DIEL TEMPERATURE AND DISSOLVED OXYGEN PATTERNS IN SITES WITH AND WITHOUT PLANKTONIC LIFE STAGE OF *THOMPSODINIUM INTERMEDIUM* IN COMAL SPRINGS, TX

A Thesis

by

CHERYL GILPIN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2012

Major Subject: Oceanography

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Approved by:

Chair of Committee,	John Wormuth
Committee Members,	Wyndylyn von Zharen
	Dan Thornton
	George Guillen
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ABSTRACT

Diel Temperature and Dissolved Oxygen Patterns in Sites with and without Planktonic Life Stage of *Thompsodinium intermedium* in Comal Springs, TX.

(May 2012)

Cheryl Gilpin, B.S., Stephen F. Austin State University, TX

Chair of Advisory Committee: Dr. John Wormuth

Between July 2009 and October 2011, a new habitat was found for a rarely reported freshwater dinoflagellate species, *Thompsodinium intermedium* - Comal Springs (Comal County), Texas. In 2011, diel *in-situ* monitoring in monospecific blooms of this species revealed previously undetected negative impacts on endangered species habitat availability associated with conditions of low flow levels, recorded at the U.S. Geological Survey gage # 08169000 on Texas Commission on Environmental Quality river segment 1811 station 12655. During a period of low springflow in the summer of 2011, late afternoon and early morning measurements of dissolved oxygen and temperature and presence of dinoflagellate blooms were monitored at six sites. Significant differences in diel fluctuations were found in all of these parameters among sites with and without the planktonic blooms. These fluctuations increased risk of hypoxia and hyperthermia conditions at sites of planktonic bloom events. Arrays of *in-situ* continuous monitoring temperature/light probes were used inside and outside of blooms. Wildlife and human

health implications are that hypoxia and hyperthermia are known to promote conditions favorable to harmful microbes which may be transported from springs to coastal bays. *In-situ* data demonstrated that *T. intermedium* blooms, hypoxia, and hyperthermia occurred in the upper Comal headwaters. These natural environmental stressors may be avoidable if adequate springflows are maintained to buffer against these impacts.

DEDICATION

To my husband, Len Gilpin and son, Mark Allen

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I thank my committee co-chairs Dr. John Wormuth, and Dr. Wyndylyn von Zharen, and my committee members, Dr. Dan Thornton and Dr. George Guillen and the University Writing Center for their guidance and support throughout the course of this research. Also I thank my friends and colleagues at the TAMU Oceanography department and TAMUG Marine Science department for making my time at Texas A&M in College Station and in Galveston a great experience. Dr. Susan Carty of Heidelberg University in Ohio provided me confirmation of species level dinoflagellate identification. Dr. Anis and Dr. Stoessel provided assistance with understanding physical processes and research design.

Dr. Antoinetta Quigg read my proposal and provided advice. Wim von Egmond of Amsterdam, Netherlands mentored me in photomicroscopy when I was his assistant at a photomicroscopy class for communication majors. I was able to demonstrate in this thesis research that photomicroscopy of live dinoflagellates is a great science tool for relating physical science to biological processes, for species level identification and for detection of multiple life stages of plankton.

Special thanks to my husband, Len Gilpin and son, Mark Allen. This was a long, hard marathon to the finish. My husband, who paddled our canoe, was a valuable resource. He is a living encyclopedia of aquatic biology. He also helped me to do a little science on the side. He made sure I had good running shoes and tread on our vehicles. Dr. Doug Biggs started me tracking live dinoflagellates in the field through gradients of density in cold core eddy rings in the Gulf of Mexico and made equipment available to

me. Dr. Wormuth provided me with equipment I needed for collecting plankton and environmental data and provided valuable comments on my written thesis. My son, the historian, gave me a perspective on the drought of the 1950s at Comal Springs and loaned me his computer and was an infinite source of encouragement and ideas. Whenever I tired along the way, Dr. von Zharen and her GR2R research team always pulled me up to continue the race. I was enabled to finally cross the line with the focused editing and expert formatting assistance of Dr. von Zharen, Amanda Solitro, Elaine Washington and Wendy Turner. I appreciate their hard work after hours from their busy careers.

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I. INTRODUCTION AND LITERATURE REVIEW

a. Hypoxia and Hyperthermia Investigations in Stream Habitats

Due to recent assumptions used in the modeling of Comal River minimum flow targets for management of pumping in the Edwards Aquifer (EARIP 2011), risk of hypoxia was removed from a list of threats Hardy et al. (1999) considered as potential stressors for the endangered fountain darter *Etheostoma fonticola*, a small fish which is used as the sentinel species for three endangered Comal Springs invertebrate species. According to Bonner et al. (1998), temperature levels below 25^{o} C are needed for success of fountain darter eggs. For this thesis research, increased risk of encountering conditions known to be associated with hypoxia (dissolved oxygen < 4 mg/L) and hyperthermia (> or = 27^{o} C) were investigated in sites of planktonic freshwater dinoflagellate blooms in the Comal River during a period of critically low aquifer levels in the summer of 2011. Investigations of spatial and temporal patterns of freshwater dinoflagellates and their interactions with physical, chemical, and biological parameters in karst springruns were not found in the scientific literature.

A few ecological studies have addressed the interactions of phytoplankton blooms in rivers (Walks 2007, Jones and Mulholland 1999, Reynolds 1991, 1994, 2000), but none included dinoflagellates.

This thesis follows the style of the Journal of Freshwater Ecology.
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Available from: http://www.savingcranes.org/guadalupe-river.html

Characteristic diel and seasonal stream hypoxia and hyperthermia patterns were previously discussed by Smale and Rabeni (1995 a and b), Giller and Mahlquist (2001) and McGinty (2003). In Boondoggle Lake, Mississippi (Canion and Ochs 2005), dinoflagellates were sampled and observed by zooplankton protocols and that led to a better understanding of their ecology since dinoflagellates are a much larger size class than most other phytoplankton which occur in bloom numbers. Spring to sea water quality studies from karst spring source "springsheds" to coastal wetlands have been reported by Jones and Mulholland (1999), Mulholland et al. (2008), Walks (2007) and Reynolds (2000). According to Branco and Torgerson (2009), Branco (2007) and Ganf (1974) strong coupling exists between diel patterns of physical processes and the dynamics of phytoplankton. These have a strong influence on patterns of dissolved oxygen changes (Gu et al., 1996, and Branco 2007).

During the day, the process of photosynthesis produces oxygen.

$$6H_2O + 6\ CO_2 \text{ -----Sunlight } \rightarrow C_6H_{12}O_6 + 6O_2$$

At night, the oxygen dissolved in the water is used up for cell respiration while no oxygen is being produced.

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O.$$

Hypoxia is a condition of dangerously low levels of dissolved oxygen. Hypoxia may result from a combination of low flow, high water temperature, algal blooms, sewage leaks and decomposing aquatic vegetation (Giller and Mahlquist 2001). As a general trend, spring headwaters with shallow riffle areas tend to have more oxygen than downstream because of turbulence (mixing with air) and higher surface to volume ratio

facilitating more diffusion of oxygen. Oxygen demand in stream biota can increase 10 times for every one degree Celsius change in water temperature. Thus, warmer water holds less oxygen and increases demand for oxygen which can compound physiological stress on stream organisms (Moss 1998).

Extreme fluctuation in dissolved oxygen was reported to occur in lab cultures of planktonic dinoflagellates from estuaries (Brownlee et al. 2005). Studies by Smale and Rabeni (1995 a and b) and by McGinty (2003) indicated that karst springflows comprised of adequate amounts of longer stored, diffuse groundwater buffer against daily and seasonal changes in stream water temperature and water level. In these springflows, water quality impacts of local pumping events adjacent to springs, hyperthermia events, hypoxia events, and diurnal springflow cessation do not occur as long as the flow is adequate to buffer the impacts of these habitat stressors.

Avoiding conditions of hypoxia and hyperthermia in streams is essential to the protection of endangered karst spring species and other wildlife adapted to habitats with little fluctuation in temperature or dissolved oxygen concentration. This thesis research adds another condition that occurs in streams that interacts with others in the field to impact the conditions of hypoxia and hyperthermia: the presence of planktonic dinoflagellate blooms.

b. Freshwater Dinoflagellate *Thompsodinium intermedium*

In July of 2009, a previously undetected planktonic dinoflagellate bloom of a rare species, *Thompsodinium intermedium*, was observed and identified by this researcher in

the upper springruns of the Comal River. In the otherwise clear Comal Spring waters, this event caused over 200m of the length of stream channel in the Comal Spring headwaters above Landa Lake to appear as opaque brown gumbo (Fig. 1).

Figure 1. Upper Comal River headwaters with appearance of "River Gumbo." Algal bloom caused by *Thompsodinium intermedium* July 2009. Photo by Woody Welch

According to Carty (2003), this is a rare species and the ecology of this species had never been investigated. According to Krakhmalny (2011), this species was also found in Lake Buchak in the Ukraine in 2009 and reported as a rare species which he had not observed in the Ukraine since it was observed in 1966 at Lake Beloye in the coastal plankton in the thicket of reeds. Lake Buchak is an abandoned quarry with a clay bottom,

around 10 m deep with transparent water. Krakhmalny (2011) reported it had been found on both warm days and cold rainy days.

c. Comal Springs: Its Location and Endangered Species

The Comal River is located in Comal County, Texas. The word "Comal" is Spanish for "shallow, bowl-shaped pan" which accurately describes the geological morphology of the Comal River riparian network and watershed. It is situated beside the steep Balcones Escarpment which rises over 100ft along the side of Landa Lake and its headwaters in New Braunfels. Usually, Comal Springs is the highest continuously-flowing spring system in the Southwest United States (Brune 2002).

From 1930-1999, the annual mean discharge was 8 m^3/sec (approximately 180 million gallons per day) (Gandara et al. 2000). There are four major springs in the Comal River. This research location is described by Hardy (2009) as the headwaters of Landa Lake and includes the fourth largest of the large Comal Springs and two medium large springs (Guyton 2004).

Landa Lake and the Comal River downstream are under the jurisdiction of the City of New Braunfels. However, most of the river upstream of Landa Lake, including most of this research location, are under the jurisdiction of the Comal County Water Recreation District No. 1 (CCWRD1).

In the Federal Register: 72 FR 39248-39283 136 July 17, 2007, in accordance with the Endangered Species Act (16 U.S.C. § 1531), the United States Fish and Wildlife Service (USFWS) designated the fountain darter (*Etheostoma fonticola*) as the

sentinel species, the canary in the coal mine, for the three endangered invertebrates that are endemic to the Comal River: Peck's Cave amphipod (*Stygobromus peckii*), Comal Springs riffle beetle (*Heterelmis comalensis*) and Comal Springs dryopid beetle (*Stygoparnus comalensis*) (Brune 1981). Field studies regarding the ecology of endangered Comal Spring species and characterization of Comal River flow and water quality were reported by Bowles et al. (2003), Falquist and Slattery (1997), Bonner et al. (1998) and Twidwell (2004). However, much about the ecology and life histories of the endangered invertebrate species in the Comal River remains unknown.

This study area is co-occupied by each of the three invertebrate species and is designated as their critical habitat (Federal Register 2007, Fed Reg 1970 and 1985). It is also occupied by their sentinel endangered species, the fountain darter. This is the only area of the Comal River occupied by all of the endangered species that reside in the Comal River. In 1995, the United States Fish and Wildlife Service USFWS established "take" and "jeopardy" levels for springflows at 5.7 m^3/sec (200 cubic feet per second (cfs)) and 4.2 m^3/sec (150cfs), respectively. However, the most recent proposed minimum springflow number for the flow measured by the USGS gage near the mouth of the Comal River is 30 cfs (EARIP 2011).

For this habitat, minimum springflow modeling assumed dissolved oxygen levels less than 4 mg/L a threat to endangered species survival. However, it also assumed that levels less than 4 mg/L were never reached during 2011 since the modeling assumed that springs continued to flow (Hardy 2009). For this study, less than 4 mg/L per liter

dissolved oxygen defines hypoxia and 27 degrees Celsius or higher defines the condition of hyperthermia. During low springflow periods, the assumption that fountain darters were present and healthy inside the aquatic plants may require adjustment when these habitats contain blooming freshwater dinoflagellates. Conditions of hypoxia and hyperthermia in the Comal River not only are concerns for aquatic life, but they can potentially impact negatively large numbers of people who recreate in the Comal River.

Harmful microbes can thrive even under temporary hypoxia. Harmful microbes can likely be transported downstream and from the bottom of springruns into aquifers and then into drinking water wells. Dinoflagellates have been previously reported as feeding on coliform bacteria (Jeong et al. 2008). Undetected and unmitigated sewage contamination is a threat to both wildlife and human health.

The dinoflagellates decrease *E. coli* from sewage contamination. Dinoflagellates have not shown that they are capable of removing the other sewage pathogens such as *Cryptosporidium* and *Giardia.* Since *E. coli*, a sewage indicator, is also an oxygen stressor, *E. coli* was monitored.

d. Climate and Springsheds

Droughts and extreme heat occur frequently in Central Texas where Comal Springs is located. The climate is characterized as drought punctuated by floods (Loaiciga et al. 2000, Firth and Fisher 1992). Prior to the 1900's more severe droughts were implicated by tree ring data than by records of streamflow data (Dunne 2002). Both

droughts and flooding events in this region have set over half of the recorded global records (Firth and Fisher 1992). The Comal and Guadalupe River watersheds and riparian networks, which include karst, dry creek beds upstream of Landa Lake, became braided during extreme flooding in June 2010, as they had during 1970s flooding as documented by Baker (1977), Caran and Baker (1986) and CH2M Hill (2002). Extreme risk of sewage contamination from the damaged wastewater facility in Gruene into these Comal River headwaters was not monitored upstream of Landa Lake during the flood of 2010 because cross contamination between watersheds was assumed to only occur during 100yr or greater flood events. In reality, these events have occurred four times (1972, 1998, 2002, 2010) within the past 40 years.

During this study period, major springs inside the research area ceased to flow in 2009 and 2011 during extreme drought conditions (Fig. 2). Record breaking heat occurred in 2011. It was the most severe drought since the 1950s.

Figure 2. Spring 5 ceased to flow.

Prior to the drought of the 1950s, the Comal Springs ecosystem had continued to maintain flows dominated by diffuse older groundwater and to support many endemic species through a history of repeated droughts. Buffering by springflow of water temperature from seasonal air temperature fluctuation (McGinty 2003, Shuster and White 1971) and a continuous supply of water may have been more adequate during past droughts (Fig. 3).

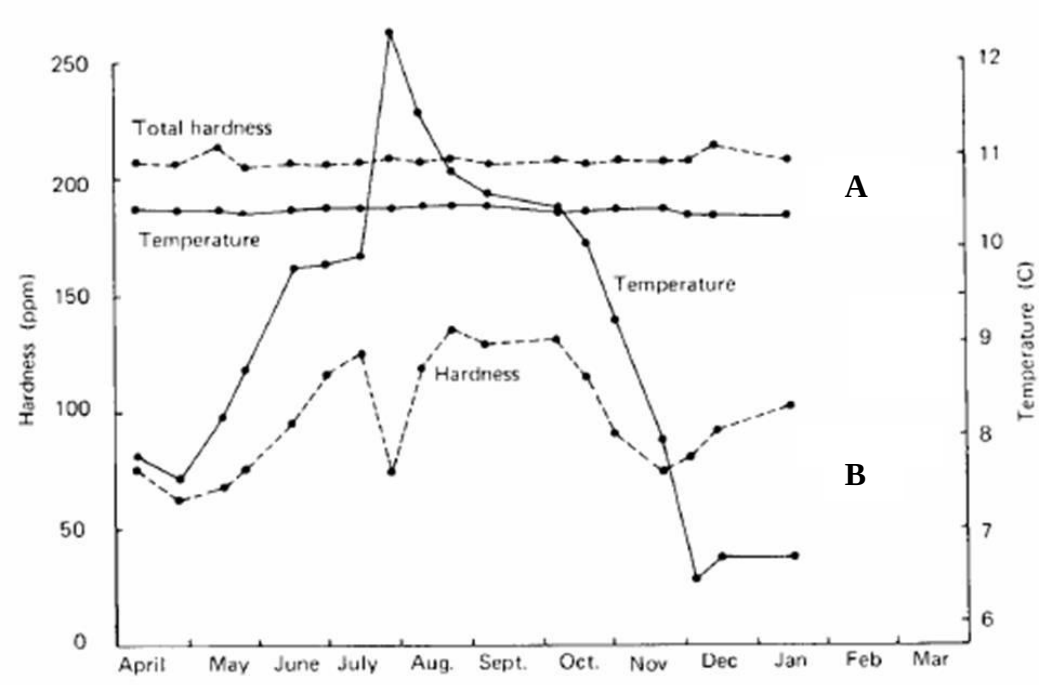


Figure 3. Buffering capacity of two types of springflow regimes in typical karst springs over one year. A. Buffering when springflow is dominated by diffuse flow of regionally stored older groundwater (top lines). B. Lack of buffering when springflow is dominated by conduit flow of local recent groundwater (bottom lines). Shuster and White 1971. Reprinted with permission from ELSEVIER.

There are freshwater and marine harmful microbes including dinoflagellates that are adapted to climate extremes, droughts punctuated by flood, watersheds and coastal inflows that primarily flow with groundwater. They also have similar mechanisms, triggers, and ecological strategies. Recently, Mulholland et al. (2008) documented that what happens in springs and neighborhood streams have profound impacts on coastal environmental health, ocean dead zones, and red tides. Human practices that alter hydrological processes can impact the ecohydrology from backyard streams to oceans. Conditions of hypoxia or "dead zones" occur in both freshwater and in oceans and may be triggered by ecohydrological patterns which are easier to determine in a karst springrun on smaller spatial and temporal scales.

The Edwards Aquifer springshed system (Fig. 4) is one of the largest in the world associated with one single aquifer. Harmful microbes that exist in the Comal River upper springrun headwaters may travel to the coastal wetlands at the end of this springshed system. Four major Texas river basins comprise this springshed including two river basins with most of their surface flows originating from the San Antonio section of the Edwards Aquifer: the San Antonio River and the Guadalupe River (ICF 2012). These unite inland before they flow into the San Antonio Bay and the coastal wetlands of the Aransas National Wildlife Refuge, which is the winter habitat of internationally migrating endangered whooping cranes (*Grus americana*).

According to Bill West, General Manager of GBRA, "while protecting endangered species is important, protecting water resources during times of drought is critical to meeting the overall needs of the region. Springflows are essential to the water

supply in the Guadalupe and San Marcos and Comal Rivers. This water is used by cities, industries and agricultural producers all the way to the Texas Coast, and is the basis for many water rights issued by the State of Texas. The springs are critical to instream flows

Figure 4. Edwards Aquifer Springshed System. Reprinted with permission from: International Crane Foundation (2012).

for the Guadalupe River and fresh water inflows to San Antonio Bay. National Weather Service reports that the period of September 2007 through May 2008 for San Antonio, TX, is the driest on record at 6.57 inches - 2 inches lower than the previous record of 8.89

inches noted in May, 1956 (GBRA 2008).” A wet summer followed the drought of fall 2007 through spring 2008. Subsequently, there was no significant rain until June 2010 when there was historically significant flooding in the basin of the Upper Comal Spring research area. Meanwhile, 23 whooping cranes that wintered at ANWR in 2008/2009 perished (Slack et al. 2009). Could harmful microbes growing in stagnant Edwards Aquifer springruns like these in the Comal River have washed down to the coast and made the cranes ill? This is the question that inspired this thesis research.

According to GBRA (2011), experts testified in a US District Court hearing in Corpus Christi Texas in 2011 that the crane deaths were not caused by the drought 2008-2009, nor were they caused by lack of food or water. However, delayed impacts from the drought of 2007-2008 may not have been detected if the water quality and microbes were not monitored on proper scales of space and time during and after the drought.

II. METHODS

a. Observations and Identification of *Thompsodinium intermedium*

In July of 2009, brown, cloudy water in the Comal River was sampled and live cells were concentrated by volume reduction using a 0.45 μ millipore filter (standard method # 10200G, APHA 2005) in a Nalgene in-line filter case (Fig.5-7). Cells were observed under an Olympus BX60 DIC Epiflouresence research microscope. By volume reduction, a small volume is always left with cells on top of the filter. Cells become damaged when water is completely drained through the filter. Pressure or suction is halted in time to prevent that. The reduced volume was poured out of the in-line filter (Fig. 5) into a 20 mL vial (Fig. 6) and the filter was put into the vial and rinsed by pumping the contents of the vial with a wide bore plastic pipette over the filter until it rinsed clean. Live concentrated cells and empty theca were observed with differential interference contrast (DIC) microscopy, photographed with a Nikon Coolpix camera, and taxonomic characteristics were recorded and used for species level identification according to taxonomic methods described in Bourrelly (1970), and Carty (1986, 1989, 2003).

Figure 5. Nalgene in-line filter case with

0.45 micron Millipore filter.

Figure 7. Cells on the filter rinse off inside the vial in a fraction of the sample volume.

Figure 6. Water sampled 0.3m below the surface before (left) and after (right) filtering.

b. Documentation of Seasonal Biotic and Abiotic Conditions at Comal Springs, Summer 2009-Fall 2011

Between July of 2009 and fall of 2011, conditions relevant to both the ecohydrology of the habitat and to dinoflagellate dynamics were documented monthly on a presence/absence basis. The following criteria were used to establish the presence/absence of abiotic and biotic conditions. Atmospheric temperature and other conditions were considered extreme if the temperature was above 38° C (100° F) or below 0° C (32° F). Afternoon hyperthermia was defined as > or = 27° C between 2-6pm, morning hypoxia was defined as < 4 mg/L dissolved oxygen before 9:30 am, rain events, vertical stratification by ΔTEMP/ΔZ (change in temperature with change in depth), USGS flow gauge downstream < 220 cfs, no springflow at either Spring 4 or Spring 5, low springflow at either Spring 4 or Spring 5, construction (road, sewer or gas pipe), lodge full (maximum water use and toilet flushing), *E. coli* present, turbid whitish plume flowing from bank, no contact recreation advised due to *E. coli* > 206 mpn, dinoflagellate plankton cells, dinoflagellate bloom > 10,000 cells/mL and brownish plumes, wet draw-down lines on bank greater than .05 m and pumping sounds. For each condition, a "1" was recorded if present, "0" was recorded if not present. These were documented to begin a long term data set from which trends might be determined in which dinoflagellate cysts may be used as a proxy for future paleohydrology research. Trends in these parameters relevant to the following investigation conducted in 2011 were recorded in Table 1.

Table 1. Comal River Upper Springrun Atmospheric and Hydrologic Conditions.

Planktonic Dinoflagellate Study 2009-2011											
1= **During** yes	**YEAR**	**2009**			**2010**				**2011**		
0=no	**Season**	**Summer**	**Fall**	**Winter**	**Spring**	**Summer**	**Fall**	**Winter**	**Spring**	**Summer**	**Fall**
Planktonic	dinoflagellate	1	1	0	0	0	1	1	0	1	1
	E. Coli	1	1	1	1	1	1		1	1	1
Springflow	Ceased short-term	0	1	0	0	0	1	0	0	1	1
Springflow	Ceased long-term	1	0	0	0	0	0	0	0	0	0
Springflow	Low	0	1	1	1	0	0	1	1	0	0
Air Temp	Extreme (hot or cold)	1	1	0	0	1	1	1	0	1	1
Air Temp	< 0 C	0	0	0	0	0	0	1	0	0	0
Air Temp	> 38 C	1	1	0	0	1	1	0	0	1	1
Stratification	Static water column	1	1	0	0	0	1	1	0	1	1
	No streamflow	1	1	0	0	0	1	0	0	1	1
critical period	stage 1	1	1	0	0	0	0	0	1	1	1

Preliminary data was collected and assessed in 2009 and 2010 (Tables 2 and 4) prior to conducting an investigation in 2011 at multiple sites in the morning and afternoons. During periods with daily blooms, *E. coli* sampling on a monthly basis took place in the early morning inside and outside of dinoflagellate bloom sites. *E. coli* cultures were analyzed by laboratories at the Guadalupe-Blanco River Authority, and the Edwards Aquifer Research and Data Center (Table 3). On August 5, 2009, environmental data was measured inside and outside of the dinoflagllate bloom site in this study area by the GBRA (Table 3). At the Klingeman St. crossing: GPS: N29.431330, W98.073132, in 2010, a two week long bloom event occurred. It was monitored to compare differences between early morning and late afternoon levels of dinoflagellate cell concentrations, dissolved oxygen and temperature (Table 4). Another bloom event occurred in February of 2011 during extreme cold weather.

Table 2. Environmental Data and Dinoflagellate Cell Counts August 5, 2009.

Environmental Parameters (morning)	**Outside Bloom**	**Inside Bloom**
Flow cfs	0	0
Flow m3/sec	0	0
Temperature C	23	24.2
pH	7.1	7.1
Dissolved Oxygen mg/L	4.8	1.8
Specific Conductance µS	584	582
E coli MPN	250	98
NO3-N	0.06	1.45
Total Phosphorous	0.05	0.05
N:P ration	1N to 1P	3N to 1P
Thompsodinium motile cells (afternoon)	20	20,000

Table 3. Indication of *E. coli* Contamination and Interference of *Thompsodinium intermedium* Blooms in *E. coli* Detection.

Year	Date MMDD	Military Time	*E.coli mpn* Outside Dinoflag. Bloom site	*E. coli* mpn Inside Dinoflag. Bloom site
2009	805	900	250	
2009	805	940		98
2009	827	800	411	
2009	827	805	548	
2009	921	800		68
2009	921	805	162	
2011	516	830	179	
2011	516	730		62
2011	523	730	461	
2011	731	730	2000	
2011	731	810		240
2011	811	800	333	
2011	811	810		160
2011	827	710	547	

Data in Tables1, 2, 3 and 4 were used to establish a monitoring design for an investigation to compare diel fluctuations in environmental conditions inside and outside of dinoflagellate blooms during the summer of 2011. Monitoring to be continued in the future with quality assurance of *E. coli* testing performed by EARDC was funded in part by the Comal County Water Recreation District 1 in 2009, and supported starting in 2011 by a partnership with Guadalupe Blanco River Authority and Texas Stream Team (RSI 2010).

Table 4. Klingeman St. Crossing 2010. Diel Fluctuations in Environmental Data and Cell Counts.

Diel Fluctuations in Environmental Data and Cell Counts at Klingeman St. Crossing 2010										
Diurnal dissolved oxygen and temperature						Thompsodinium bloom at Klingeman				
Bold "=" < 4mg/l DO										"> or =" 27° C
Inside Location			DO	DO	DO	Depth	Flo	Temp	Temp	Dino Cells
Date	Time		mg/L	mg/L	mg/L	m-samp	cfs	C	F	cells/ml
Sample 1	6:00		**2.8**	**2.8**	**3.2**	0.3	0	23	73.4	**0**
11-Sep	5:30	PM	10.4	10.4	10.8	0.3	0	**27**	80.6	182,000
Sample 2	6:00	**AM**	**2.6**	**2.4**	**2.4**	0.3	0	23	73.4	0
12-Sep	6:00	PM	8.8	9	9	0.3	0	26	78.8	160,000
Sample 3	7:30	AM	**2.8**	**2.8**	**2.8**	0.3	0	23	73.4	0
13-Sep	3:00	PM	10.4	10.4	10.4	0.3	0	26	78.8	164,000
Sample 4	9:00	**AM**	**3.0**	**3.0**	**3.0**	0.3	0	23	73.4	0
15-Sep	2:00	PM	11	10.6	10.6	0.3	0	**27**	80.6	140,000
Sample 5	7:30	**AM**	**3.6**	**3.6**	**3.8**	0.3	0	24	75.2	0
16-Sep	3:30	PM	11	11	12	0.3	0	26	78.8	180,000
Sample 6	9:00	**AM**	**3.8**	**3.8**	**4.6**	0.3	0	24	75.2	0
17-Sep	7:00	PM	10	10	10	0.3	0	26	78.8	140,000
Sample 9	9:00	**AM**	**4.4**	**4.2**	**4.2**	0.3	0	23	73.4	0
20-Sep	7:00	PM	9.2	9.2	9.2	0.3	0	24	75.2	60,000
Sample 18	7:10	**AM**	7.2	**4.6**	**4.3**	0.3	0	23	73.4	60,000
29-Sep	4:20	PM	12	11.8	11.8	0.3	0	26	78.8	180,000
Sample 19	7:30	AM	7.4	7.2	7.2	0.3	0	17	62.6	0

c. Comal River Environmental Investigation 2011

Monitoring was designed to detect low early morning dissolved oxygen and high late afternoon temperature levels at sites with and without monospecific blooms of the dinoflagellate *Thompsodinium intermedium*. These data were used to determine the frequency (% of samples) morning hypoxia (< 4 mg/L dissolved oxygen) and afternoon hyperthermia (> or = 27^{o} C) and fluctuation intensity (difference between PM and AM levels). Differences between morning and afternoon readings of dissolved oxygen and temperature were also compared at sites with and without monospecific blooms of *T. intermedium*.

During this study, the Comal River exhibited other conditions known to affect dissolved oxygen, such as of lack of flow and presence of fecal contamination as indicated by *E. coli*. This allowed determination of whether the dinoflagellate blooms made a significant difference in diel dissolved oxygen and temperature fluctuations when they occurred with these other stressors.

Time Period: Discrete sampling of dissolved oxygen, temperature, and dinoflagellate cells was done at least three days per week between June and October of 2011. Early morning samples were taken between 6:30 and 9:30 am, and late afternoon samples were taken between 2:00 and 6:00 pm. Since diel fluctuations in hyperthermia, hypoxia, and planktonic *T. intermedium* were observed at one site in the fall bloom of 2010, multiple sites were tested for similar trends in summer 2011. Fluctuations in these parameters were compared between sites with and without dinoflagellate bloom events in the late afternoon of 24 hr sampling periods.

Location: According to Bowles, et al. (2003), Comal Springs located in Comal County, Texas, issues from the Edwards Aquifer along the Balcones Fault zone (Garmin GPS 45 coordinates are 29° 42’49.6”N, 98°8’2.6”W). This research was conducted in the area of the Upper Comal Springruns where streamflow was absent during the summer of 2011. This area is defined as a Comal River TCEQ Stream Segment 1811(Twidwell 2004) upstream to the Klingemann Street Crossing. The location is defined as part of the critical habitat of 3 invertebrate species. Further background on the Comal River was included in the Introduction. BioWest (2010) shows the collection location for Comal Springs threatened and endangered invertebrates during the Edwards Aquifer Authority variable flow study.

Sampling sites: Sites 2-4 had dinoflagellate blooms during most of this sampling period. Site 1 had no bloom during this sample period while sites 3, 5 and 6 only had dinoflagellate blooms for a few weeks and were also monitored as sites without blooms when the afternoon bloom events did not occur (Table 5).

Table 5. Sampling Locations 2011.

Sites	**Sampling Locations**	
1	N29.720366	W98.125784
2	N29.720627	W98.125956
3	N29.720869	W98.127136
4	N29.719602	W98.129089
5	N29.719416	W98.129454
6	N29.718242	W98.130741

Sampling depth: Water samples for measurement of cells and temperature were obtained at 0.3 m below the surface. Dissolved oxygen samples were taken directly underwater by opening and closing the dissolved oxygen testing bottles from the kits at depth of 0.3 m below the surface. Probes were set to monitor temperature and light at depths 0.3 and 0.6 m below the surface.

Continuous monitoring: An array of Onset brand HOBO® temperature/light intensity probes (Figs.8-10) was deployed for periods between 4 days and 2 weeks. Discrete temperature measurements with a hand held thermometer were made (standard method # 2550-B APHA 2005) in the morning and evenings and used to calibrate these probes by adjusting their readout tables.

Presence/absence of dinoflagellate bloom: A drop of water from 0.3 m depth was transferred by a wide bore plastic pipette into a Palmer-Maloney counting chamber (standard method #10200F, APHA 2005). A bloom was recorded present if a concentration was greater than 10,000 cells/mL. The counting chamber was attached to a fiber optic field scope with 100 x magnification.

Presence/absence of dinoflagellate cells: Dinoflagellate cells were considered absent if less than 0.1 cells/mL. A liter of water was reduced to 10 mL (standard method # 10200G APHA 2005). The image in Fig. 11 was counted (standard method #10200F, APHA 2005) and bloom status, (over 10,000 cells/mL) was documented.

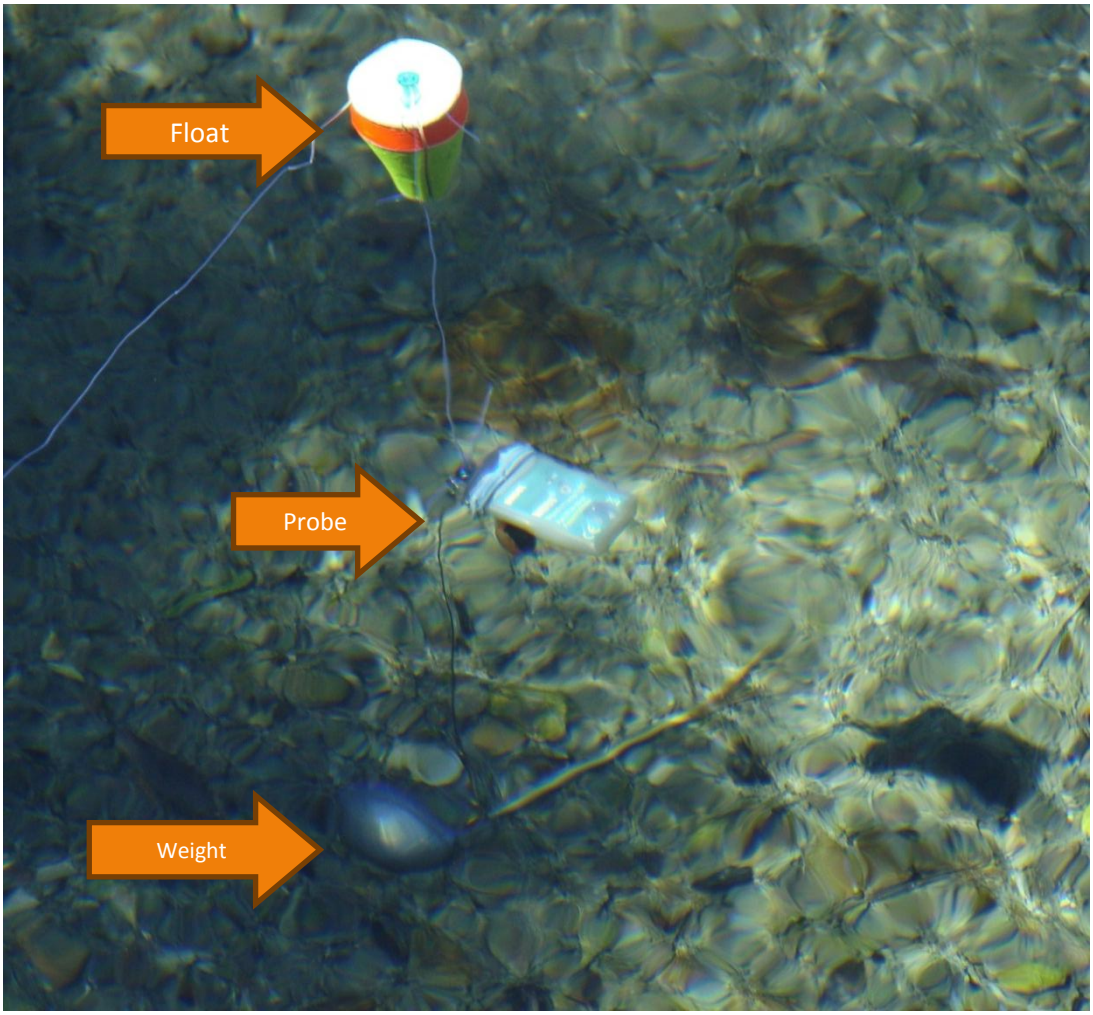


Figure 8. HOBO® light intensity /temperature probes. Probes were deployed attached to floats that were attached to weights so that they stayed a constant depth beneath the surface.

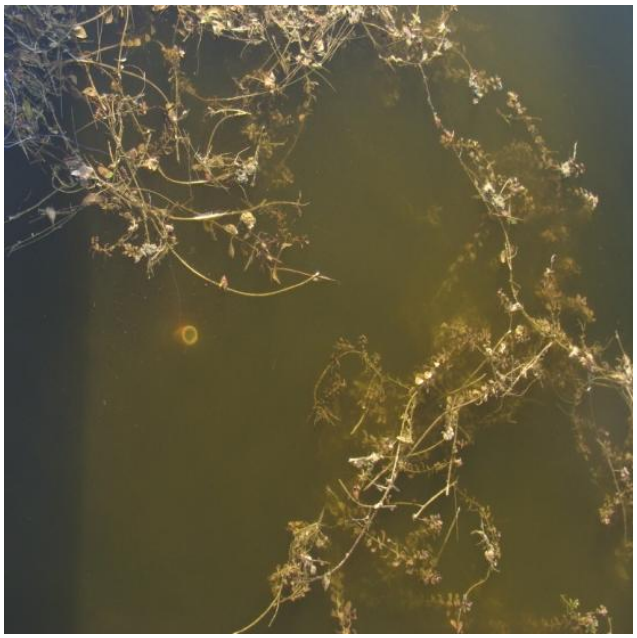


Figure 9. New bloom site in late afternoon.

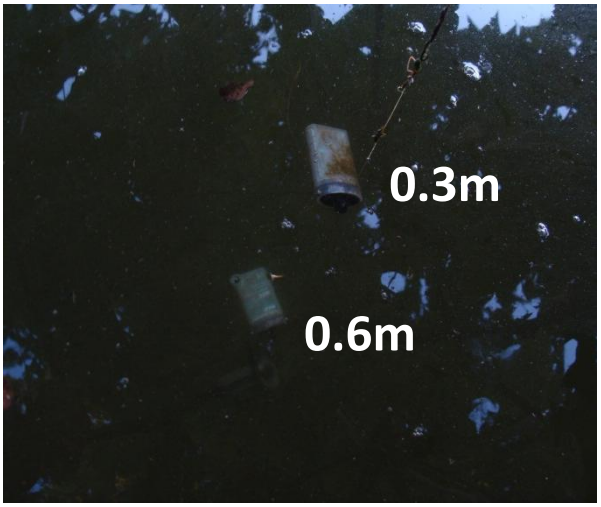


Figure 10. Morning at two-week old bloom site.
Depths at 0.3 m and 0.6 m below surface.

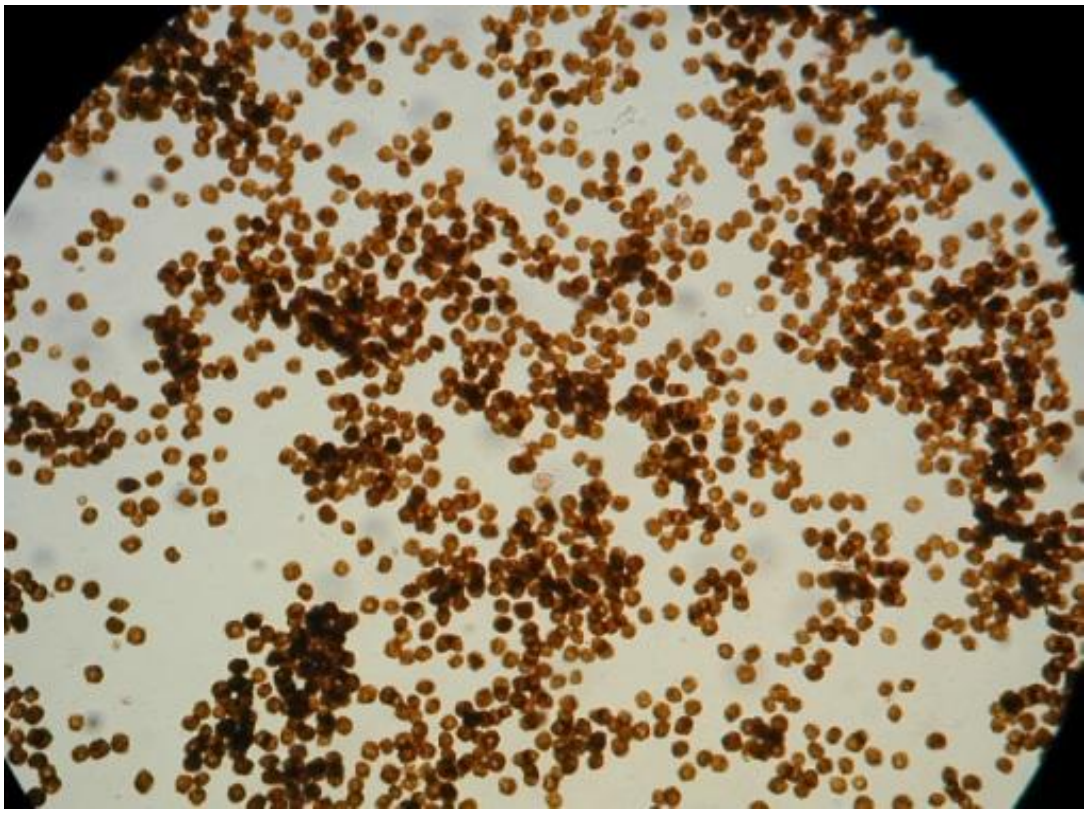


Figure 11. *Thompsodinium intermedium*, 10k cells/mL. Photographed at 100x under a field scope at the point of collection. (photo by Cheryl Gilpin).

In-situ dissolved oxygen measurement: Dissolved oxygen was measured using a La Motte Dissolved Oxygen testing kit (standard methods #4500-O C APHA 2005). The azide modification of the Winkler Method, standard method # 4500-O C (APHA 2005) was used in this study to measure dissolved oxygen concentration. The azide modification Winkler Method is a simple and very accurate reduction-oxidation reaction that fixes and measures the amount of dissolved oxygen in water at the time it is sampled. This method was developed by Lajos Winkler in 1886 and has been a reliable standard method of measuring *in-situ* dissolved oxygen ever since. Today, the Winkler Method of

measuring dissolved oxygen is used to calibrate *in-situ* dissolved oxygen probes to maintain their accuracy (APHA 2005).

Two samples were collected at each site and checked to ensure they were not more than 0.5 mg/L different for quality control. Samples were fixed in the field. The titration method used in this study differs from that used by the Texas Stream Team only with respect to the addition of the color indicator. "The Texas Stream Team Manual" (River Systems Institute 2010) requires the fixed sample to be partially titrated until it is a very pale yellow color before adding the starch indicator. However, early morning samples were already very pale yellow when they were fixed (Fig. 12) and this technique would cause the endpoint of titration to be overestimated. Therefore, in this study the starch indicator was added to the fixed sample vial prior to any titration and very small drops were left at the tip of the titration syringe (equipped with a detachable micro tip). The tiny drops were then tapped off into the sample and it was mixed in the capped titration vial between each tiny drop. This technique is suggested for samples very low in dissolved oxygen (standard methods #_4500-O C APHA 2005). Stream Team Manual (RSI 2010) directions worked best on the afternoon samples (Fig. 13). All dissolved oxygen readings below 4 mg/L were defined as hypoxia events. Frequencies of hypoxia events defined as < 4mg/L dissolved oxygen and hyperthermia events defined as > or = to 27º C were compared between samples collected at sites with and without dinoflagellate bloom events. The intensity of these events was determined by the amount of fluctuation measured between morning and afternoon dissolved oxygen concentrations. For statistical analysis, one composite set of differences in PM-AM levels from samples

collected at the sites and times when the daily afternoon dinoflagellate bloom events occurred were compared to a composite data set of samples from sites and times with no daily dinoflagellate bloom present. The significant difference between the intensity of the daily fluctuation between sites with and without dinoflagellates was statistically assessed by a 2 tailed t-test at a confidence level of 99%.

Figure 12. Dissolved oxygen sampling bottles from early morning samples. Pale color indicates low dissolved oxygen concentrations.

Figure 13. Dissolved oxygen sampling bottles from afternoon samples. Dark color indicates high dissolved oxygen concentrations.

III. RESULTS AND DISCUSSION

a. Taxonomic Descriptions

Differential Interference Contrast (DIC) microscopy resulted in the following images used for the identification of *Thompsodinium intermedium* based upon the morphology and arrangement of armored plates (Figs. 14-17):

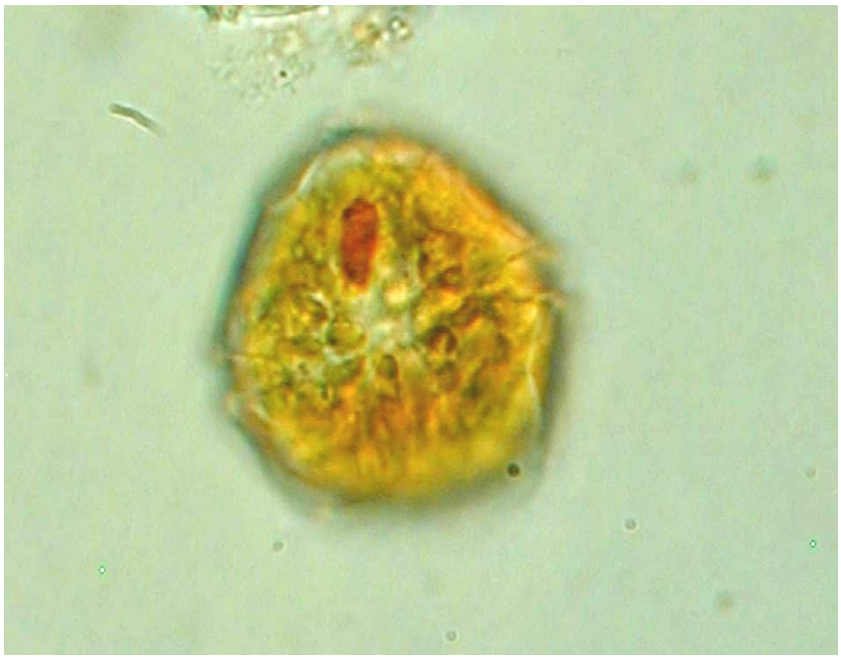


Figure 14. Armored swimming life-cycle stage of *Thompsodinium intermedium.* Note red eyespot and golden chloroplasts. Image by Cheryl Gilpin copyright 2009.

Part of this chapter, Fig. 17, was reprinted with permission from Dr. Susan Carty, Heidelberg University, Ohio for use of plate 49 from Carty, S. 1986 PhD Dissertation Texas A&M University, College Station, Texas.

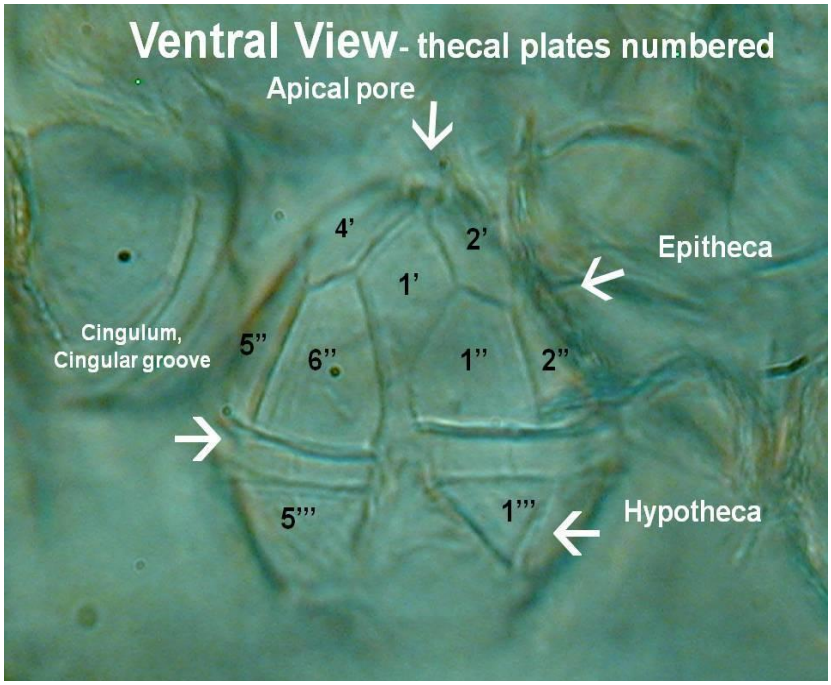


Figure 15. Front of armored plate stage of *Thompsodinium intermedium*. Image and labels by Cheryl Gilpin copyright 2009.

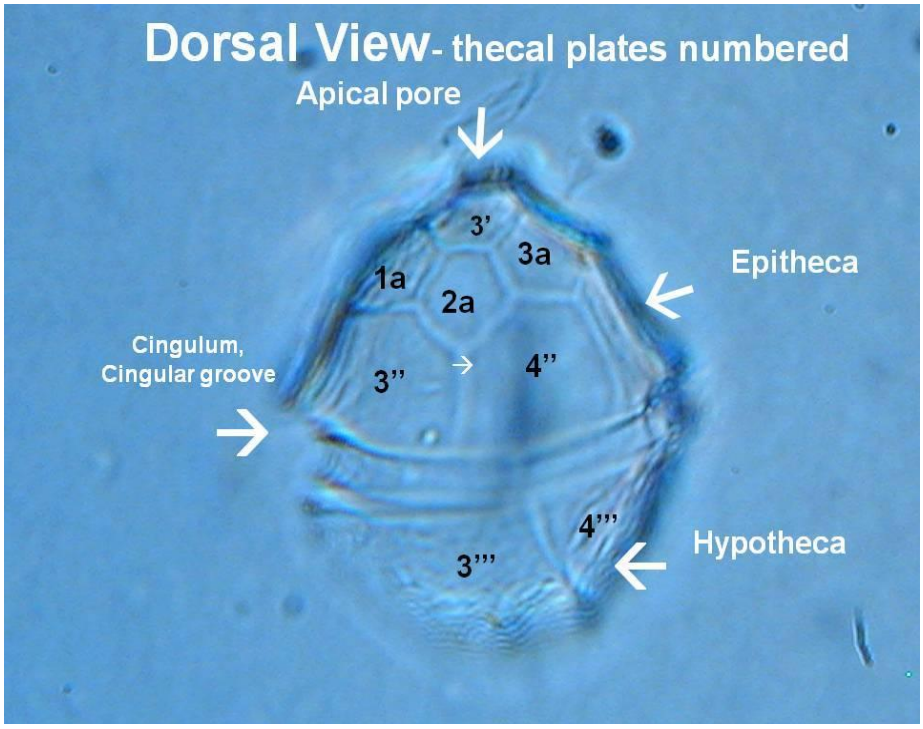


Figure 16. Back of armored cell stage of *Thompsodinium intermedium.*
Image and labels by Cheryl Gilpin copyright 2009.
Plate tabulation of 4', 3a, 6", 5"', lp, 2""

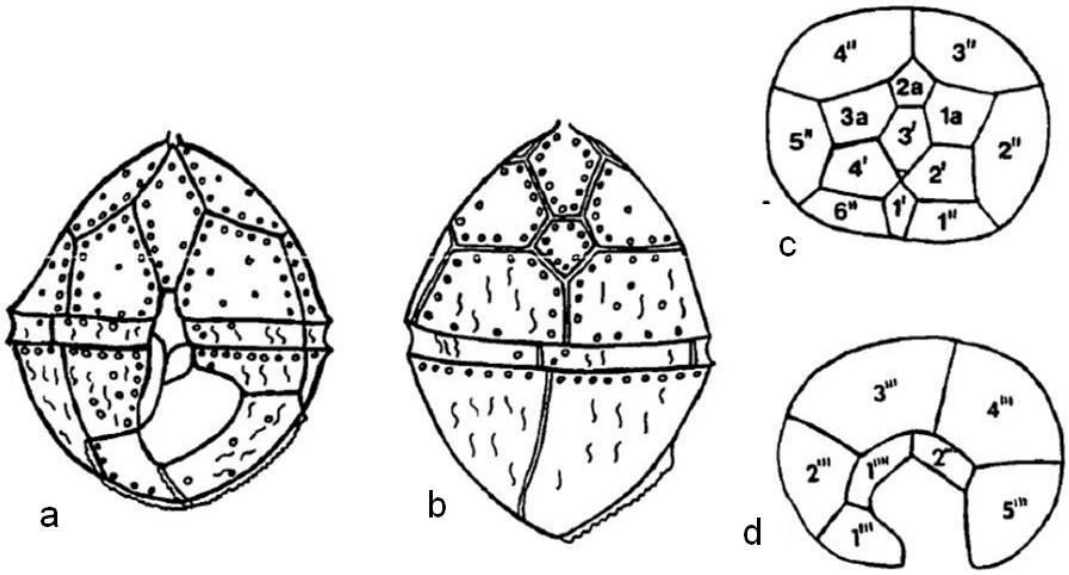


Figure 17. Line drawings of a *Thompsodinium intermedium* cell, by Susan Carty. 1986. Reprinted with permission from Susan Carty Texas A&M PhD Dissertation. Plate 49.

a. ventral (front) view: cingulum-horizontal groove, sulcal groove-vertical
b. dorsal (back) view
c. epitheca (top of cell) with apex in middle: plates numbered counterclockwise from sulcal groove
d. hypotheca (bottom of cell) with cell bottom in middle: plates numbered clockwise from sulcal groove.

The following morphological characteristics were observed. There were four single prime plates. There were six plates next to the cingulum. There were 3 back plates 1a-3a. There were five triple prime plates. There was 1 winged sulcal plate. There were 2 quadruple prime plates. These plate tabulations were listed in taxonomic keys by this code: 4′, 3a, 6″, 5‴, lp, 2‴′. Other taxonomic characteristics that were not part of this code, but needed for identification, include: 1 eyespot, 2 flagella, photosynthetic golden chloroplasts, a fringed list; 4 sulcal plates; the 2a plate is variable in shape. Cell dimensions were up to 43 microns long by up to 40 microns in diameter with slight dorsoventral compression; cingulum in the middle. By using dinoflagellate keys (Bourrelly 1970 and Carty 1986, 1989, 2003 and Wehr and Sheath 2003), the species level identification was determined to be *Thompsodinium intermedium.*

Although *Thompsodinium intermedium* was cited as a rarely reported freshwater dinoflagellate species, it may be more widely distributed and occur more frequently than previously recorded due to difficulties in identification as well as spatial and temporal patchiness (Carty 2003). In July 2011, video microscopy documented swimming behaviors of *Thomspodinimum intermedium.* Stratification in the Comal River was indicated by temperature probes at depths of 0.3 and 0.6 m. The swimming behavior of the cells indicated that the static water may have allowed dinoflagellates to swim, and to facilitate transfer of nutrients, food sources and dissolved gasses without bumping into each other. In July 2009, it was observed that if cells collided at a very minimal force, they would stick together, start to aggregate and start to transform into benthic stages.

b. Observations of Seasonal Biotic and Abiotic Conditions at Comal Springs, Summer 2009-Fall 2011

Table 1 documents the key hydrologic and atmospheric conditions in the presence/absence of dinoflagellate blooms and *E. coli* in the Comal River upper spring run between the summer of 2009 and the fall of 2011. Combined conditions of no streamflow and extreme temperature existed whenever *T. intermedium* events re-occurred in the Comal Springs. Occasionally, this species bloomed outside of critically low aquifer levels in the fall of 2010 and once during extreme cold in 2011. However, lack of flow in the stream occurred at these times. In 2011, oxygen levels were below 4 mg/L in 41% of the samples from sites with dinoflagellate events. Temperature levels equaled or exceeded 27° C in 68% of the samples with the bloom. These sites were also under previously existing influences of no streamflow and *E. coli* contamination. Differences in variation of dissolved oxygen and temperature between morning and late afternoon were compared between sites with and without planktonic dinoflagellate events by T-tests at the 99% confidence level. Sites with *T. intermedium* blooms showed significantly different amounts of diel fluctuation in levels of dissolved oxygen, temperature and planktonic cell concentration compared to sites without the bloom.

Planktonic dinoflagellate events re-occurred in the Comal River during periods of extreme air temperature along with springflow cessation in the upper Comal Springs. The following criteria were used to establish the presence/absence of abiotic and biotic conditions in Table 1. Atmospheric temperature was considered extreme if the temperature was above 38° C (100° F) or below 0° C (32° F). Upon the initial 2009

observation of the dinoflagellate bloom, springs 4 and 5 had previously ceased to flow altogether (until November 2009). In 2011, both spring 4 and spring 5 often temporarily ceased to flow.

In the summers of 2009 and 2011, the springflows determined by the USGS gage near the confluence of the Comal River with the Guadalupe River, were lower than half the long term average springflow. The aquifer levels were also critically low. These periods were referred to as the "critical period" for regional management of pumping. Currently, the stage 1 critical period is triggered by either San Antonio J-17 index well levels of 650 above sea level or Comal River streamflows of 225 cfs as mandated by Senate Bill 3 section 12, 2007. Minimum springflow model outcomes that were reported in the proposed Edwards Aquifer Habitat Conservation Plan, predicted that at Spring 4, flow would be 42.9 cfs when 150 cfs is measured at the USGS gage downstream. Hydrologic observations recorded in Table 1 during this research revealed that while the USGS gage averaged 150 cfs in the Comal River in July 2009 and 2011, springflows at spring 4 and spring 5 (Fig. 2) were ceasing in the upper springruns.

c. Comal River Environmental Investigation 2011

Between June and October 2011, in the Comal River, sites with events of the planktonic stage of *T. intermedium* caused increased frequency and intensity of hypoxia and hyperthermia compared to sites without these events. Summer diel patterns were monitored in 2009 and 2011 and it was determined that diurnal late-afternoon blooms of *T. intermedium* changed from benthic to planktonic for 2-3 hours each afternoon over a

period of several weeks to months in Comal Springs. However, some severe environmental impacts of these blooms occurred in a diel pattern through parts of the 24 hour period when the planktonic cells were not in the water column. HOBO® probes documented these life stage patterns which occurred simultaneously with abnormally high peaks in temperature and light intensity. Relationships between physical processes and blooms are discussed in Donaghay and Osborn (1997), Kudela (2010) and D'Ovidio et al. (2010) on larger scales.

The following is a physical science based explanation of the stratification/bloom triggering processes tracked with HOBO® light/temperature probes in the Comal River: Stratification or an end to mixing occurs when temperature changes with depth which happens independent of time but dependent upon spatial gradients in temperature and density. When the water column is layered, it is resistant to shear and mixing. This provides a velocity shelter required for planktonic dinoflagellates to exist in the water column since their cells are very sensitive to shear stress (Walks 2007, Maldonado and Latz 2007). Vertical mixing is dependent upon time. Phytoplankton blooms triggered by mixing can be influenced by nutrient uptake rates (also dependent upon time). But in the karst Comal Springs, dinoflagellate life stage transitions and hypoxia events were associated with occurrence of vertical stratification. In this case, the key factors were spatial gradients in temperature and density. Both of these factors were possibly influenced by the lack of flow and extreme atmospheric temperature which were found in this study to exist when these daily cycling bloom events recurred (Table 1).

A HOBO® probe readout from a probe positioned at 0.3 m below the surface in a site with blooms demonstrates the afternoon bloom and temperature peaks occurred during light intensity peaks. Declines in dissolved oxygen occurred during the light intensity valleys which began before sunset and persisted after sunrise until the late morning increases light intensity (Fig. 18).

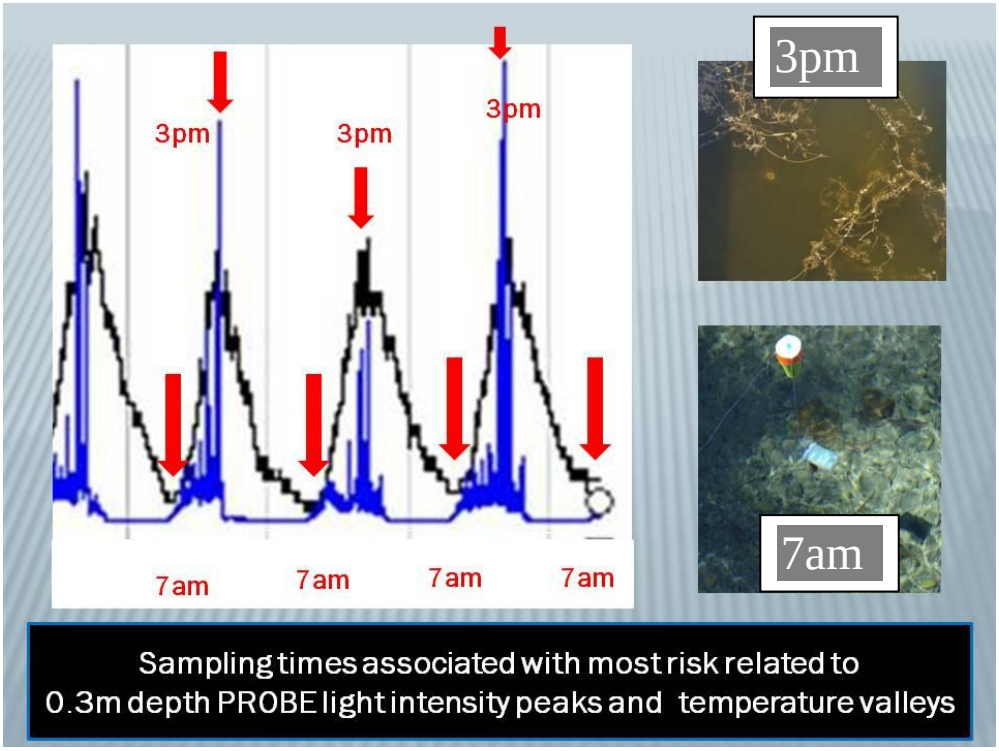


Figure 18. Diel temperature and light intensity patterns. In sites with and without blooms, sampling times were determined by the HOBO® probe at 0.3 m depth. Blue lines show peaks in light intensity (lux) and black lines show peaks in temperature ^{o}C.

The HOBO® probe readout for light intensity and temperature during one 24 hour period was isolated in Fig. 19. The observed period of the bloom is documented on this figure based on microscopic and visual observations in the field. Probes at 0.3 m and 0.6 m measured different temperatures only during the periods when the blooms occurred. It was at the onset of the temperature difference that the abnormal peak in light intensity occurred at the 0.3 m probe due to light scattering by planktonic *T. intermedium* cells (Fig. 20).

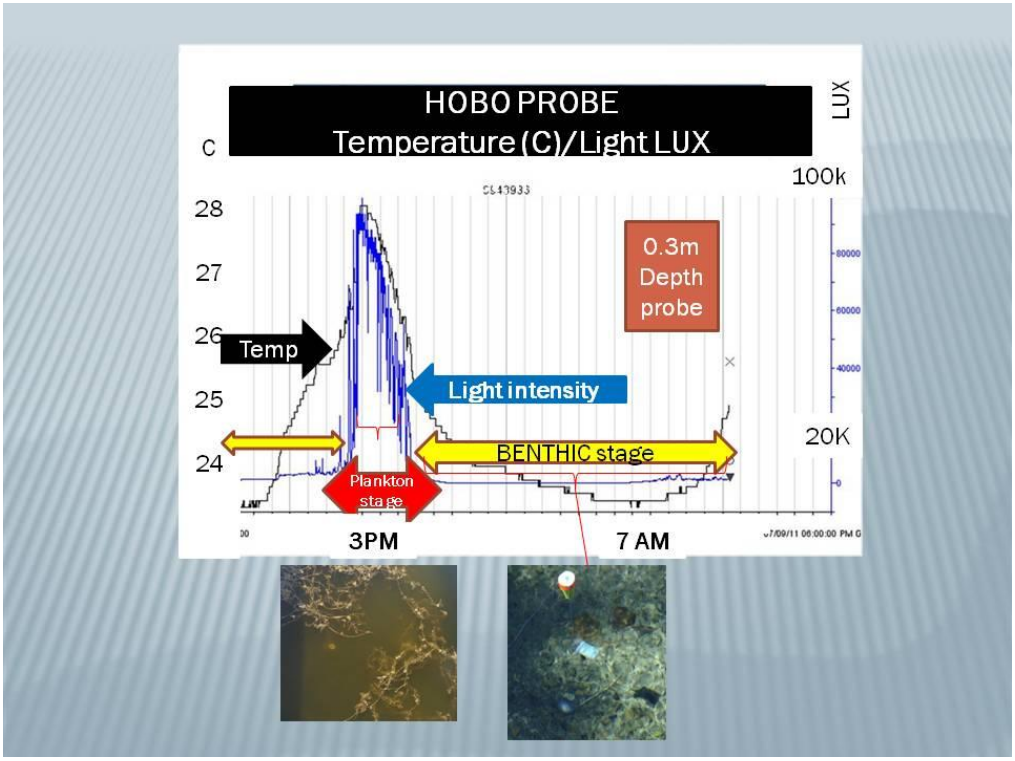


Figure 19. Typical HOBO® Probe readout for a 24 hour period. Site with a dinoflagellate bloom. Images of contrasting water clarity in the late afternoon (plankton stage) and early morning (benthic stage) at a bloom site.

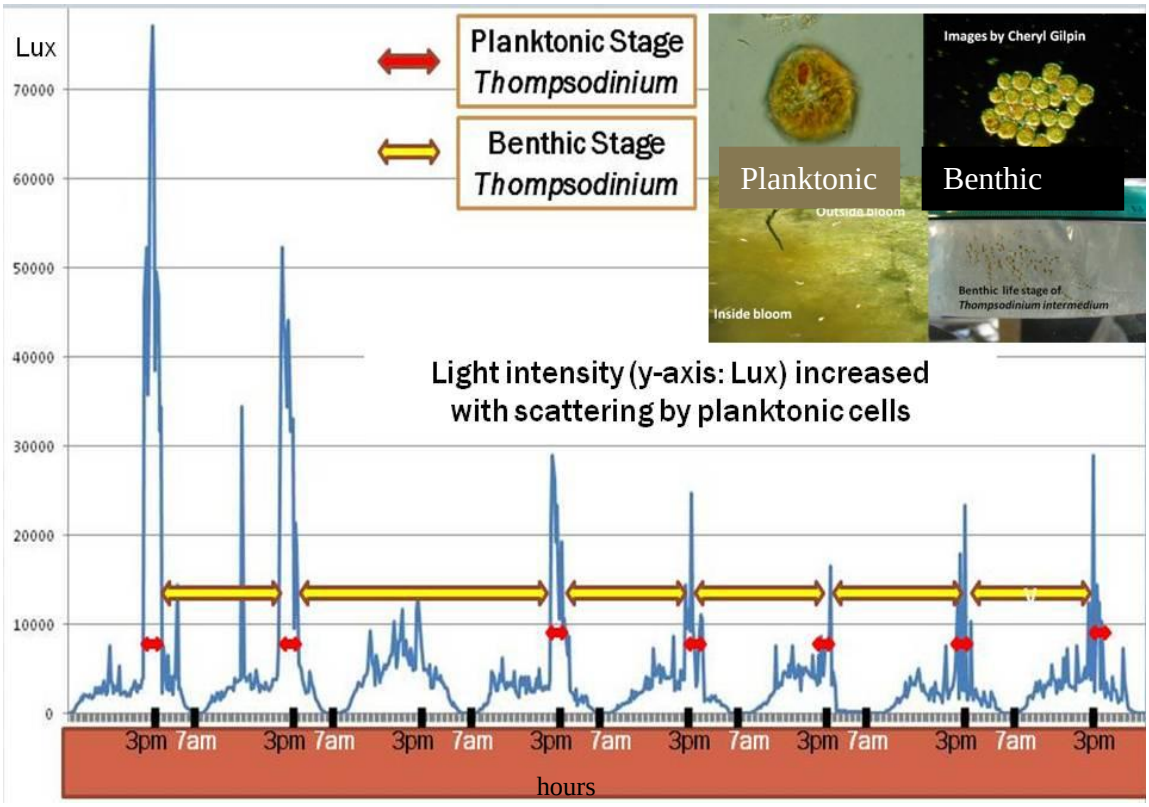


Figure 20. Light intensity measured in lux shows extreme change at the same time the brown cloudy water appeared.

Sites 2, 3 and 4 had the longest lasting blooms with the most concentrated cell counts and covered the widest area. The widest area bloom was at site 2, it was over 20 square meters with late afternoon cell counts over 100,000 cells per ml between mid July and late August. In the 28 days sampled during that intense bloom period at site 2, 23 days had late afternoon dissolved oxygen levels over 10 mg/L, 4 of those days were over

15 mg/L and the highest measurement was 16.8mg/L. During that period at site 4, morning dissolved oxygen levels were the lowest. For six weeks in 20 of 20 samples collected in the morning at site 4, dissolved oxygen was below 4 mg/L. During four of those mornings dissolved oxygen was less than 2 mg/L. The lowest dissolved oxygen level at site 4 during that period was 1.4 mg/L. Dinoflagellate cell concentrations at site 4 in the late afternoon were between 10,000 and 40,000 cells per ml. At site 4, risk of hypoxia in the early morning was 100% for six weeks with afternoon dinoflagellate blooms present.

The total number of morning samples with dissolved oxygen readings < 4 mg/L from days and sites with dinoflagellate blooms was divided by the total number of days and sites with blooms sampled. Table 6 depicts the sharp contrast between risks of hypoxia and hyperthermia inside the blooms compared to outside the blooms. Fig. 21 depicts the sharp increase in risk of hypoxia inside the blooms compared to outside the blooms. Fig. 22 depicts the sharp increase in risk of hyperthermia inside the blooms compared to outside the blooms.

Differences in variation of dissolved oxygen and temperature between morning and late afternoon were compared between sites with and without planktonic dinoflagellate events by T-tests. Sites with *T. intermedium* blooms showed significantly different amounts of diel fluctuation in levels of dissolved oxygen, temperature and planktonic cell concentration compared to sites without the bloom. This was demonstrated by a T-test at the 99% confidence level (Figs.23-24).

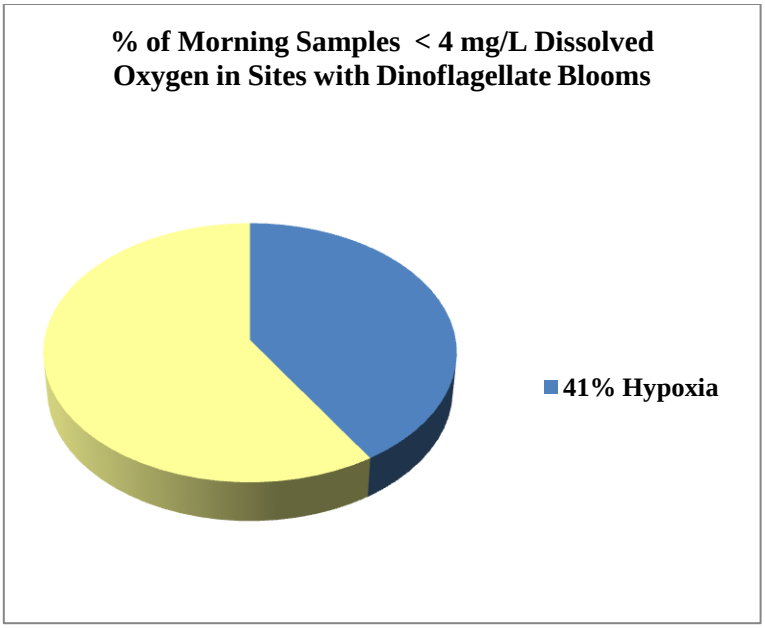
Table 6. Frequency (Risk) of Hypoxia and Hyperthermia 2011. Sites and %Days (a) with and (b) without events of daily cycling *T. intermedium* blooms.

Days When Afternoon > 10K cell/mL = w/Bloom			
Site	# of Days with blooms	# of Days <4 mg/L Hypoxia	# of Days ≥ 27 °C Hyperthermia
2	28	6	14
3	14	5	13
4	20	20	15
5	13	10	11
6	24	0	14
Total	99	41	67
%		41	68

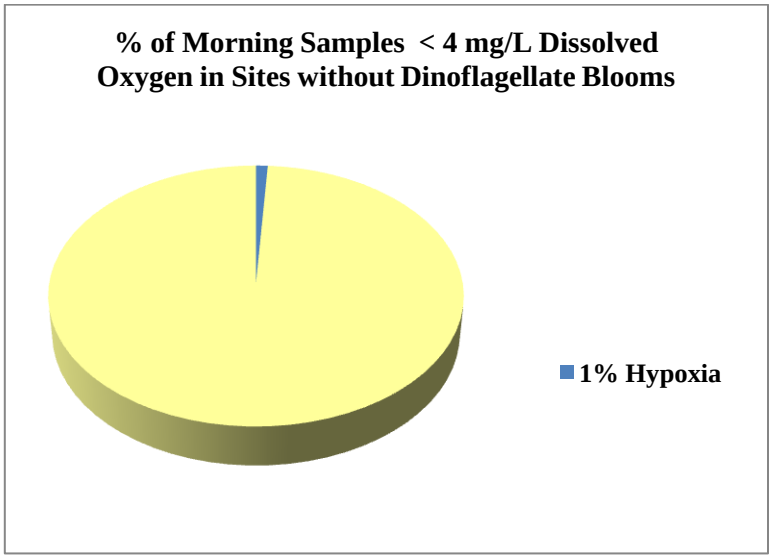
(a)

Days When Afternoon Dinoflagellates < 0.1 cells/mL = w/o Bloom			
Site	# of Days without Blooms	# of Days <4 mg/L Hypoxia	# of Days ≥ 27 °C Hyperthermia
1	23	0	0
3	23	1	4
4	15	0	0
5	16	0	0
6	24	0	7
Total	101	1	11
%		1%	11%

(b)

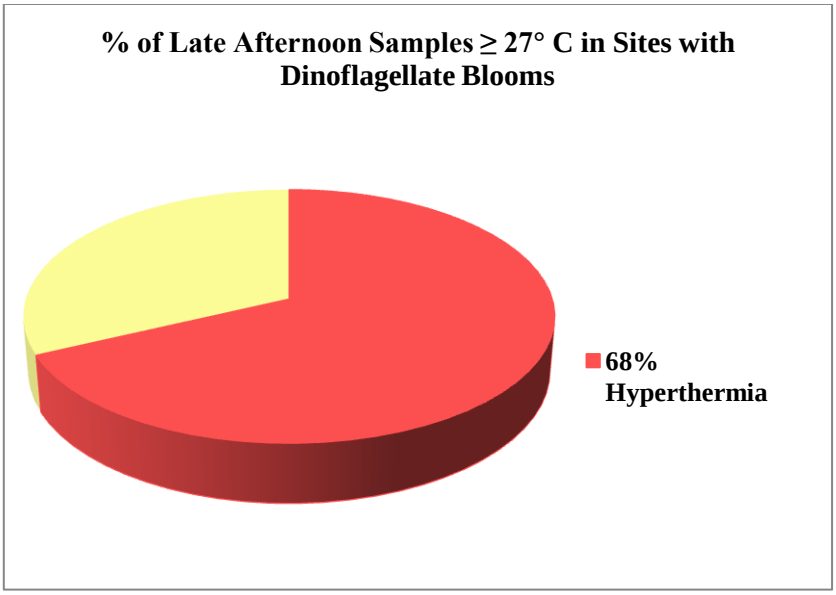


(a)

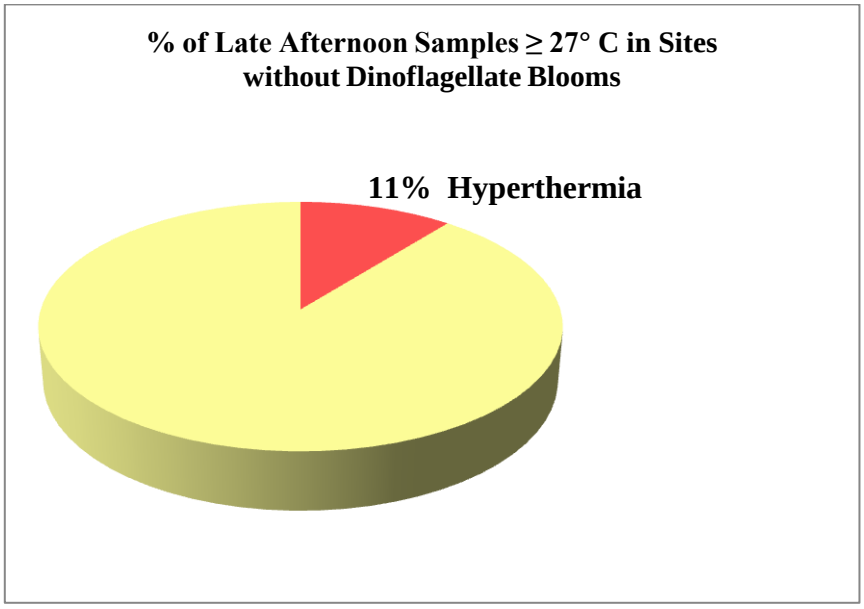


(b)

Figure 21. Risk of hypoxia as dissolved oxygen < 4 mg/L. Risk of hypoxia is defined here as the chance of encountering hypoxia determined by the percent of samples in which dissolved oxygen < 4 mg/L.



(a)



(b)

Figure 22. Risk of hyperthermia inside (a) dinoflagellate blooms compared to outside (b) the bloom sites is compared in the Comal River, Summer 2011.

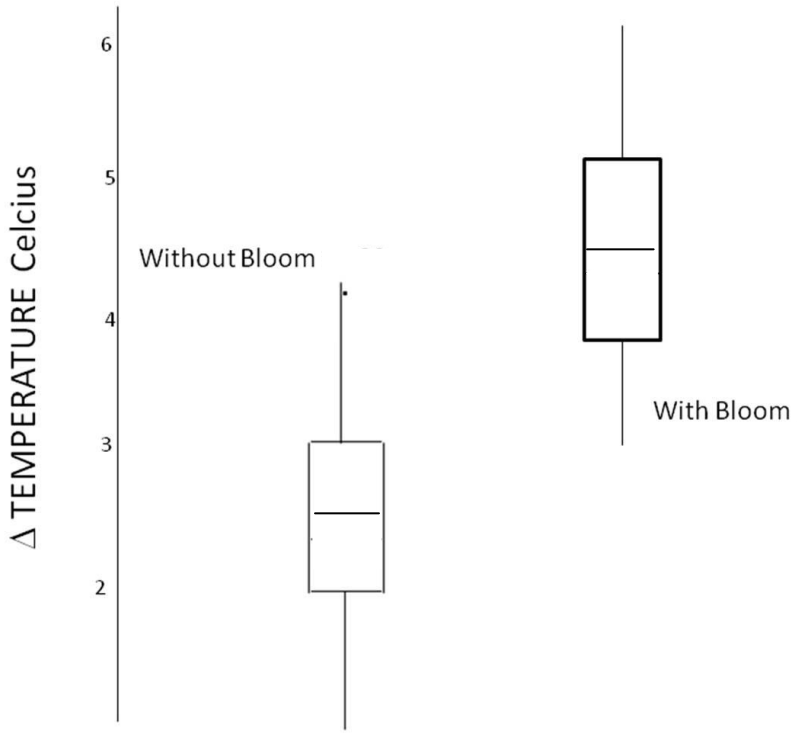


Figure 23. Confidence interval box plots for fluctuation in temperature in sites with and without *T. intermedium* blooms. No overlap of boxes: therefore, there is a significant difference in temperature fluctuation between groups of samples with and without blooms. T test with confidence level of 99% and p value of 2.58 showed significant difference between groups with and without blooms.

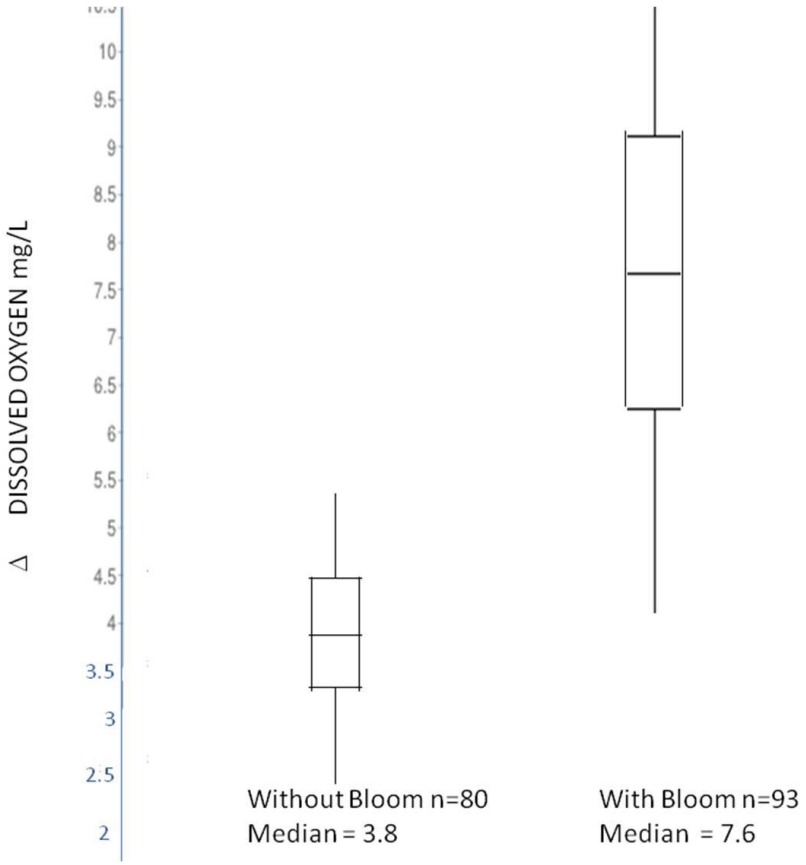


Figure 24. Confidence interval box plots for fluctuation in dissolved oxygen in sites with and without *T. intermedium* blooms. No overlap of boxes: therefore, there is a significant difference in dissolved oxygen fluctuation between groups of samples with and without blooms. T test with confidence level of 99% and p value of 2.58 showed significant difference between groups with and without blooms.

IV. APPLICATION TO CRITICAL PERIOD MANAGEMENT

The increased frequency and intensity of hypoxia and hyperthermia events shown in this study indicate the following actions may be required to satisfy agreements to implement the 2011 draft Edwards Aquifer Habitat Conservation Plan (HCP) (EARIP 2011) in the upper Comal Spring headwaters and local index wells during and after periods of atmospheric temperature extremes and no streamflow which occur during and in between periods of critically low aquifer levels in the Upper Comal River springruns:

1. Continuous *in-situ* monitoring of temperature, dissolved oxygen, flow, salinity, *E. coli*, and dinoflagellates. An array of light/temperature *in-situ* probes at two depths helped determine when and where stratification, planktonic dinoflagellates and low dissolved oxygen occurred for establishing sampling designs.
2. Early morning and late afternoon testing of these parameters if continuous monitoring is not possible.
3. Hypoxia should be considered a major determinant of habitat suitability, not just for adaptive management, but in determination of issuing a United States Fish and Wildlife Service (USFWS) “take” permit for management of critical habitat at Comal Springs in accordance to the Endangered Species Act, as amended 16 U.S.C. §§ 1531 Section 10 (a)(2).
4. Implement localized pumping management when old diffuse groundwater springflow is not adequate to buffer changes.
5. Implement stormwater pollution monitoring to determine the delayed impacts of drought and low flow periods.

In future droughts when springflows are lower than 150 cfs and the upper Comal River springruns have no flow during extreme heat, local pumping and water use practice changes may be required such as changing daily water use routines of lawn watering to only take place between 10 pm and 1 am in the evening and other non-emergency domestic, commercial and industrial water uses to only take place between 10 am and 1 pm from the late morning to early afternoon and 10 pm and 1am in the late evening. This would minimize draw downs near the springs in the early morning and late afternoon when low dissolved oxygen and peaks in temperature in sites with dinoflagellate blooms are most likely to occur.

If localized pumping management changes are inadequate to provide mitigation on as short a time scale as harmful hypoxia and hyperthermia events occur, then a minimum flow rate above 150 cfs must be determined that will buffer against formation of static water conditions and hypoxia in these upper spring areas. This may be a more effective minimum springflow management strategy for protection of wildlife and human health as well as for preventing harm to endangered species from springruns to coastal wetlands. According to the Texas Edwards Aquifer Act, SB 1477, 73rd legislative session, agency policies and management strategies must demonstrate that the quality of aquifer water will be protected (EAA Act, Section 1.14(a)(2)) and that adequate flows for instream uses, bays and estuaries will be ensured (EAA Act, Section 1.14(a)(8)) (Votteler 2000).

V. CONCLUSIONS

Many factors interact in the field which may increase the intensity and frequency of hypoxia and hyperthermia events beyond what simplified models can derive or assume. That is why most investigations to determine how multiple stress factors influence fish species survival or geographical habitat distributions rely heavily upon large sets of data collected in the field at proper spatial and temporal scales (Hubbs 1995 and 2001, Feminella and Matthews 1984, Matthews and Matthews 2003, Magoulick 2000, Schenck and Whiteside 1976, Smale and Rabeni 1995 a and b). Implications from this field based assessment during low flow periods in a karst springrun are very different from those based upon laboratory assessments which assume little variation in environmental changes that are buffered by adequate springflow. However, the 2011 draft HCP relies heavily upon laboratory demonstrations such as BioWest (2002) and assumed and derived environmental conditions where there was inadequate field data from the Comal River. In this study, it was shown that during periods of low flow only by measuring dissolved oxygen and temperature responses *in-situ* throughout 24 hour periods or by continuous monitoring can interacting karst stream stress factors that influence hypoxia and hyperthermia show their combined impacts on species in the habitat. In the summer of 2011, the planktonic stage of *Thompsodinium intermedium* in the Comal River increased risk of hypoxia and hyperthermia at springflow levels previously assumed to buffer against hyperthermia in the upper springrun of the Comal River. The lack of buffering of impacts from these factors could be detected more easily in a karst habitat than in an estuary with tidal fluxes. This study demonstrated the

findings of Brownlee et al. (2005) that planktonic dinoflagellates should be considered as an agent of severe oxygen depletion. Hypoxia, hyperthermia, and the retention of stormwater pollution each create conditions that support harmful disease causing microbes that can impact human and wildlife health in springruns and coastal wetlands during and after periods of low streamflow in springruns.

Typically, adequate springflows comprised of older groundwater buffer changes in water temperature, even as air temperature varies. Fluctuation in water temperature at sites with and without afternoon dinoflagellate blooms at the Comal Springs location indicated that the flow of older groundwater was not adequate to buffer water temperature change and may have led to static, stratified water conditions. The instantaneous response of vertical stratification repeating in a predictable diurnal pattern at exactly the same time and place as the diurnal life stage transitions to planktonic *Thompsodinium intermedium* strikingly demonstrated that significant negative environmental impacts must be monitored, assessed and managed within the same short time scales. These data indicate that it may not be reasonable to expect localized management of water quality in the Comal River to sustain endangered species instead of managing regionally to maintain adequate springflow to buffer against habitat conditions of hyperthermia and hypoxia. The risks of not doing so threaten not only endangered species survival but also the health and survival of other wildlife and humans that depend upon the springflows. The impacts and risks may potentially extend temporally and spatially from springruns to the coast during low springflows and for extended periods of time after springflows resume as harmful microbes under hypoxic and hypothermic

conditions in springruns are nurtured and spread within the springshed system. This research demonstrated new microbial and water quality monitoring designs and their application to improve assessment of hypoxia and hyperthermia in karst springruns in order to help future monitoring and modeling efforts coordinated between stakeholders and agencies better understand, assess, predict and mitigate to reduce harmful low springflow impacts on wildlife and humans.

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