegraeff 1993, Smith 2003, Smith and Schindler 2009). HABs are proliferations of algae that have deleterious effects on other organisms, often via production of toxins (Hallegraeff 1993, Landsberg 2002, Granéli and Turner 2006). The global frequency and severity of HABs has ignited interest in assessing their consequences for human health and local economies (Granéli and Turner 2006). Moreover, a subset of HABs have been described as ecosystem disrupting algal blooms (EDABs) because the species involved respond to disturbances, such as increased nutrients, and become a disturbance themselves by altering ecosystem structure and function (Sunda et al. 2006).

Beyond the negative effects of HABs in general, EDABs are characterized by their adverse direct effects on fishes and herbivorous invertebrate grazers and their indirect effects on nutrient and foodweb dynamics, which create feedbacks that can enable bloom persistence (Sunda et al. 2006). One EDAB species is *Prymnesium parvum* (Carter 1937), a toxigenic marine haptophyte that has invaded freshwater systems worldwide causing widespread fish kills (Edvardsen and Imai 2006, Lutz-Carrillo et al. 2010). Recent studies have revealed other substantial negative effects on herbivorous zooplankton, including reduced survivorship, growth rates, and fecundities (Michaloudi et al. 2009, Remmel et al. 2011), primarily via release of contact glycolipid toxins, which probably evolved to support heterotrophy (Hen-

E-mail addresses: [4]rmzamor@ou.edu; [5]Present address: Department of Biology, University of New Mexico, Albuquerque, New Mexico 87131 USA, nrfranssen@gmail.com; [6]ouporter9@yahoo.com; [7]tpatton@se.edu; [8]dhambright@ou.edu

DOI: 10.1086/675508. Received 08 February 2013; Accepted 14 October 2013; Published online 05 March 2014.
Freshwater Science. 2014. 33(2):390–401.

rikson et al. 2010, Remmel and Hambright 2012). *Prymnesium parvum* blooms have substantial impacts on abundances and diversity of other unicellular eukaryotes and bacteria (Michaloudi et al. 2009, Jones et al. 2013). In North America, the first record of a *P. parvum* EDAB is from a fish kill in 1985 in the Pecos River system of southern Texas, USA (James and De La Cruz 1989). In the subsequent 2 decades, *P. parvum* has gradually expanded its range and has caused fish kills in reservoirs and rivers throughout much of the southern USA from California to Florida and north to Wyoming and West Virginia (Hambright et al. 2010, Roelke et al. 2010, Zamor et al. 2012).

EDAB-related fish kills have been documented many times in the literature (reviewed by Landsberg 2002), but fish are rarely integrated into analyses of bloom dynamics and few data are available for the fish kills themselves (e.g., species affected and extent of mortality). Thus, little is known of the longer-term response, e.g., the resilience and recovery of fish assemblages to EDAB-related fish kills. Our goal was to document the response of a fish assemblage to an EDAB. We sampled fishes in 2 coves of a southern Great Plains reservoir during a *P. parvum* EDAB. One cove has experienced previous recurrent *P. parvum* blooms, whereas the other cove has experienced *P. parvum* presence but no blooms. Our study covered an annual cycle, including samples before, during, and after a bloom and provides insight into the extirpation and subsequent reassembly and recovery of a fish assemblage from the effects of the bloom.

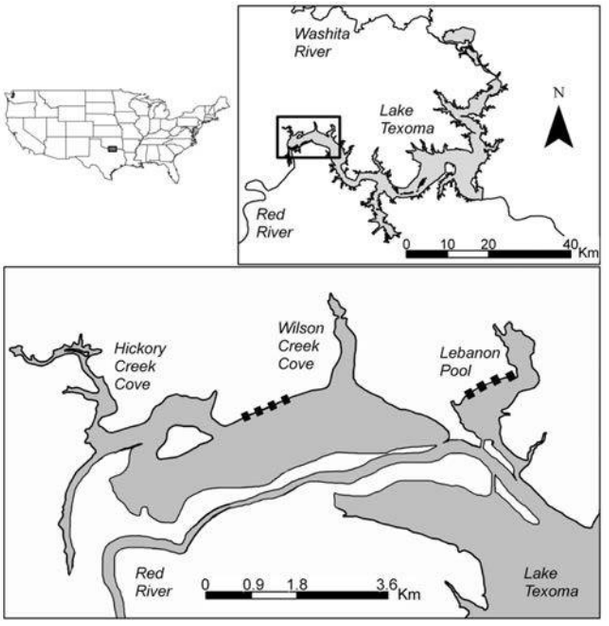

Figure 1. Map of Lebanon Pool and Wilson Creek Cove collection sites in the Red River arm of Lake Texoma on the Oklahoma–Texas border, USA. Black bars indicate reaches of beach sampled during near- and offshore collections at each site.

METHODS

Study site

Lake Texoma, constructed in 1944, was formed by the impoundment of the Red and Washita Rivers on the border of Oklahoma and Texas, USA (Fig. 1). Its drainage basin encompasses 87,500 km^2 and, at normal lake elevation, Lake Texoma is the 12th-largest reservoir in the USA. At least 50 species of fish inhabit Lake Texoma, including many recreationally important species (e.g., Striped Bass, *Morone saxatilis* (Walbaum); Matthews et al. 2004). *Prymnesium parvum* first bloomed in Lake Texoma in winter 2004 and caused a massive fish kill in many of the shallow areas of the Red River arm of the reservoir (Hambright et al. 2010). Including this 1st bloom, *P. parvum* has bloomed during 7 of the 9 winters through 2012. The strongest blooms occurred in Lebanon Pool (LP), an ~130-ha embayment formed by the confluence of Hauani Creek and Lake Texoma (Fig. 1). Extensive sedimentation by the Red River has blocked much of the mouth of LP, and connectivity to Lake Texoma is usually maintained through 1 or 2 narrow channels to the main reservoir body (Fig. 1) when water levels are at or above the normal conservation pool (188.1 m asl). Water levels in Lake Texoma are managed primarily for flood control, so the seasonal pool is drawn down during much of the winter in expectation of late spring–early summer rains (Fig. 2A). During these drawdown periods, LP and other similar coves can become isolated from the main reservoir body, and evaporation can lead to increased salinities. Fueled by the inherently high nutrient availability in Lake Texoma, *P. parvum* blooms can occur during these seasonal increases in salinity (Hambright et al. 2010). Blooms of *P. parvum* do not always produce fish kills (e.g., winter 2007–2008), and the environmental conditions that trigger increased *P. parvum* toxicity are unclear. The closest reservoir embayment upstream is ~1050-ha Wilson Creek Cove (WCC; Fig. 1), which also can lose its connection to the reservoir during seasonally low water levels (Fig. 1) and has water chemistry similar to that of LP. However, WCC has never experienced a toxic bloom or fish kill, although *P. parvum* is often present in WCC at low abundances during blooms (Hambright et al. 2010, Zamor et al. 2012; Fig. 2B). Therefore, we focused on fish in LP as an affected assemblage and fish in WCC as an unaffected assemblage. Our comparison is naturally limited because *P. parvum* blooms in only 1 cove in Lake Texoma. Nevertheless, the literature is replete with unreplicated natural and large-scale experiments that have proven useful to our understanding of how populations respond to variation in important environmental factors, including those that lead to local extinction (Likens 1985, Carpenter 1990). Thus, we think our study will be useful for understanding how fish populations respond to EDABs, and how fish pop-

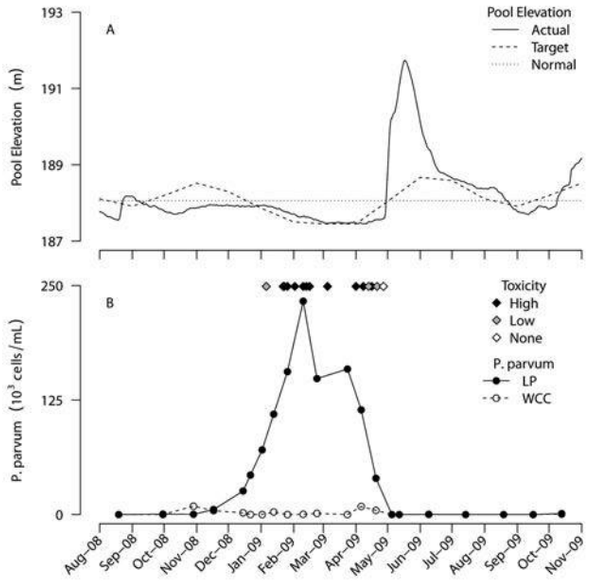

Figure 2. A.—Mean daily lake elevation during the study period. Lake elevation is managed for flood control and recreation. A dashed line indicates the target elevation. Normal conservation pool is indicated by a dotted line at 188.1 m asl. Below this is the level at which both Lebanon Pool (LP) and Wilson Creek Cove (WCC) become separated from Lake Texoma. B.—*Prymnesium parvum* cell densities in LP and WCC during the study period (08 = 2008, 09 = 2009). Results from toxicity bioassays are depicted above the line graphs on their test dates. High toxicity indicates that test fish died in <4 h, low toxicity indicates fish died in >4 h, and no toxicity indicates that fish did not die in bioassays.

ulations may respond to any number of factors that might lead to local extinctions.

Fish sampling

We assessed the temporal effects of the *P. parvum* EDAB on fish by sampling near- and offshore fish assemblages in LP and WCC, before, during, and after a toxic bloom in LP. We sampled nearshore fish monthly from October 2008–March 2009 and during June, July, and October 2009. At each sampling event, we collected nearshore fish by conducting four 25-m seine hauls (1.83 × 4.57-m seine, mesh = 3.18 mm) along four 100-m reaches of shoreline that were separated by ≥100 m (Fig. 1). We surveyed the same four 100-m reaches monthly. Evidence from repeated monthly sampling in other locales in Lake Texoma suggests that no bias or depletion arises from these methods (Mathews et al. 2004). We pooled fish from the seine hauls within each 100-m survey and immediately euthanized and preserved them in 10% formalin before returning to the laboratory for identification and ultimate accession into the Sam Noble Oklahoma Museum of Natural History. We completed 16 seine hauls per sampling trip at each site regardless of the number of individuals collected (e.g., no fish during the *P. parvum* bloom at LP). In the laboratory, we identified and measured individual fish (total length [TL]) for up to 100 arbitrarily selected individuals of each species from each reach. We counted any remaining individuals for species numbering >100 and then stored all fish in 50% isopropyl alcohol.

We sampled offshore fish monthly from November 2008–October 2009 with gill nets. At each cove in each month, we set 2 gill nets for a single night. Nets were 61 × 1.8 m and were composed of eight 7.6-m panels with mesh ranging from 1.27 to 10.16 cm. Mesh size changed at each panel in 1.27-cm increments (i.e., panel 1 = 1.27-cm mesh, panel 2 = 2.54-cm mesh, etc.). We set a shallow net horizontally with the float line just below the surface and a deep net horizontally with the lead line along the bottom in the evenings between 1600 and 1900 h and retrieved nets the next day between 1000 and 1400 h. We removed fish from nets and weighed and measured them (TL). Previous studies using repeated monthly sampling in other locales in Lake Texoma suggest that no bias or depletion arises from these methods (Gido et al. 2000).

Algae monitoring

We assessed densities of *P. parvum* at least monthly in WCC and LP with microscope- and quantitative polymerase chain reaction (qPCR)-based counts (Zamor et al. 2012) as part of a larger ongoing *P. parvum* monitoring project on Lake Texoma (Hambright et al. 2010). We report qPCR results because they offer better resolution of cell numbers with lower error rates (Zamor et al. 2012). We defined blooms as periods when *P. parvum* densities were >10,000 cells/mL because densities above this level frequently result in fish kills (Roelke et al. 2010).

Fish toxicity bioassays

No method exists for quantifying directly the amount of *P. parvum* toxin that could be affecting fish in a water body, in part, because several *P. parvum* toxins have been identified and which of these toxins is directly causing toxicity is hotly debated (e.g., Igarashi et al. 1998, Henrikson et al. 2010, Bertin et al. 2012a, b). Furthermore, which toxic metabolites are detected differ between culture and field samples (Henrikson et al. 2010). The toxins are released either in direct contact with fish or during cell lysis (Remmel and Hambright 2012) and can break down quickly under natural conditions, such as exposure to sunlight (Parnas et al. 1962, James et al. 2010). Thus, assessing the amount of toxin available to kill fish at any given time is complex, and the best available method for quantifying toxicity of water containing *P. parvum* is a bioassay, e.g., with larval Fathead Minnows (*Pimephales*

promelas (Rafinesque); USEPA 2002). We conducted bioassays at both sites to assess the toxicity of the water to fish throughout the bloom period (for detailed methods see Remmel and Hambright 2012). These tests consisted of 3 treatments: 100% lake water, 50% lake water:50% tap water, and 100% aged tap water.

We tested the possibility of species-specific differences in susceptibility to *P. parvum* of Lake Texoma fish by conducting toxicity bioassays with young-of-year (YOY) of 4 Lake-Texoma fishes (Striped Bass, *Morone saxatilis* (Walbaum); Inland Silverside, *Menidia beryllina* (Cope); Gizzard Shad, *Dorosoma cepedianum* (Lesueur) and, for reference, juvenile and adult Fathead Minnows, *P. promelas*). Little evidence that is not anecdotal is available (see Rhodes and Hubbs 1992) regarding differential susceptibility of fishes to *P. parvum* toxins, so we chose these species for their wide phylogenetic and ecological ranges and because they are among the most common fishes in the lake (Matthews et al. 2004). We measured time to death of lake fish relative to time to death of 10- to 14-d-old Fathead Minnows instead of using standard acute toxicity bioassays (USEPA 2002) so that we could make useful inferences regarding *P. parvum* toxicity to Lake Texoma fish without the high level of sacrifice required in standard median lethal concentration (LC_{50}) bioassays. We exposed all fish in each bioassay to concentrations of laboratory-cultured *P. parvum* (for culturing methods see Zamor et al. 2012) that exceeded a previously measured LC_{50} concentration for 10- to 14-d-old fathead minnows by 50 to 150% (KDH, unpublished data) for 24 h. In each assay, we paired 6 test fish with 6 fathead minnow larvae, exposed 3 of each species to *P. parvum*, and used the rest as controls (except for 2 of 8 Gizzard Shad assays in which only 2 and 4 test fish were used because of availability). We examined toxicity in 1st-y Striped Bass (number of experiments [N] = 8, number of fish exposed to *P. parvum* [n] = 24, 2.84 ± 1.25 g; mean wet mass ± SD), Gizzard Shad (N = 8, n = 21, 3.02 ± 2.56 g), Inland Silversides (N = 9, n = 27, 0.85 ± 0.55 g), and similarly sized adult Fathead Minnows (N = 7, n = 21, 2.74 ± 1.09 g). We collected all lake fish from the lake by shoreline seining during early and mid-spring and allowed them to acclimate to laboratory conditions for 48 h before using them in experiments. Adult fathead minnows were laboratory-reared individuals <1 y old. We conducted bioassays in 3.8-L (larger lake fish) or 100-mL (10- to 14-d-old fathead minnows) aerated jars containing 3 individuals per jar, with separate jars used for each species. We added *P. parvum* grown in 15‰-salinity culture medium to half of the jars at final concentrations of 200,000 to 400,000 cells/mL and the same volume of 15‰ culture medium without *P. parvum* to the rest. The volume of culture used varied with cell density, and bioassay salinities ranged between 1 and 3‰. We recorded time to death after addition of *P. parvum* for each fish in each experiment. We excluded experiments without mortality of all fish exposed to *P. parvum* at the end of 24 h from data analysis to eliminate possible confounding by low-toxicity *P. parvum* cultures. We did Shapiro–Wilk analyses of normality on time to death relative to fathead minnow larvae for each species with the *shapiro.test* function in R (version 2.15.3; R Project for Statistical Computing, Vienna, Austria). Data for Gizzard Shad were not normally distributed, so we used a Kruskal–Wallis rank–sum test (*kruskal.test*) in R to compare relative species susceptibility to *P. parvum*.

Data analysis

We assessed the effects of the *P. parvum* EDAB on the temporal dynamics of fish assemblages by comparing total fish abundances, species richness, and assemblage structure in both coves before, during, and after the bloom. We analyzed the seine and gill-net data separately because of differences in sampling times and methods.

Nearshore fish: abundance and species richness We assessed variation in nearshore fish abundance ($\log_{10}$[total fish abundance] in each reach; n = 4) between coves during the sampling period with repeated-measures analysis of variance (rmANOVA). We assessed effect sizes of independent variables (time and cove) via partial η^2. We also assessed species richness over time in each cove with rmANOVA. The number of individuals collected varied among reaches, so we estimated species richness in each reach with individual-based rarefaction (Hurlbert 1971) using the *rarefy* function in R. We rarified species richness in each reach to 45 individuals (i.e., the smallest number of individuals collected among all reaches). We ran both rmANOVAs in PASW (version 18; SPSS, Chicago, Illinois), and assumptions of sphericity were met in both analyses. No fish were caught in LP during the bloom period (see Results below), so we excluded these 3 mo from analyses.

Offshore fish: abundance and species richness We set 2 gill nets in each cove during a given month, and tabulated the fish removed in a single count. Therefore, we could not assess within-month variation in total fish abundance or species richness. Accordingly, we investigated trends in total fish abundance and species richness qualitatively. However, we did rarefy species richness in each month using 8 individuals (i.e., the smallest number of individuals collected in a cove in 1 mo) to control for differences in the abundance of individuals captured between coves and amongst months.

Fish assemblage structure We used nonmetric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarities to assess fish assemblage structure for each 100-m reach in each cove before (October–December 2008) and

after the bloom (June, July, and October 2009). We $\log_{10}(x + 1)$-transformed the data to reduce the effects of superabundant species (e.g., *Menidia beryllina*). We used only species that occurred in >10% of collections in the analysis to avoid skewing the analysis with rare species. We removed the data for the 3 mo during the bloom (January–March 2009) because no fish were caught in LP (see Results). After the NMDS ordinations, we used a multiresponse permutation procedure (MRPP) with Euclidean distances and 10,000 permutations to assess whether groups (before and after bloom, within and between coves) exhibited significant clustering in 2-dimensional NMDS space. Significant clustering was assessed via comparisons of the expected and observed MRPP statistic δ (the overall weighted mean of within-group means of the pairwise dissimilarities among sampling units). Significance of δ is assessed similarly to a *p*-value. Cut-off values for significance of δ were adjusted with a Bonferroni correction for multiple comparisons ($\alpha = 0.0125$). If MRPP indicated significant clustering of groups, we ran indicator species analysis (ISA) with 10,000 iterations. We considered species with significant indicator values >0.50 to be drivers of group separation. We used the *vegan* package in R for NMDS and MRPP (Oksanen et al. 2012) and the package *labdsv* for ISA (Roberts 2012).

RESULTS

Lake water levels decreased to below the conservation pool at 188.1 m asl during late August 2008 and remained below the conservation pool until May 2009 (Fig. 2A). During this time, salinities and *P. parvum* densities increased (Fig. 2B). *Prymnesium parvum* cell densities >10,000 cells/mL were first detected on 15 December 2008 in LP (25,000 cells/mL), peaked on 10 February 2009 (233,000 cells/mL) and remained >10,000 cells/mL until 1 May 2009. Bioassays done with 10- to 14-d-old Fathead Minnows (either lakeside or in the laboratory with water returned from LP) revealed extremely high toxicities from 6 January–21 April 2009. No toxicity was observed on or after 27 April. Cell densities did not reach 10,000 cells/mL in WCC nor was toxicity detected. The decline in cell densities and toxicity in late April–May 2009 coincided with increases in the lake elevation after spring rains.

Abundance and species richness

Nearshore fish Most individuals (33,385 of 35,660 fish; Table S1) were collected in our nearshore samples. Twenty species were collected in LP, and 21 were collected in WCC. Fish were collected in both coves in October, November, and December 2008. From January–April 2009, fish were caught in WCC but not in LP (Fig. 3A). Fish reappeared in the catch in LP in June 2009.

Time had the strongest significant effect on total fish abundance (effect size = ~0.80), followed by cove (~0.52), and their interaction (~0.32; Table 1). Fish abundance was higher in LP than WCC before the bloom, but abundances in both coves declined to equivalent levels in December. After the EDAB, abundances increased in both coves and reached levels higher than levels observed before the bloom by July. Fish abundance was slightly higher in WCC than in LP in June, but then returned to a pattern similar to that seen before the bloom. Fish were more abundant in LP than in WCC on the last 2 sampling dates (Fig. 3A).

Variation in rarefied species richness depended on cove (effect size = ~0.96), followed by time (~0.88), and their interaction (~0.79) (Table 1). As with abundance, species richness was higher in LP than in WCC prior to December. However, in December, richness was slightly higher in WCC. Following the EDAB, species richness returned to similar levels as observed before the bloom in LP, but increased substantially in WCC in June and July before returning to prebloom levels in October (Fig. 3C).

Offshore fish A total of 2275 fish were collected in our offshore samples. Sixteen species were caught in LP, and 18 species were caught in WCC (Table S1). Fish were collected in both coves in October, November, and December 2008, but from January–April 2009, fish were caught only in WCC (Fig. 3B). Both total fish abundance and species richness rebounded rapidly in LP starting in May 2009. Offshore fishes qualitatively demonstrated dynamics similar to nearshore fishes for total fish abundance over the sampling period (Fig. 3B). No noticeable quantitative differences in rarefied species richness between the 2 coves were evident from June–October 2009 (Fig. 3D).

Assemblage structure: nearshore fish

A stable NMDS ordination was obtained for the nearshore fish assemblage (stress = 15.5%; Fig. 4A–D). Between-site comparisons indicated that the nearshore fish assemblage differed between LP and WCC before the bloom (MRPP, significance of δ = 0.0002; Bonferroni adjusted $\alpha = 0.0125$; Fig. 4A, Table 2), but not after the bloom (MRPP, significance of δ = 0.0797; Fig. 4B, Table 2). Mosquitofish (*Gambusia affinis* (Baird and Girard)) were indicative of the before-bloom assemblage in WCC, whereas Inland Silverside, Threadfin Shad (*Dorosoma petenense* (Gunther)), and Red Shiner (*Cyprinella lutrensis* (Baird and Girard)) were indicators of the before-bloom assemblage in LP (Table 3).The nearshore fish assemblage within sites differed before and after the bloom in WCC (MRPP, significance of δ = 0.0001; Fig. 4D, Table 2), but not in LP (MRPP, significance of δ = 0.0451; Fig. 4C, Table 2). Mosquitofish

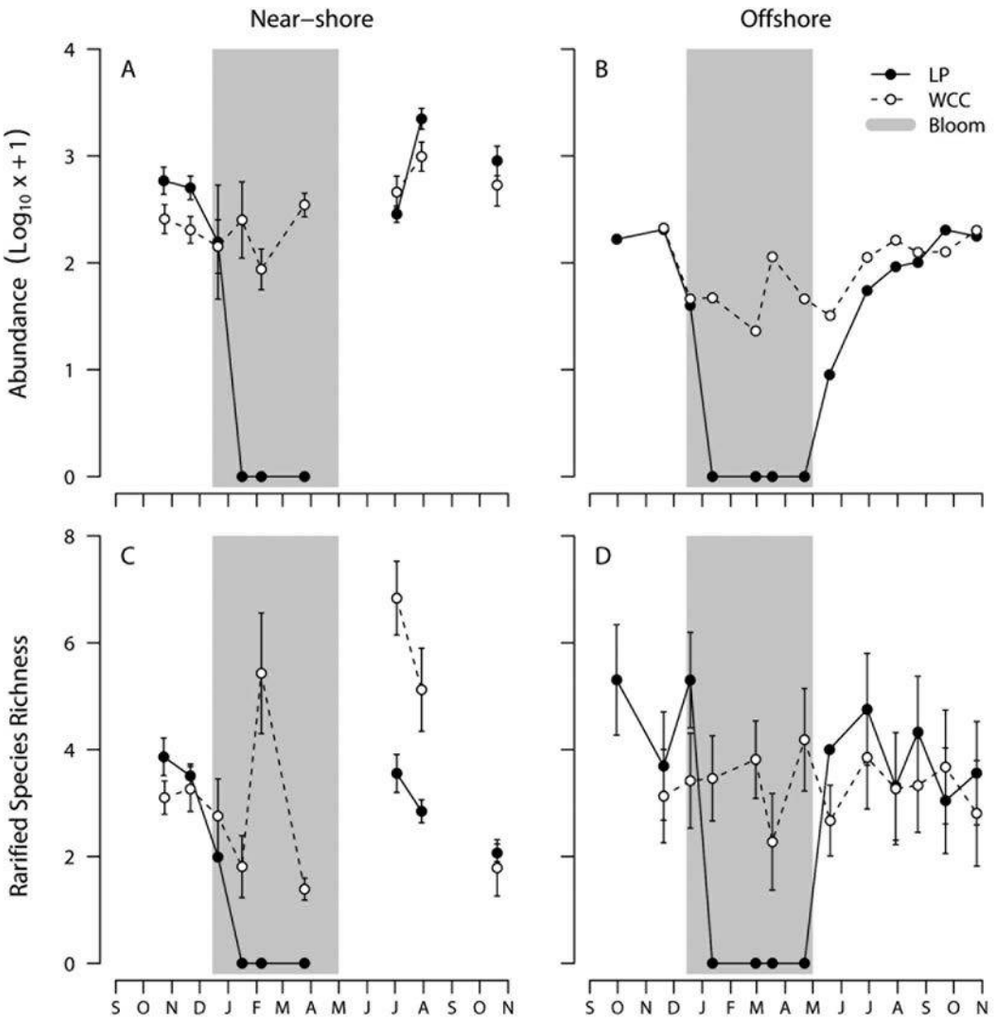

Figure 3. Fish abundance (A, B) and rarefied species richness (C, D) in nearshore (A, C) and offshore samples (B, D). Abundances are $\log_{10}(x + 1)$ transformed for ease of comparison.

Table 1. Results from 2 repeated-measures analyses of variance (rmANOVAs) with fish abundance ($\log_{10}[x + 1]$-transformed) and rarified species richness as dependent variables. df = degrees of freedom (hypothesis, error). Effect sizes of independent variables (time and cove) were assessed via partial η^2.

Dependent	Source	df	p	Effect size
Abundance	Time	5,30	<0.001	0.795
	Cove	1,6	0.042	0.524
	Time × cove	5,30	0.032	0.321
Richness	Time	5,30	<0.001	0.884
	Cove	1,6	<0.001	0.961
	Time × cove	5,30	<0.001	0.785

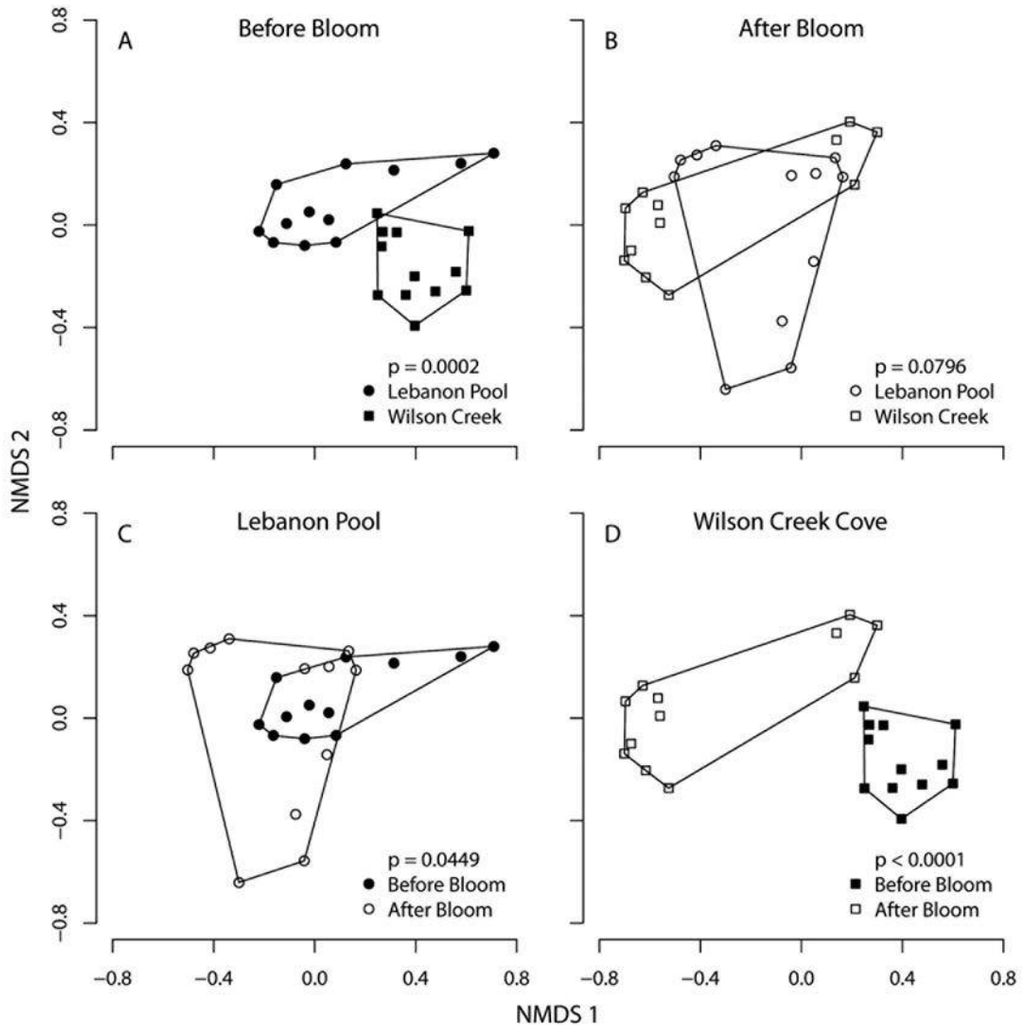

Figure 4. Results of nonmetric multidimensional scaling (NMDS) analysis of the before-bloom (A) and after-bloom (B) nearshore fish assemblages between sites and before-bloom (C) and after-bloom (D) assemblages within sites.

Table 2. Results from multiple response permutation procedure (MRPP) comparing before-bloom and after-bloom nearshore fish assemblages within sites and before-bloom and after-bloom assemblages between sites. Significance of δ is equivalent to a *p*-value. Significance of δ was adjusted using a Bonferroni correction for multiple comparisons (α = 0.0125). LP = Lebanon Pool, WCC = Wilson Creek Cove.

Comparison	Expected δ	Observed δ	Significance of δ (α = 0.0125)
Within sites			
Before-bloom LP vs after-bloom LP	0.4825	0.4608	0.0451
Before-bloom WCC vs after-bloom WCC	0.6454	0.3998	<0.0001
Between sites			
Before-bloom LP vs before-bloom WCC	0.4154	0.3183	0.0002
After-bloom LP vs after-bloom WCC	0.5680	0.5422	0.0797

Table 3. Results from indicator species analysis. The indicator value (IV) and the associated *p*-value indicate species that contributed to discriminating between before-bloom and after-bloom assemblages within or between sites. WCC = Wilson Creek Cove, LP = Lebanon Pool.

Comparison	Species	IV	*p*
Within sites – WCC			
Before bloom	*Gambusia affinis*	0.8174	0.0001
After bloom	*Dorosoma petenense*	0.8597	0.0003
After bloom	*Dorosoma cepedianum*	0.7218	0.0009
After bloom	*Ictiobus bubalus*	0.6667	0.0010
After bloom	*Morone saxatilis*	0.6667	0.0012
After bloom	*Notropis atherinoides*	0.6554	0.0232
After bloom	*Pomoxis annularis*	0.6365	0.0028
After bloom	*Morone chrysops*	0.5833	0.0043
After bloom	*Menidia beryllina*	0.5496	0.0010
After bloom	*Cyprinus carpio*	0.5000	0.0143
Between sites – before bloom			
LP	*Menidia beryllina*	0.7310	0.0008
LP	*Cyprinella lutrensis*	0.7209	0.0233
LP	*Dorosoma petenense*	0.5556	0.0218
WCC	*Gambusia affinis*	0.9367	0.0001

were indicative of the before-bloom assemblage in WCC, but a new suite of species, primarily consisting of species that can obtain larger body sizes, were indicative of the after-bloom assemblage. These species included Threadfin Shad and Gizzard Shad, Smallmouth Buffalo (*Ictiobus bubalus* (Rafinesque)), Striped Bass and White Bass (*Morone chrysops* (Rafinesque)), White Crappie (*Pomoxis annularis* (Rafinesque)), and carp (*Cyprinus carpio* (Linnaeus)) (Table 3).

Fish toxicity bioassays

Relative susceptibilities to *P. parvum* of the 4 species tested were similar (Kruskal–Wallis, $\chi^2 = 3.317$, df = 3, $p = 0.345$), and death occurred in ~5 to 7× the amount of time required for death of 10- to 14-d-old fathead minnows (Fig. 5).

DISCUSSION

HABs are receiving increased societal attention primarily because of their negative anthropocentric impacts, which range from aesthetic concerns including beach fouling and discolored or distasteful water to severe consequences including damage of fisheries or recreational resources, or even human fatality. These negative effects are known primarily for marine and coastal systems, and freshwater HABs and their impacts have received far less attention. Research on freshwater HABs has focused primarily on cyanobacteria and their aesthetic effects. However, since the mid-1980s, the EDAB-forming species, *P. parvum*, has been a source of concern for scientists and resource managers of freshwater bodies of the southern USA, where it is now known as a notorious fish killer.

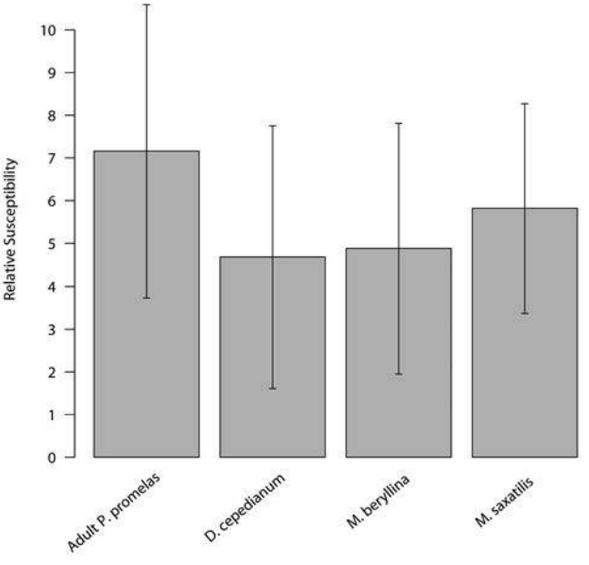

Figure 5. Mean (±1 SE) susceptibility of common Lake Texoma fishes to *Prymnesium parvum* as time to death relative to time to death of fathead minnow larvae. Mean time to death for all Fathead Minnow larvae ($n = 96$) across experiments ($N = 32$) was 100.4 ± 80.9 min.

For nearly 3 decades, blooms of *P. parvum* and their impacts have been discussed in terms of fish loss (including numbers, biomass, and dollars; Southard et al. 2010). Few investigators have quantified the recovery of fish assemblages after the disturbance caused by the invasion, establishment, and proliferation of *P. parvum* populations. Like numerous other inland systems worldwide that have experienced *P. parvum* blooms, Lake Texoma experienced a nearly lake-wide fish kill in 2004, and annually since then, local fish kills in isolated coves and backwaters (Edvardsen and Paasche 1998, Hambright et al. 2010). Since the initial 2004 fish kill, most kill events have been limited to the northern and western shores of Lake Texoma on the Red River arm of the reservoir (Hambright et al. 2010). The winter of 2008–2009 was no different. *Prymnesium parvum* bloomed in LP and reached densities up to 232,000 cells/mL by 10 February 2009. Intensive seine and gill-net sampling combined with results from toxicity bioassays confirmed the fish kill. The 2008–2009 bloom severely reduced abundances and species richness of both near- and offshore fish assemblages at LP, and water samples taken during the bloom from this site were always toxic to fish in bioassays, whereas the fish assemblages at WCC showed typical seasonal variation in abundance and richness throughout the sampling period, and water samples taken from this site were never toxic to fish.

No fish were collected in LP during the bloom, and water from LP was toxic to fish, but few dead fish were observed onshore during this period. Large flocks of White Pelicans (*Pelecanus erythrorhynchos* (Gmelin)) and Turkey Vultures (*Cathartes aura* (Linnaeus); RMZ, NRF, CP, J. D. Easton [University of Oklahoma], personal observation) were present in and around LP during the bloom and, presumably, were feeding on dead or dying fish. Similarly high densities of scavenging birds have been witnessed during other bloom years at LP (J. D. Easton, personal communication). To date, no dead birds have been associated with *P. parvum* blooms in Lake Texoma, although a massive bird kill, including pelicans (*Pelecanus crispus* (Bruch)) and other water birds, was reported during a *P. parvum* bloom and fish kill in Lake Koronia, Greece (Moustaka-Gouni et al. 2004, Michaloudi et al. 2009). Another potential, though unlikely, explanation for the lack of dead fish is a mass emigration from LP before the bloom period. Two shallow outlets on the southern end of LP were barely connected to the reservoir (≤10 cm depth) from August 2008–March 2009 because of unseasonably low autumn and winter precipitation (Fig. 2A). Any major fish emigration would have had to occur prior to that time, but our samples in December 2008 showed that fish were still present, albeit at lower abundances than in November 2008, before any *P. parvum* were detected. A 3rd potential cause of fish mortality could be that fish were killed because of deterioration of water quality once the coves were disconnected. However, water quality differed little between LP and WCC.

Recovery of fish assemblages following EDABs appears to depend on spatial heterogeneity of EDAB effects coupled with connectivity to source populations. The *P. parvum* EDAB resulted in an apparently complete kill in LP, but fish assemblages quickly recovered once the bloom subsided. The reappearance of fish in LP coincided with increased spring rains, water level, and the reconnection of LP and WCC to the main reservoir body. Connectivity and flooding can be instrumental in maintaining fish assemblages because fish can colonize isolated or disturbed habitats when high water removes barriers to movement (Franssen et al. 2006). In general, fish in connected freshwater systems (rivers and reservoirs) have high resilience and rapid recovery to both natural and anthropogenic disturbances (Olmstead and Cloutman 1974, Matthews 1986, Peterson and Bayley 1993, Matthews and Marsh-Matthews 2003). In systems with extremely high heterogeneity in environmental conditions and fish assemblages, e.g., in the Pecos River, Texas, recovery can be delayed substantially (up to 18 mo) because nearby habitats and their resident assemblages, which serve as sources of initial immigrants, can be quite different from the habitat in which the fish kill occurred (Rhodes and Hubbs 1992). However, in Lake Texoma, spatial variation in fish assemblages is low and variation in EDABs across coves is substantial. Thus, many refuge populations can serve as sources of new immigrants following an EDAB. Studies of the extirpation and recolonization of Red Shiners in Lake Texoma provide evidence that reservoir-wide fish metapopulation dynamics are important to maintaining community stability in large, fragmented, spatially complex river–reservoir systems (Matthews and Marsh-Matthews 2007, Marsh-Matthews et al. 2011). Thus, at the community level a reservoir-wide, homogeneous fish metacommunity (sensu Leibold 2004) enabled rapid recovery of local fish assemblages after a spatially heterogeneous EDAB. We suspect that the main channel and other nearby tributaries and coves provided refugia that prevented complete extirpation of the fish metacommunity and a source of emigrants that re-established the fish assemblage after the *P. parvum* bloom once connectivity was reestablished.

Prymnesium parvum EDABs affected the fish assemblages in LP in previous winters (2003–2004 through 2007–2008), and it is unclear whether the recovered fish assemblages had the same composition as the assemblage that existed before the 1st *P. parvum* bloom in winter 2003–2004. Potentially, the LP fish assemblage could always be composed of new immigrants and their offspring following blooms. Both near- and offshore fish assemblages in Lake Texoma are relatively stable across years, probably because of their depauperate species richness and the dy-

namic environment created by widely fluctuating water levels (Gelwick and Matthews 1990, Gido et al. 2000, Matthews et al. 2004, Eggleton et al. 2005). However, the fish assemblages in LP and WCC differed from each other before the bloom, and the assemblage composition in WCC differed before and after the EDAB. We suspect the differences are related to previous blooms and a general reset after floods. In 2007, the lake experienced a 100-y flood, with lake levels that averaged 194.5 m asl (conservation pool = 188.1 m asl) in July, that probably reset all assemblages (Gelwick and Matthews 1990, Gido et al. 2000, Matthews et al. 2004, Marsh-Matthews et al. 2011). In spring 2009, water levels rose rapidly to an average of 190.1 m asl in May (Fig. 2A). This rise completely flooded all backwaters and coves and probably reset the assemblages as in 2007. Length–frequency distributions for 2 nearshore fish species common in both coves before and after the bloom (Inland Silverside and Emerald Shiner [*Notropis atherinoides* (Rafinesque)]), suggest that YOY individuals colonized both coves similarly after the bloom and grew during the following months (e.g., Figs S1, S2). In winter 2007–2008, a major *P. parvum* bloom developed in LP with smaller blooms downstream, but not in WCC. Unlike in 2007 and 2009, water level in spring 2008 barely topped the conservation-pool level, and averaged 188.9 m asl in April. Thus, before the 2008 bloom, assemblages in LP and WCC differed because of local effects and cove-specific history (e.g., prior fish kill in LP) known to cause the structure of littoral assemblages in Lake Texoma to differ (Gido et al. 2002).

Our results are consistent with those from previous work in Lake Texoma and from other studies examining fish responses to disturbance. However, our study is unique because, to our knowledge, it is the only study in which recovery of fish assemblages from an EDAB was documented in a reservoir. Our results suggest a possible mechanism for maintenance of assemblage stability in large, complex reservoirs in terms of patch-dynamics and mass-effects following a small-scale stochastic extinction. An influx of individuals from the greater reservoir metacommunity (Leibold 2004) during periods of high connectivity associated with high water levels overwhelmed any local effects that caused differences in cove fish assemblages before the bloom. Hence, our results suggest that the general fish assemblage structure is rather resilient to localized *P. parvum* EDABs when connected to viable source populations. However, situations in which an entire lake or reservoir is affected by a bloom merit further study because a lack of nearby source populations could translate into longer-term, and potentially dire, consequences to fish assemblages. Little is known about the chronic effects of repeated EDABs on fish assemblages, both in terms of repeated exposure to toxins and the effect of repeated exhaustive fish kills. In extremely disturbed ecosystems that experience chronic blooms, we might expect to find lower species diversity and an assemblage made up of opportunistic (colonizing) individuals (Connell 1978). Indeed, Lake Texoma, like many other aquatic ecosystems of the Southern Great Plains, is well known for its relatively high levels of disturbance (Matthews 1988, Dodds et al. 2004) and relatively low-diversity assemblages (but not necessarily low richness; Gido et al. 2000, Eggleton et al. 2005). Investigations into chronic effects of EDABs on fish assemblages would be particularly useful in systems where the fauna is not pre-adapted to disturbance. Last, because the rapid loss and then recovery of fishes in response to EDABs could produce significant feedbacks in both grazer and nutrient dynamics that are characteristic of EDABs, we suggest that assessment of both fish and connectivity beyond the cursory mention of their occurrence should be included when studying EDABs and their implications in affected ecosystems.

ACKNOWLEDGEMENTS

We thank Emily Remmel of the Plankton Ecology and Limnology (PEL) Laboratory and the Fisheries Techniques class at Southeastern Oklahoma State University for help in fish collection and identification, W. J. Matthews for laboratory space and supplies, and fellow members of the PEL Lab and the Zoology and Ecology and Evolutionary Biology journal club for helpful discussions and comments on earlier versions of this manuscript. The study also benefited from comments by L. J. Weider, M. Kaspari, B. Stephenson, and J. Hawthorne. This study was conducted in partial fulfillment of the requirements for a PhD degree for RMZ from the University of Oklahoma. Funding was provided by a grant from the Oklahoma Department of Wildlife Conservation through the Sport Fish Restoration Program (grant F-61-R) to KDH and the Oklahoma Alliance for Minority Participation (through National Science Foundation grant HRD 090-2027) to TMP. RMZ was supported through the ODWC grant and a University of Oklahoma Biological Station Graduate Research Fellowship. Fish collections were conducted under University of Oklahoma Institutional Animal Care and Use Committee (OU-IACUC) protocol R08–004, and all toxicity bioassays were conducted under OU-IACUC protocol R08–021.

LITERATURE CITED

Bertin, M. J., P. V. Zimba, K. R. Beauchesne, K. M. Huncik, and P. D. R. Moeller. 2012a. Identification of toxic fatty acid amides from the harmful alga *Prymnesium parvum* Carter. Harmful Algae 20:111–116.

Bertin, M. J., P. V. Zimba, K. R. Beauchesne, K. M. Huncik, and P. D. R. Moeller. 2012b. The contribution of fatty acid amides to *Prymnesium parvum* Carter toxicity. Harmful Algae 20: 117–125.

Carpenter, S. R. 1990. Large-scale perturbations: opportunities for innovation. Ecology 71:2038–2043.

Carter, N. 1937. New or interesting algae from brackish water. Archiv für Protistenkunde 90:1–68.

Connell, J. H. 1978. Diversity in tropical rain forests and coral reefs. Science 199:1302–1310.

Davis, M. D., P. Grime, and K. Thompson. 2000. Fluctuating resources in plant communities: a general theory of invasibility. Journal of Ecology 88:528–534.

Dodds, W., K. Gido, M. R. Whiles, K. M. Fritz, and W. J. Matthews. 2004. Life on the edge: the ecology of Great Plains prairie streams. BioScience 54:205–216.

Edvardsen, B., and I. Imai. 2006. The ecology of harmful flagellates within Prymnesiophyceae and Raphidophyceae. Pages 67–79 *in* E. Granéli and J. T. Turner (editors). Ecology of harmful algae. Ecological Studies. Volume 189. Springer, Berlin, Germany.

Edvardsen, B., and E. Paasche. 1998. Bloom dynamics and physiology of *Prymnesium* and *Chrysochromulina*. Pages 193–208 *in* D. M. Anderson, A. D. Cembella, and G. M. Hallegraeff (editors). Physiological ecology of harmful algal blooms. NATO ASI Series G. Volume 41. Springer-Verlag, Heidelberg, Germany.

Eggleton, M. A., R. Ramírez, C. W. Hargrave, K. B. Gido, J. R. Masoner, G. D. Schnell, and W. J. Matthews. 2005. Predictability of littoral-zone fish communities through ontogeny in Lake Texoma, Oklahoma-Texas, USA. Environmental Biology of Fishes 73:21–36.

Franssen, N. R., K. B. Gido, C. S. Guy, J. A. Tripe, S. J. Shrank, T. R. Strakosh, K. N. Bertrand, C. M. Franssen, K. L. Pitts, and C. P. Paukert. 2006. Effects of floods on fish assemblages in an intermittent prairie stream. Freshwater Biology 51:2072–2086.

Gelwick, F. P., and W. J. Matthews. 1990. Temporal and spatial patterns in littoral-zone fish assemblages of a reservoir (Lake Texoma, Oklahoma-Texas, U.S.A.). Environmental Biology of Fishes 27:107–120.

Gido, K. B., C. W. Hargrave, W. J. Matthews, G. D. Schnell, D. W. Pogue, and G. W. Sewell. 2002. Structure of littoral-zone fish communities in relation to habitat, physical, and chemical gradients, in a southern reservoir. Environmental Biology of Fishes 63:253–263.

Gido, K. B., W. J. Matthews, and W. C. Wolfinbarger. 2000. Long-term changes in a reservoir fish assemblage: stability in an unpredictable environment. Ecological Applications 10: 1517–1529.

Granéli, E., and J. T. Turner. 2006. An introduction to harmful algae. Pages 3–7 *in* E. Granéli and J. T. Turner (editors). Ecology of harmful algae. Ecological Studies. Volume 189. Springer, Berlin, Germany.

Hallegraeff, G. M. 1993. A review of harmful algal blooms and their apparent global increase. Phycologia 32:79–99.

Hambright, K. D., R. M. Zamor, J. D. Easton, K. L. Glenn, E. J. Remmel, and A. C. Easton. 2010. Temporal and spatial variability of an invasive toxigenic protist in a North American subtropical reservoir. Harmful Algae 9:568–577.

Henrikson, J. C., M. S. Gharfeh, A. C. Easton, J. D. Easton, K. L. Glenn, M. Shadfan, S. L. Mooberry, K. D. Hambright, and R. H. Cichewicz. 2010. Reassessing the ichthyotoxin profile of cultured *Prymnesium parvum* (golden algae) and comparing it to samples collected from recent freshwater bloom and fish kill events in North America. Toxicon 55:1396–1404.

Hurlbert, S. H. 1971. The nonconcept of species diversity: a critique and alternative parameters. Ecology 52:577–586.

Hutchinson, G. E. 1953. The concept of pattern in ecology. Proceedings of the Academy of Natural Sciences of Philadelphia 105:1–12.

Igarashi, T., S. Aritake, and T. Yasumoto. 1998. Biological activities of Prymnesin-2 isolated from a red tide alga *Prymnesium parvum*. Natural Toxins 6:35–41.

James, S. V., T. W. Valenti, K. N. Prosser, J. P. Grover, D. L. Roelke, and B. W. Brooks. 2010. Sunlight amelioration of *Prymnesium parvum* acute toxicity to fish. Journal of Plankton Research 33:265–272.

James, T. L., and A. De La Cruz. 1989. *Prymnesium parvum* Carter (Chrysophyceae) as a suspect of mass mortalities of fish and shellfish communities in western Texas. Texas Journal of Science 41:429–430.

Jones, A. C., T. S. V. Liao, F. Z. Najar, B. A. Roe, K. D. Hambright, and D. A. Caron. 2013. Seasonality and disturbance: annual pattern and response of the bacterial and microbial eukaryotic assemblages in a freshwater ecosystem. Environmental Microbiology. doi:10.1111/1462-2920.12151

Landsberg, J. H. 2002. The effects of harmful algal blooms on aquatic organisms. Reviews in Fisheries Science 10:113–390.

Leibold, M. 2004. The metacommunity concept: a framework for multi-scale community ecology. Ecology Letters 7:601–613.

Likens, G. E. 1985. An experimental approach for the study of ecosystems. Journal of Ecology 73:381–396.

Lockwood, J., M. Hoopes, and M. Marchetti. 2007. Invasion ecology. Blackwell Publishing Ltd., Malden, Massachusetts.

Lutz-Carrillo, D., G. M. Southard, and L. T. Fries. 2010. Global genetic relationships among isolates of golden alga (*Prymnesium parvum*). Journal of the American Water Resources Association 46:24–32.

Marsh-Matthews, E., W. J. Matthews, and N. R. Franssen. 2011. Can a highly invasive species re-invade its native community? The paradox of the red shiner. Biological Invasions 13: 2911–2924.

Matthews, W. J. 1986. Fish faunal structure in an Ozark stream: stability, persistence and a catastrophic flood. Copeia 1986: 388–397.

Matthews, W. J. 1988. North American prairie streams as systems for ecological study. Journal of the North American Benthological Society 7:387–409.

Matthews, W. J., K. B. Gido, and F. P. Gelwick. 2004. Fish assemblages of reservoirs, illustrated by Lake Texoma (Oklahoma-Texas, USA) as a representative system. Lake and Reservoir Management 20:219–239.

Matthews, W. J., and E. Marsh-Matthews. 2003. Effects of drought on fish across axes of space, time and ecological complexity. Freshwater Biology 48:1232–1253.

Matthews, W. J., and E. Marsh-Matthews. 2007. Extirpation of red shiner in direct tributaries of Lake Texoma (Oklahoma–Texas): a cautionary case history from a fragmented river–reservoir system. Transactions of the American Fisheries Society 136:1041–1062.

Michaloudi, E., M. Moustaka-Gouni, S. Gkelis, and K. Pantelidakis. 2009. Plankton community structure during an ecosystem disruptive algal bloom of *Prymnesium parvum*. Journal of Plankton Research 31:301–309.

Moustaka-Gouni, M., C. M. Cook, S. Gkelis, E. Michaloudi, K. Pantelidakis, M. Pyrovetsi, and T. Lanaras. 2004. The coincidence of a *Prymnesium parvum* bloom and the mass kill of birds and fish in Lake Koronia. Harmful Algae News 26:1–2.

Oksanen, J. F., G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. M. Stevens, and H. Wagner. 2012. vegan: community ecology package. R package version 2.0-3. R Project for Statistical Computing, Vienna, Austria. (Available from: http://CRAN.R-project.org/package=vegan)

Olmstead, L. L., and D. G. Cloutman. 1974. Repopulation after a fish kill in Mud Creek, Washington County, Arkansas following pesticide pollution. Transactions of the American Fisheries Society 103:79–87.

Parnas, I., K. Reich, and F. Bergmann. 1962. Photoinactivation of ichthyotoxin from axenic cultures of *Prymnesium parvum* Carter. Applied Microbiology 10:237–239.

Peterson, J. T., and P. B. Bayley. 1993. Colonization rates of fishes in experimentally defaunated warmwater streams. Transactions of the American Fisheries Society 122:199–207.

Remmel, E. J., and K. D. Hambright. 2012. Toxin-assisted micropredation: experimental evidence shows that contact micropredation rather than exotoxicity is the role of *Prymnesium* toxins. Ecology Letters 15:126–132.

Remmel, E. J., N. Kohmescher, J. H. Larson, K. D. Hambright. 2011. An experimental analysis of harmful algae–zooplankton interactions and the ultimate defense. Limnology and Oceanography 56:461–470.

Rhodes, K., and C. Hubbs. 1992. Recovery of Pecos River fishes from a red tide fish kill. Southwestern Naturalist 37:178–187.

Roberts, D. W. 2012. labdsv: ordination and multivariate analysis for ecology. R package version 1.5-0. R Project for Statistical Computing, Vienna, Austria. (Available from: http://CRAN.R-project.org/package=labdsv)

Roelke, D. L., J. P. Grover, B. W. Brooks, J. Glass, D. Buzan, G. M. Southard, L. Fries, G. M. Gable, L. Schwierzke-Wade, M. Byrd, and J. Nelson. 2010. A decade of fish-killing *Prymnesium parvum* blooms in Texas: roles of inflow and salinity. Journal of Plankton Research 33:243–253.

Smith, V. H. 2003. Eutrophication of freshwater and coastal marine ecosystems. Environmental Science and Pollution Research 10:126–139.

Smith, V. H., and D. W. Schindler. 2009. Eutrophication science: where do we go from here? Trends in Ecology and Evolution 24:201–207.

Southard, G. M., L. T. Fries, and A. Barkoh. 2010. *Prymnesium parvum*: the Texas experience. Journal of the American Water Resources Association 46:14–23.

Sunda, W. G., E. Granéli, and C. J. Gobler. 2006. Positive feedback and the development and persistence of ecosystem disruptive algal blooms. Journal of Phycology 42:963–975.

USEPA (US Environmental Protection Agency). 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. 5th edition. US Environmental Protection Agency, Washington, DC.

White, P. S., and S. T. A. Pickett. 1985. Natural disturbance and patch dynamics: an introduction Pages 3–13 *in* S. T. A. Pickett and P. S. White (editors). The ecology of natural disturbance and patch dynamics. Academic Press, Orlando, Florida.

Zamor, R. M., K. L. Glenn, and K. D. Hambright. 2012. Incorporating molecular tools into routine HAB monitoring programs: using qPCR to track invasive *Prymnesium*. Harmful Algae 15:1–7.