Golden Algae & the Health of Okla. Lakes

David Hambright

Introduction

The golden alga *Prymnesium parvum* is a toxigenic marine haptophyte that now occurs in and is prevalent in many freshwater systems throughout the world. Golden algae were first recorded in North America in the 1980s in the Pecos River system of southern Texas. Since then, golden algae have been reported in watersheds to the north, including the Red River (2001), and the Canadian River (2003) basins in Texas and Oklahoma. The present U.S. distribution of golden algae known today includes coastal and inland waters of at least 20 states (Figure 1). This apparent range expansion over the past three decades would mark a notably fast spread for an invasive species, but could also indicate an environmental trend that is fostering species replacement. The simple occurrence of golden algae in a given system is not the primary concern for lake and fisheries managers, environmentalists, or the general public. Rather, its toxigenic nature, its ability to bloom (i.e., to produce massive outgrowths), and its typical effect – widespread and massive fish kills (Figure 2) – represent clearly the more pressing issues with respect to ecosystem health, water quality, recreational status of lakes, and even the economic well-being of communities dependent on our lakes and reservoirs. New research reveals that golden algae also can cause considerable harm to zooplankton (the primary food base for fish), suggesting the potential for more subtle, longer-term effects on lake food webs following the invasion and establishment of golden algae populations.

In today's climate of uncertainty with respect to water supply and climate change, Oklahoma recently unveiled its updated 2012 Comprehensive Water Plan (http://www.owrb.ok.gov/supply/ocwp/ocwp.php) which assesses current

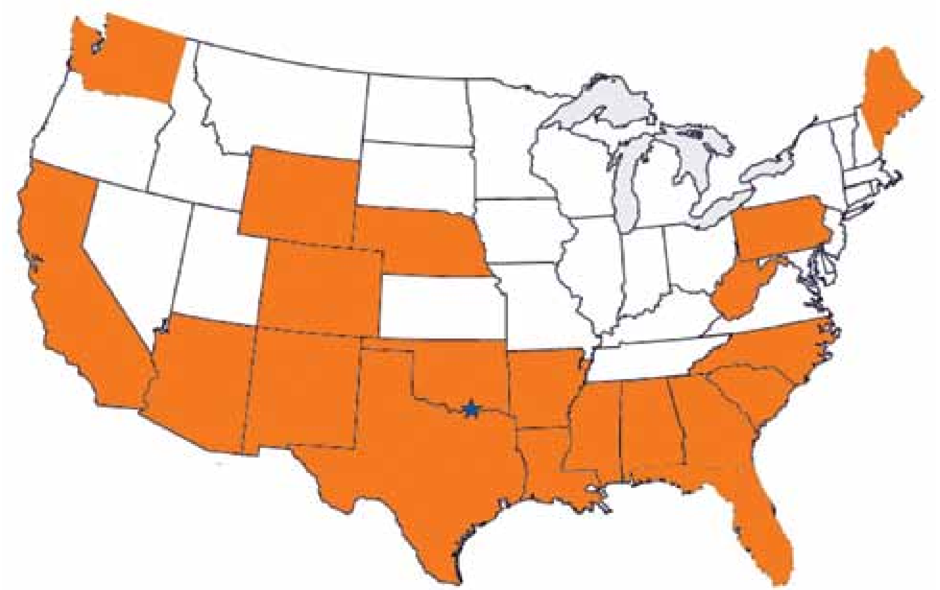

Figure 1. U.S. states reporting golden algae blooms and related fish kills. Blue star indicates location of Lake Texoma.

Figure 2. Photo from Lebanon Pool (northwestern Lake Texoma) taken during January 2004 fish kill.

statewide water supplies and projections of future demand and deficits of fresh water. Although the water plan is focused on water quantity, it is certain that the quality of Oklahoma's water resources will be a priority in the decades to come. Although the same argument could be made for groundwater resources, the quality of surface waters (lakes, reservoirs, ponds, and streams) is clearly linked to the health and well-being of the biological communities (including humans) inhabiting those waters and their surrounding watersheds. Golden algae, like other better-studied toxigenic algae, such as the common cyanobacterial species *Microcystis*, pose a serious threat to the overall health of these freshwater systems. However, unlike cyanobacteria and their toxins, reliable methods for detecting and quantifying golden algae are only recently available, and we have yet to develop reliable methods for identifying, detecting, and quantifying what appears to be a complex suite of multiple golden algal toxins.

For the past nine years, the Plankton Ecology and Limnology Lab at the University of Oklahoma, with funding through the Oklahoma Department of Wildlife Conservation (ODWC), has investigated the ecology and toxicology of golden algae in Lake Texoma, an impoundment of the Red and Washita Rivers on the Oklahoma-Texas border. Our proximate objectives have been to identify factors conducive to golden algae growth, toxicity, and bloom formation. Ultimately our goal is to develop a series of management recommendations to enable lake managers to better combat the spread and bloom formation of golden algae, thus preventing extensive fish kills. Results thus far of a dual monitoring and research program have revealed both predictable patterns and stochastic events that contribute to golden algal dynamics in Lake Texoma, as well as in golden algae toxicity to fish and zooplankton.

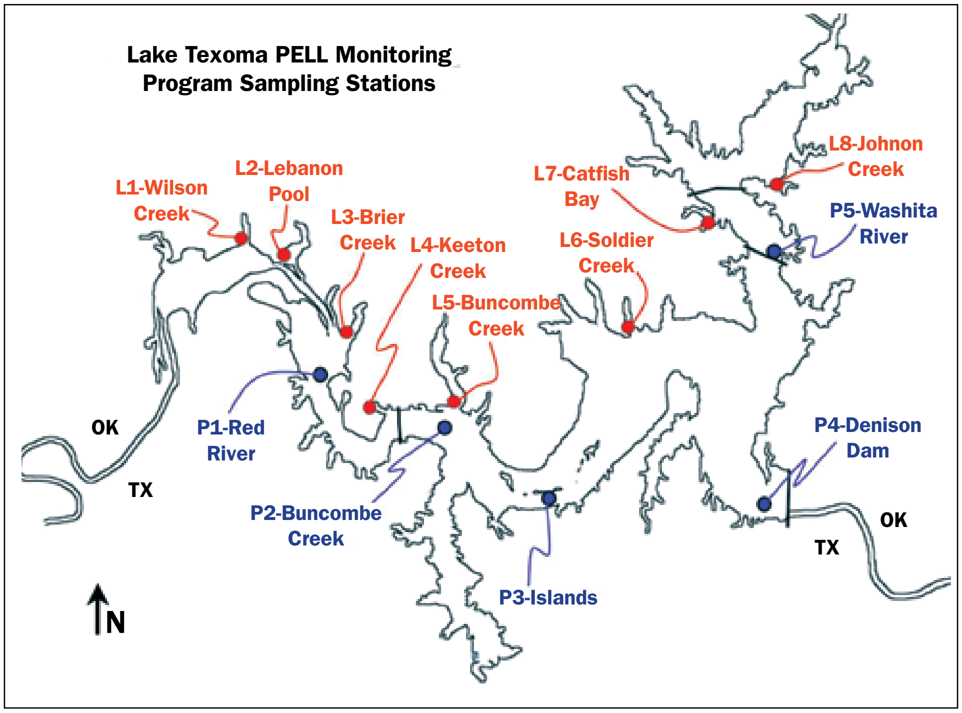

Figure 3. Diagram of the Plankton Ecology and Limnology Lab's (PELL) monitoring program sampling locations on Lake Texoma. Stations L1-L8 are near-shore (littoral) sites and P1-P5 are off-shore (pelagic) sites. Samples for temperature, dissolved oxygen, pH, specific conductance, chlorophyll, phycocyanin, golden algae, particle size distributions, zooplankton, and nutrients, have been collected approximately 32 times per year since 2006. Heavy black lines across channels indicate highway and railway bridges.

Golden Algae in Lake Texoma

As with lakes in Texas, *P. parvum* blooms in Lake Texoma have generally been limited to the cooler months of winter (Figure 3) when water temperatures are between 10 and 15°C. Abundances of *P. parvum* are site-dependent and typically higher near shore. The heaviest blooms (>100,000 cells / mL) have been restricted to Lebanon Pool, an often isolated cove on the western Red River arm of the lake, while minor blooms (up to 50,000 cells / mL) have occurred in other western littoral and pelagic sites. In central and eastern near-shore and pelagic sites, particularly on the Washita River arm of the lake, golden algae rarely exceed 5,000 cells / mL. This west-to-east, Red River-to-Washita River pattern appears to be driven primarily by salinity, and secondarily by nutrients, especially N:P ratios (Hambright et al. 2010). However, it should be pointed out that maximum salinities during the golden algal blooms in Lebanon Pool have averaged only ~ 1.8 PPT, and that high nutrients are expectedly a requisite of any algal bloom (Table 1).

In practical terms, a large die-off of fishes during winter months currently embodies one of the first warning signs that golden algae may be in a particular lake, reservoir, or pond. Given the large number of water bodies in Oklahoma that potentially could be invaded by golden algae, and the critical importance that many of these reservoirs play with respect to water supply, fisheries, and recreation, there is clearly pressing need for a more sensitive and more precise means of detection of golden algae and their toxins.

One of the more serious limitations to the ecological study of golden algae in natural systems is related to the general limitations of microscopic analyses of free-living, single-celled organisms. Golden algae are typically 8-12 µm in diameter and are very similar morphologically to a number of other related algae. Even with very high resolution microscopy that allows clear visualization of key descriptive structures, quantifying golden algae in a natural algal assemblage is incredibly laborious and time consuming. To relieve this problem, a quantitative real-time polymerase chain reaction approach (qPCR) for detecting, amplifying, and quantifying a targeted DNA sequence specific to *Prymnesium parvum* (Zamor et al. 2012) was developed. Using this approach, sample processing and analysis time relative to microscopic-based techniques has been reduced by more than 90 percent.

Table 1. Peak Golden Algal Population Sizes, Salinities (November-April Averages and Maxima), and Nutrient Concentrations and Ratios in Lebanon Pool During Nine Winters.*

Winter	Peak golden algae density (cells / mL)	Salinity (PPT) Average	Salinity (PPT) Maximum	TN (µg / L)	TP (µg / L)	TN:TP
2003/04	144,000	n.d.	n.d.	n.d.	n.d.	n.d.
2004/05	15,000	1.29	1.55	n.d.	n.d.	n.d.
2005/06	161,000	1.36	1.72	1,437	300	4.8
2006/07	183,000	1.82	2.68	999	158	6.3
2007/08	162,000	1.74	2.73	1,304	166	7.9
2008/09	198,000	2.38	3.01	1,071	109	9.8
2009/10	2,000	0.76	1.74	836	126	6.6
2010/11	56,000	1.35	1.57	905	84	10.7
2011/12	85,000	0.67	1.02	1,695	183	9.3

*total nitrogen, TN; total phosphorus, TP; ratios of TN to TP by weight, TN:TP; n.d. = No data.

The detection limit has been improved dramatically, down to 6 cells / mL for a 1-L sample or to 28 cells / mL if 200 mL of sample are processed. Finally, the error and uncertainties associated with microscopy and taxonomic identification are virtually eliminated. Thus, qPCR can be a very effective tool for monitoring golden algae, particularly during non-bloom periods when densities may be low or in systems in which golden algae have not yet been observed.

Toxins and Toxicity

Toxicity and the extent of fish kills have varied annually in Lake Texoma, with the most extensive kill to date occurring in winter 2003-2004 in Lebanon Pool (Figure 4) on the Oklahoma shore and along the Texas shoreline from Slickum Slough to Preston Point. There was no bloom or fish kill in 2004-2005 or 2009-2010, and moderate to large blooms and fish kills in 2005-2006, 2006-2007, and 2007-2008 were restricted to Lebanon Pool and Buncombe Creek, as well as a few western sites along the Texas shore. Interestingly, the 2008-2009 bloom was the second-largest bloom in Lake Texoma to date, but it was not accompanied by a noticeable fish kill, even though laboratory bioassays revealed high toxicity, and shoreline seining and gill netting efforts resulted in no fish collected during the bloom (Zamor, pers. comm.). Presumably, birds and other scavengers consumed the dead and dying fish before they were noticed. For large complex systems like Lake Texoma, the future is not, however, wholly grim. Fish assemblages do seem quite capable of strong recovery following a golden algal bloom, as countless unaffected coves provide a rich source of immigration.

It is important to point out that our studies have also uncovered a more subtle, potentially longer-term problem associated with the presence of golden algae. While fish kills receive the major press coverage during a golden algal bloom, we have documented a more serious ecological threat – the potential modification of food-web dynamics through negative impacts on herbivorous zooplankton, particularly large herbivores, such as *Daphnia*. In a study of golden algae-zooplankton interactions, Remmel et al. (2011) found that golden algae could be eaten by zooplankton at similar rates by which green algae were consumed with no apparent short-term (one day) side effects. However, in longer-term exposures to golden algae, daphniid survivorship, juvenile growth rates, and fecundity were severely harmed after ~ three days of exposure (Table 2), and, more importantly, even at very low concentrations of golden algae (25 percent golden algae in the diet in these experiments was equivalent to ~7,500 cells / mL).

It has been generally thought that fish kills do not occur at golden algal densities below 20-50,000 cells / mL. So, even if golden algae are present at relatively low densities, detrimental effects on herbivorous zooplankton could result in important changes to a lake's food web.

In all of the experiments by Remmel at al., few negative effects occurred when cell-free filtrate was used. Cell-free filtrate is culture medium from which all golden algae cells had been removed by filtration. This observation led to the hypothesis that toxins were actually cell-bound, rather than being released as exotoxins into the water, as had been thought for nearly six decades (Remmel and Hambright 2012). Indeed, in an experimental series in which fish were separated from golden algal cells by a permeable membrane, we discovered that previous findings of

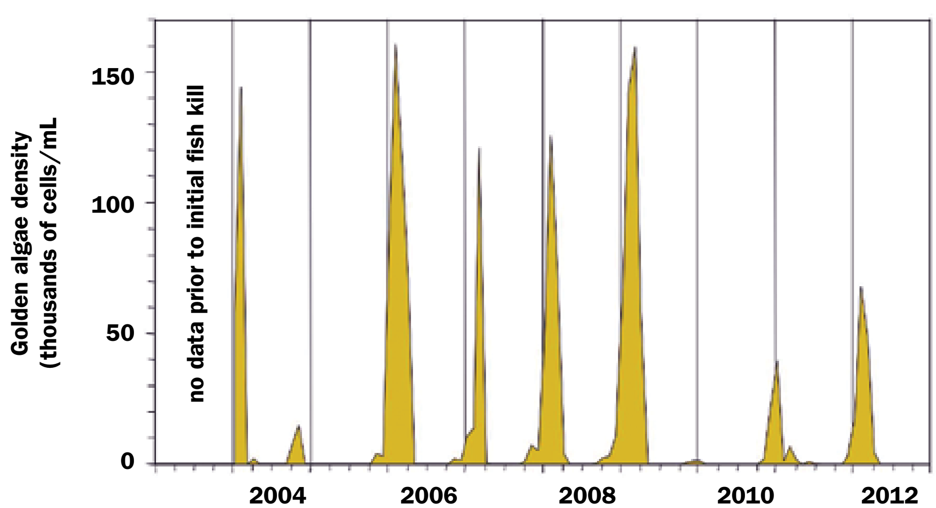

Fig. 4. Densities of golden algae in Lebanon Pool (Lake Texoma) during the past nine winters. The first fish kill was reported on 22 Jan 2004; the golden algae population peaked at 144,000 cells/mL on 25 Feb 2004.

Table 2. Summary of Life History Consequences in Two Species of *Daphnia* Fed Golden Algae (GA) and *Scenedesmus* (S, a high food quality alga) in Varying Proportions for Ten Days (10 *Daphnia* per species per food treatment).

Daphnia	Food source		Life history characteristic				
	% GA	% S	% alive after day 3	Average days survived	Juvenile growth rate (mm/d)	Age at first reproduction (d)	1st clutch (no. of eggs)
D. pulex	0	100	100	5.7	0.191	8	6
	25	75	60	3.5	0.081	7	4
	75	25	40	2.2	0.039	9	4
	100	0	0	0.5	0	-	0
D. pulicaria	0	100	90	7.6	0.162	9	3
	25	75	70	5.4	0.139	-	-
	75	25	20	1.7	0.086	-	-
	100	0	20	2	0	-	-

Data taken from Remmel et al. (2011).

exotoxins were likely influenced by the artificial generation of "exotoxins" by the procedures used to produce the cell-free filtrate. This hypothesis was supported in another experimental series in which ichthyotoxicity of golden algal toxins decreased from being very high when live cultures were used, to moderate when cell-free filtrate was produced by vacuum-filtration, to very low and negligible when cell-free filtrate was produced by gentle gravity filtration (Remmel and Hambright 2012). This result is logical within the context of evolutionary theory. In addition to being photosynthetic, golden algae are also heterotrophic, capable of ingestion of detritus, bacteria, and other protists (Granéli and Johansson 2003; Tillmann 2003). They are also likely capable of uptake of dissolved organic carbon, vitamins, and other organic nutrients, particularly those made available by cell membrane-disrupting toxins. So, rather than releasing toxins into the water, our studies suggest that golden algae encounter gill and other sensitive tissues in fish and zooplankton, and the chemicals involved in heterotrophic processes cause cellular lysis and other symptoms observed in fishes, such as changes in gill permeability, hemorrhaging, heavy mucus secretion, and ultimately death.

One of the more surprising results of our investigations to date has been the inability to detect the presumed cyclic polyether "prymnesins" that are believed to be responsible for fish deaths. On the other hand, we have characterized other toxins, including multiple fatty acids and a still-to-be characterized group of highly-labile chemicals, produced by golden algae that cause fish mortality (Henrikson et al. 2010). These studies also revealed that the toxins produced by golden algae grown in cultures were strikingly different than the toxins produced during a bloom in Lake Texoma. Initial screening of some of these substances also has revealed that a few of them are toxic to human cells with direct application to cell cultures. However, we do not know yet the fate and efficacy of golden algal toxins if they are inhaled, ingested, or generally contacted, such as through the handling of fish or swimming in infested waters. In light of the increasing probability of encounter with golden algae as its distribution range continues to expand and the intriguing complexity of golden algal toxins thus far described, the current lack of concern regarding human contact with golden algae infested waters may require reassessment.

Why Should You Care?

According to the Oklahoma Water Atlas, Oklahoma has 146 major water reservoirs that were impounded between 1902 and 1997 by the US Army Corps of Engineers (USACE), the Bureau of Reclamation, the Grand River Dam Authority, Natural Resources Conservation Service, and various municipalities, private corporations, and others. Most reservoirs serve multiple purposes, including flood control, municipal, industrial, and agricultural water supply, power generation, navigation, hydroelectric power production, and conservation; most reservoirs support fish and wildlife habitat and recreation as ancillary benefits. All reservoirs play a significant role in the economic and social well-being of the state.

Today, golden algal blooms in Oklahoma have been limited to Lake Texoma on the OK-TX border and a few western ponds and reservoirs in Jackson county (all within the Red River drainage basin), including the Altus City water supply reservoir. Given the extent of golden algae occurrence across Texas and beyond, as well as the fact that golden algae are already in the Canadian River basin in the Texas Panhandle, the future appearance of golden algae in other Oklahoma water bodies seems likely. Indeed, qPCR screening has revealed golden algal presence within the Canadian River basin in western Oklahoma (Zamor, Pers. Comm.). More recently, golden algae were found in water samples taken from Fort Gibson Lake (Grand River) and Tenkiller Ferry Lake (Illinois River), both in eastern Oklahoma, during the summer 2012 cyanobacterial monitoring by the US Army Corps of Engineers (T. Clyde, pers. comm.). Continued high nutrient loadings to lakes and streams, coupled with salinization of our nation's fresh waters due to exploitation and climate and land use changes, undoubtedly increase the likelihood that golden algae will continue to spread across the nation.

While fish kills are certainly troubling, our research indicates that blooms and even the mere presence of golden algae should cause a much deeper level of concern – concern for the health of our lakes' food webs (Figure 5). Major portions of the economic engines of Oklahoma's lakes are driven by recreational fisheries, which are predominantly based on top predators (e.g., striped and largemouth basses, crappies, catfishes). While our experience in Lake Texoma indicates recovery of fish populations in the short term following a golden algal bloom, the longer-term

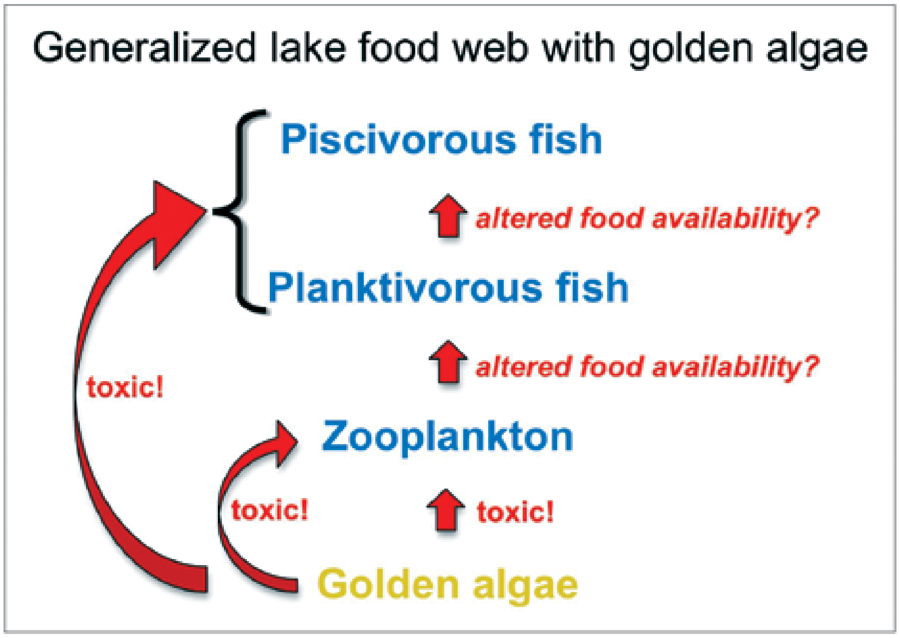

Fig. 5. Documented (!) and potential (?) effects of golden algae on a lake food web. Golden algal toxins harm fish and zooplankton via release of toxins upon contact with gills and other exposed tissues. Zooplankton are also harmed by ingesting golden algae. In addition to direct toxicity, golden algae may also harm fishes indirectly via changes in food resource availabilities.

impacts on lake food webs are still unknown. Like many cyanobacteria, golden algae are a poor and toxic food source for zooplankton, resulting in low growth and reproduction. Changes in zooplankton abundances or species composition following the establishment of golden algae in a system could lead to alterations in food availabilities for juvenile and planktivorous fishes, which could ultimately affect fish at the top of the food web, fishes on which many local economies are based.

What Can You Do?

Reservoirs are notoriously susceptible to invasion by exotic species, as reservoirs are characterized by higher physical disturbance regimes, higher and more variable fertility, higher salinity, and higher connectivity than natural lakes. Reservoirs also tend to be more accessible to humans and experience higher boating activity, and both of these factors promote species invasions (Johnson et al. 2008). For large invasive taxa like hydrilla, Eurasian watermilfoil, or zebra mussels, boaters can play a major role in reducing the movement of propagules (e.g., sprigs, seeds, individuals, etc., that can begin new populations) by following simple inspection and de-fouling procedures. However, for microbial species such as golden algae, the importance of human-supported dispersal is less certain, though considering that densities of golden algae during the winter in Lake Texoma can approach 200,000 cells / mL, extremely large populations of golden algae can be transported in even small amounts of water. Even during non-bloom conditions of a few thousand cells / mL, a 20-L (=5 gal) bucket containing your prize largemouth bass that is destined for your local pond could result in an inoculation of your pond by millions of golden algal cells!

Many researchers believe that microbial species are different from macrobial (large) species with respect to distributional ranges and invasion. Initially used to describe bacterial systems, but now generally applicable to most unicellular species, including algae, the microbiologists' credo, "Everything is everywhere, but the environment selects" hypothesizes that microbes in general have few geographic barriers to dispersal. Instead, microbial species are thought to be potentially everywhere and rather than being absent from a given habitat at a given time, they are thought simply to be very rare (and undetectable using routine methods) under non-optimal environmental conditions. This so-called "rare biosphere" may confer ecological resilience to a system's functioning by providing ecological and biogeochemical "backfilling" of important trophic roles and processes when environmental conditions no longer favor some of the abundant taxa (Caron and Countway 2009). Thus, when environmental change occurs, some or all of the dominant members of a community are simply replaced by others from the rare biosphere (hence, "the environment selects"). From this finding, one might hypothesize that the perceived invasion and range expansion by golden algae in the United States is a response of the microbial communities in lakes to an environmental change, such as increased salinization and excessive nutrient loading. Under this scenario, prevention of the spread of golden algae is much less simple than washing or drying one's boat. Instead, much larger scale solutions are required – conservation and preservation of water resources and abatement of high nutrient loading to our streams and lakes.

Limnologists and lake managers have been fighting the seemingly uphill battle for nutrient abatement since at least the 1970s, and we have not had much better success with preserving water resources, in general. Nevertheless, with the support of an informed and willing public, and the continuing efforts of agencies such as the ODWC, we can continue to protect and conserve both the quantity and the quality of our water resources, while still enjoying the benefits of flood control, water and hydroelectric supplies, rich wildlife and fisheries resources, and the many other recreational benefits to Oklahomans and visitors alike whose patronage supports directly so many local economies.

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