# ABSTRACT

Hypolimnetic Oxygen Decline and the Hypolimnetic Bacterial Community

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This study reports on bacterial cell size and abundance change during oxygen depletion of Belton and Stillhouse Hollow reservoir. Bacterial cell size and abundance are compared with amount of heterotrophic nanoflagellates, dissolved oxygen, temperature, and time. The Relative Areal Hypolimnetic Oxygen Demand (RAHOD) was calculated for Belton and Stillhouse Hollow reservoir. Bacterial abundance in Belton and Stillhouse Hollow Reservoir was not greater than bacterial abundance in natural, northern lakes. Bacterial abundance's were not correlated with time, dissolved oxygen, temperature, and nanoflagellate abundance. Bacteria cell sizes in Belton and Stillhouse Hollow Reservoir were smaller than bacterial cell sizes in natural, northern lakes. Bacteria cell sizes were not correlated with temperature or nanoflagellate abundance. The RAHOD of Belton and Stillhouse Hollow Reservoir were lower than the RAHODs of natural northern lakes.

# Hypolimnetic Oxygen Decline and the Hypolimnetic Bacterial Community

A Thesis Submitted to the Faculty of

Baylor University

in Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

By

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Waco, Texas

August, 1998

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# ACKNOWLEDGMENTS

I thank Dr. Owen T. Lind for his guidance, support, and for giving me the opportunity to learn about Limnology. I am thankful to my field and laboratory helpers: Dr. Steve K. Alexander, Eli Barcena, Christie Benson, Katie Booker, Mark Duncan, David Fleming, Steve Fleming, Kirk J., Dr. Owen Lind, Bradley Meyers, Helen Pittman, Mawbe Rendon, Harvey Richey, and Michelle Trahan. I thank Tom Parks for his statistical consulting.

I am grateful to my family and friends for their encouragement and support for the last three years. Special thanks to the Brazos River Authority for the use of their Hydrolab DataSonde unit. Funding for this project was provided by the Brazos River Authority, the Jack G. and Norma Jean Folmar Research Fund, and Dr. Owen T. Lind.

# CHAPTER ONE

## Introduction / Literature Review

Reservoir eutrophication is a water quality problem that affects decisions made by reservoir managers, biologists, and land developers. My study considered three different, yet related, possible consequences of reservoir eutrophication: formation of oxygen deficits, bacterial abundance, and bacterial cell size. Oxygen deficits have been used to assess and monitor eutrophication of lakes (Hutchinson 1957; Wetzel 1983). Bacteria are important to eutrophication of reservoirs because they play three essential roles in reservoir ecosystems (Chróst and Siuda 1977). Bacteria: use dissolved organic matter and consume oxygen (Wright and Hobbie 1966; Wright 1975; Williams and Yentsch 1976), recycle nutrients (Hobbie and Crawford 1969; Hoppe 1976; Williams et al. 1976; Azam et al. 1983; Gude et al. 1985), and convert dissolved substrates into particulate matter. Consumption of bacteria brings back particulate matter to the food web (Kuznetsov 1970; Williams 1983; Scavia D. and Laird G. 1987; Sherr and Sherr 1988).

### Stratification and dissolved oxygen

Some reservoirs stratify. Stratification and anoxic water occur in central Texas at Belton, Possum Kingdom, Hubbard Creek, Granbury, Whitney (Flugrath and Chitwood 1982), and probably other reservoirs.

Stratification occurs when the surface waters increase in temperature and become less dense. The lake separates or stratifies into three layers: epilimnion (upper part), metalimnion (middle), hypolimnion (bottom part) (Rigler 1964). Resistance to mixing increases, resulting in stratification (Wetzel 1983).

The epilimnion is usually warm and oxygen rich during the summer. The metalimnion is an area with rapidly decreasing temperature. (Atlas and Bartha 1993). Oxygen from the epilimnion is unable to diffuse into the isolated hypolimnion. Organic matter produced by photosynthesis in the epilimnion or allochthonous sources sink into the hypolimnion where it will decompose and consume oxygen (Hutchinson 1957). This oxygen depletion produces a different and changing environment for the biota, including bacteria. It is important to our understanding of lake dynamics and eutrophication to know how bacteria respond to oxygen depletion, and there is uncertainty regarding this response.

## Bacteria

### Bacterial abundance

Abundance and density are important ecological parameters in the study of bacterial populations. Various studies have found that bacterial abundance is directly proportional to a lake or ocean's trophic state (Silvey and Roach 1964; Straskrabova 1968; Kuznetsov 1970; Jones 1972, 1977; Faust and Correll 1976; Godlewska-Lipowa 1976, 1979; Ferguson and Palumbo 1979;

Hobbie and Wright 1979; Rae et al. 1979; Fuhrman et al. 1980; Saunders 1980; Azam et al. 1983; Bird and Kalff 1984).

Determining bacterial abundance. Traditionally spread plate counts determined bacterial abundance. Incubations of inoculated agar plates allowed bacteria to grow colonies. Colony counts and the dilution factor determined the bacterial abundance. This method was selective for bacteria that grew on enriched agar and at specific incubation temperatures (Atlas and Bartha 1993). Spread plate counts underestimated bacterial abundance by a factor of ten to one hundred (ZoBell 1946; Daley 1979).

Currently, direct microscopy counts are used to calculate bacterial abundance (Jones and Mollison 1948; Frederick 1965; Gray 1967; Gray et al. 1968; Harris et al. 1972; Daley and Hobbie 1975; Byrd and Colwell 1992; Atlas and Bartha 1993). Direct count methods include electron microscopy, epiflourescence microscopy, flourochrome-labeled antibodies, and flow cytometry (Hobbie et al. 1977; Schmaljohann et al. 1987; Hoff 1993; Button and Robertson 1993). Epifluorescence microscopy is the most widely direct count method used (Hobbie et al. 1977; Choi et al. 1996).

Hypolimnetic bacterial abundance. Research on bacterial abundance has accumulated during the past five years. Cole et al. (1993) was a catalyst to later papers on the subject of hypolimnetic bacterial populations. They studied twenty natural, northern lakes, and found that bacterial cell abundance was greater in anoxic hypolimnetic water than oxic epilimnetic or oxic hypolimnetic waters. Bacteria in anoxic waters were twice as numerous

as bacteria in oxic waters (Cole et al. 1993). Jones (1978) and Ochs et al. (1995) found more bacteria in anoxic hypolimnia than in other layers. Bacterial densities stabilize after a hypolimnion is anoxic. Rod and vibrio-shaped cells are more abundant than coccus-shaped cells (Ochs et al. 1995). Lind and Dávalos-Lind (personal communication), in contrast, observed that in Douglas Lake, Michigan the bacterial abundance changed very little during stratification.

Bacterial cell size

Bacterial biomass, another description of bacterial density, is the mass of living bacteria, and is a function of abundance and cell size (Bratbak 1993). Calculations of bacterial biomass involve estimates of number and volumes of coccus and bacillus shaped bacteria (Cole et al. 1993).

Bacterial biomass is an ecologically important parameter, because it estimates the amount of energy stored in bacteria (Atlas and Bartha 1993). An important use of bacterial biomass data is estimating the amount of transferable energy to higher trophic levels (Atlas and Bartha 1993).

Hypolimnetic bacterial cell size. Bacteria in anoxic (0 mg / L $O_2$) waters were found to be bigger than bacteria from oxic water. Anoxic water bacteria were twice the size of oxic water bacteria (Cole et al. 1993). Coccus-shaped cells increased in size during stratification, while rod and vibrio-shaped cells remained the same size (Ochs et al. 1995).

Lind and Dávalos-Lind (personal communication) in contrast to Cole et al. (1993), Cole and Pace (1995), and Ochs et al. (1995) found larger cell sizes

in a low oxic (2-3 mg / L $O_2$) hypolimnion. Mean bacterioplankton cell size increased during stratification.

Possible explanations for larger bacterial cells. Cole et al. (1993) proposed four possible explanations for increased cell size: species shift, reduced respiratory metabolism in cooler water, reduced predation on larger cells, and greater availability of nutrients and organic substrates for growth.

The first explanation of Cole et al. (1993) proposes a change in species composition from aerobic to anaerobic organisms. Examples of anaerobic organisms include sulfur and nitrifying bacteria. Lind and Dávalos-Lind (personal communication) have data that did not support a change to anaerobic organisms because they found larger cells when the hypolimnion oxygen concentration was 2-3 mg per liter.

The second explanation of Cole et al. (1993) proposes that colder temperatures in the hypolimnion could cause larger cell size. In Cole et al.'s (1993) data, cell size and temperature were not related. Large bacteria were in warm anoxic hypolimnions not in colder oxic hypolimnions (Cole et al. 1993). Lind and Dávalos-Lind (personal communication) had similar results in that hypolimnia with large bacteria were not colder than hypolimnia without large bacteria.

The third explanation of Cole et al. (1993) proposes lower bacterial predation rates by bacteriovores. Nanoflagellates are major predators of bacteria (Haas and Webb 1979; Fenchel 1982; Azam et al. 1983; Linley et al. 1983; Sherr et al. 1984; Porter et al. 1985; Berman et al. 1987). Nanoflagellates

may be less abundant and less active in anoxic hypolimnia (Cole et al. 1993). Some specialized protozoa inhabit anoxic waters, but their abundance is usually low (Fenchel et al. 1990). Lind and Dávalos-Lind (personal communication) found large bacterial cells in oxic hypolimnia (2-3 mg $l^{-1}$). Nanoflagellates would be present in such oxic hypolimnia.

The fourth explanation of Cole et al. (1993) proposes that greater availability of inorganic and organic nutrients increase bacterial cell size. In anoxic hypolimnia, nutrients are abundant (Atlas and Bartha 1993). Internal loading from the sediments can cause abundance of nutrients when a critical low Redox potential is reached. As long as there is oxygen overlying the lake sediments, the redox potential is high (> 200 mv), and the sediments will retain their oxidized nutrients. The redox potential decreases with low concentrations of oxygen. When the redox potential reaches 0 mv the sediments release their nutrients; e.g., internal loading (Mortimer 1942; Wetzel 1983).

Greater total phosphorus and ammonium levels correlate with larger bacterial cell size (Cole et al. 1993). Lind and Dávalos-Lind (personal communication) in contrast to Cole et al. (1993) found large bacterial cell size in low oxic hypolimnia. Two to three mg of oxygen per liter does not cause 0 mv redox potentials. They also point out that if internal loading of nutrients was a factor in producing large bacterial cells, one would expect to find the largest bacteria near the sediments. They found large bacterial cells 2 m away from the sediments.

Thesis questions

Previous studies of bacterial abundance and cell size used natural northern lakes. Reservoirs, which are artificial lakes, will tend to biologically respond differently than natural, northern lakes (Thornton et al. 1990). This portion of my study tried to answer the following questions:

- Will bacterial sizes and bacterial abundance be greater in Belton and Stillhouse Reservoirs than in northern lakes because of the presumed heavier sediment / nutrient loads to reservoirs?
- As oxygen decreases in the hypolimnion, will bacterial abundance or cell size change?
- Does bacterial abundance or cell size correlate with temperature?
- Does bacterial abundance or cell size correlate with ammonia nitrogen?
- Are nanoflagellates (bacteriovores) present in the anoxic hypolimnion and how does their abundance correlate with a change in oxygen?
- Does the presence of nanoflagellates affect the bacterial size and bacterial abundance?

Oxygen deficits

Oxygen deficits can be used to assess trophic states of lakes (Hutchinson 1957; Wetzel 1983). Oxygen depletion rates relate to trophic states (Lind 1987; Lind and Dávalos-Lind 1993). The basic assumption of oxygen depletion rate

studies is that organic matter produced by photosynthesis in the epilimnion sinks into the hypolimnion where it decomposes and consumes oxygen. Measuring the rate of oxygen depletion is an indirect way of measuring a lakes' photosynthetic production (Hutchinson 1957; Wetzel 1983; Lind and Dávalos-Lind 1993). Hypolimnetic oxygen deficits correlate with primary productivity of phytoplankton and with phosphorus loading (Welch and Perkins 1979; Wetzel 1983).

Methodology development for determining oxygen deficits

Modifications of the method for calculating oxygen depletion rates have occurred over the past 70 years. In 1928, Thienemann used actual deficits to measure production (Hutchinson 1957). An actual deficit is the difference between the observed oxygen content and the saturation concentration of that water at the water sample's temperature and atmospheric pressure. A problem with this method was that it assumed the water was saturated during spring turnover at the observed temperature.

In 1927 and 1929, Alsterberg used absolute deficits to measure production (Wetzel 1983). Absolute deficit is the difference between the observed oxygen content and the saturation value at 4 $^{0}$C at the atmospheric pressure of the lake. The problem with this method was that it assumed the water was saturated at observed temperature (4 $^{0}$C) during spring turnover (Hutchinson 1957; Wetzel 1983).

In 1931, Strøm developed the relative deficit method (Wetzel 1983). Relative deficit is the difference between the oxygen content of the

hypolimnion and the oxygen content measured at the end of spring turnover (Hutchinson 1957; Wetzel 1983). The problem with this method was that it did not account for lakes of differing hypolimnetic volumes (Thienemann 1926; 1928; Hutchinson 1957; Wetzel 1983).

In 1931, Strøm developed the hypolimnetic areal deficit and in 1938 and 1957 Hutchinson modified it (Wetzel 1983). The hypolimnetic areal deficit is the average oxygen deficit below one $cm^2$ of hypolimnetic surface and consists of the sum of each layer's deficit. The problem with this method was that it did not account for lakes of shallow volumes or hypolimnion temperature differences (Cornett and Rigler 1979; Cornett and Rigler 1980; Charlton 1980b).

Charlton (1980b) found that shallow hypolimnia (< 50 m) do not completely oxidize all the organic matter that enters. The organic matter in shallow hypolimnia settles onto and into the sediment and does not add to the oxygen depletion rate. All organic matter in deep hypolimnia (greater than 50 m) decomposes and therefore contributes to the oxygen depletion rate (Charlton 1980b; Lind and Dávalos-Lind 1993).

Temperature correction is necessary because temperature affects the rate of metabolism, i.e., the Van't Hoff principle (Charlton 1980a; Lind and Dávalos-Lind 1993). Lake Erie's rate of oxygen depletion has increased over the years. When the increase in hypolimnetic temperature was accounted for, the rate of oxygen depletion was not as great (Charlton 1980a).

Charlton (1980b) proposed the relative areal hypolimnetic oxygen deficit (RAHOD) method. This method calculates the mean oxygen deficit below one $cm^2$ of hypolimnetic surface. The method then corrects the oxygen deficit to a standard temperature (4 $^0C$) and to a standard hypolimnetic thickness (5000 cm).

Oxygen deficits and trophic states

Oxygen depletion rates can indicate trophic status of lakes. The two prominent oxygen depletion rate trophic indices are Hutchinson (1957) and Mortimer (1941). Wetzel (1983) states that most researchers use Mortimer's oxygen depletion rate index. Mortimer's index (1941) uses the following oxygen depletion rates mg $O_2$ $cm^{-2}$ $day^{-1}$: oligotrophic < 0.017, mesotrophic 0.017 to 0.033, eutrophic > 0.033. Hutchinson's index (1957) uses the following oxygen depletion rates mg $O_2$ $cm^{-2}$ $day^{-1}$: oligotrophic < 0.025, mesotrophic 0.025 to 0.055, eutrophic > 0.055.

Two important points to remember about Hutchinson's and Mortimer's indices: they were developed using the hypolimnetic areal deficit method, not the relative areal hypolimnetic oxygen deficit method, and they used natural, northern lakes, not reservoirs.

Value of the RAHOD

The Relative Areal Hypolimnetic Oxygen Deficit (RAHOD) can assess trophic status of lakes (Charlton 1980; Lind and Dávalos-Lind 1993). The RAHOD method can assess and monitor eutrophication over time in a lake or between sections of a lake (Lind and Dávalos-Lind 1993). Lind and

Dávalos-Lind working on Douglas lake in Michigan used the RAHOD method to show localized cultural eutrophication on three sections of that lake (1993). The RAHOD method is not expensive to conduct and yields valuable data.

RAHOD and reservoirs. The RAHOD method was developed, published, and used on natural, northern lakes, not reservoirs. There are many differences between natural, northern lakes and reservoirs that may affect the usefulness of the RAHOD method on reservoirs. Natural, northern lakes differ from reservoirs in the following areas: water residence time, nutrient dynamics, primary production, temperature, and oxygen depletion (Wetzel 1993; Cole and Hannan 1990).

Reservoirs have shorter and more variable water residence times (days to several weeks) than natural lakes (Wetzel 1990). Shorter water residence time could affect the calculation of oxygen depletion rates. For example, how does one account for water flowing into and out of the hypolimnion or transport of organic matter through the reservoir without oxygen demand?

Reservoirs have both horizontal and vertical gradients of nutrients whereas natural lakes have primarily vertical gradients. In a reservoir, nutrients are more concentrated up-reservoir than down-reservoir (Wetzel 1990; Kennedy and Walker 1990). Nutrient gradients directly affect primary production, and primary production directly affects oxygen deficits. Nutrient gradients therefore, indirectly affect oxygen deficits.

Reservoirs have horizontal and vertical gradients of primary production whereas natural lakes have primarily vertical gradients (Lind 1984; Kimmel et al. 1990; Lind et al. 1993). Primary production in reservoirs is greater in the transitional zone than in the riverine and lacustrine zones. Will RAHOD be greater up-reservoir as compared to down-reservoir? Can the RAHOD detect horizontal gradient differences?

Reservoir water temperatures differ from water temperatures of natural lakes. Reservoir water temperatures tend to be higher because of a more southern latitude whereas natural lake's water temperatures are lower because most are in northern regions (Wetzel 1990).

Finally reservoirs differ in hypolimnetic oxygen depletion patterns. In natural lakes hypolimnetic oxygen depletion occurs first at the sediment water interface and then moves upward (Cole and Hannan 1990). The hypolimnetic oxygen depletion pattern is spatially and temporally variable in reservoirs. Generally, oxygen depletion occurs first in the transition zone and then develops lengthwise up-reservoir and down-reservoir (Hannan et al. 1979; Cole and Hannan 1990).

The rapid depletion of hypolimnetic oxygen in the transitional zone is affected by volume and temperature factors. The transitional zone has less hypolimnetic volume of water than the lacustrine zone. The transitional zone has higher hypolimnetic temperatures than the lacustrine zone (Cole and Hannan 1990). The higher hypolimnetic temperature causes higher

respiration rates and lower dissolved oxygen solubilities. Will RAHOD be greater up-reservoir as compared to down-reservoir?

Thesis questions

This portion of my study tried to answer the following questions:

- What are the Relative Areal Hypolimnetic Oxygen Deficits (RAHOD) of Belton and Stillhouse Hollow Reservoirs?
- How do the RAHOD of Belton and Stillhouse Hollow Reservoirs compare with RAHOD of northern lakes?
- Can RAHOD be used to make intra-reservoir region comparisons?
- Should the RAHOD method be used on reservoirs to assess trophic state?

# CHAPTER TWO

## Materials and Methods

### Sample reservoirs

Two Central Texas reservoirs, Belton and Stillhouse Hollow, were the studied (Fig. 1). Belton Reservoir is 5.6 km north of Belton. It is an elongate reservoir (32 km), with two main arms, Cowhouse Creek and the Leon River. The majority of the water inflow is from the Leon River. Belton Reservoir with a surface area of $5.01 \times 10^7$ $m^2$ and a storage capacity of $5.36 \times 10^8$ $m^3$ was built in 1954 for flood control, conservation and recreation purposes (Texas Water Development Board 1994). It is a prime site for a study of hypolimnetic processes. Belton Reservoir stratifies in late February or early March, and stratification persists until September or October (Mendieta and Pate 1983). The cliffs of this reservoir protect it from wind that would affect stratification. The hypolimnion becomes anoxic in late spring.

I added another reservoir to my study, Stillhouse Hollow, late in the sampling period. It is 12.8 kilometers southwest of Belton. Stillhouse Hollow Reservoir with a surface area of $2.6 \times 10^7$ $m^2$ and a storage capacity of $2.9 \times 10^8$ $m^3$ was built in 1968, for flood control, conservation and recreation purposes (Texas Water Development Board 1995).

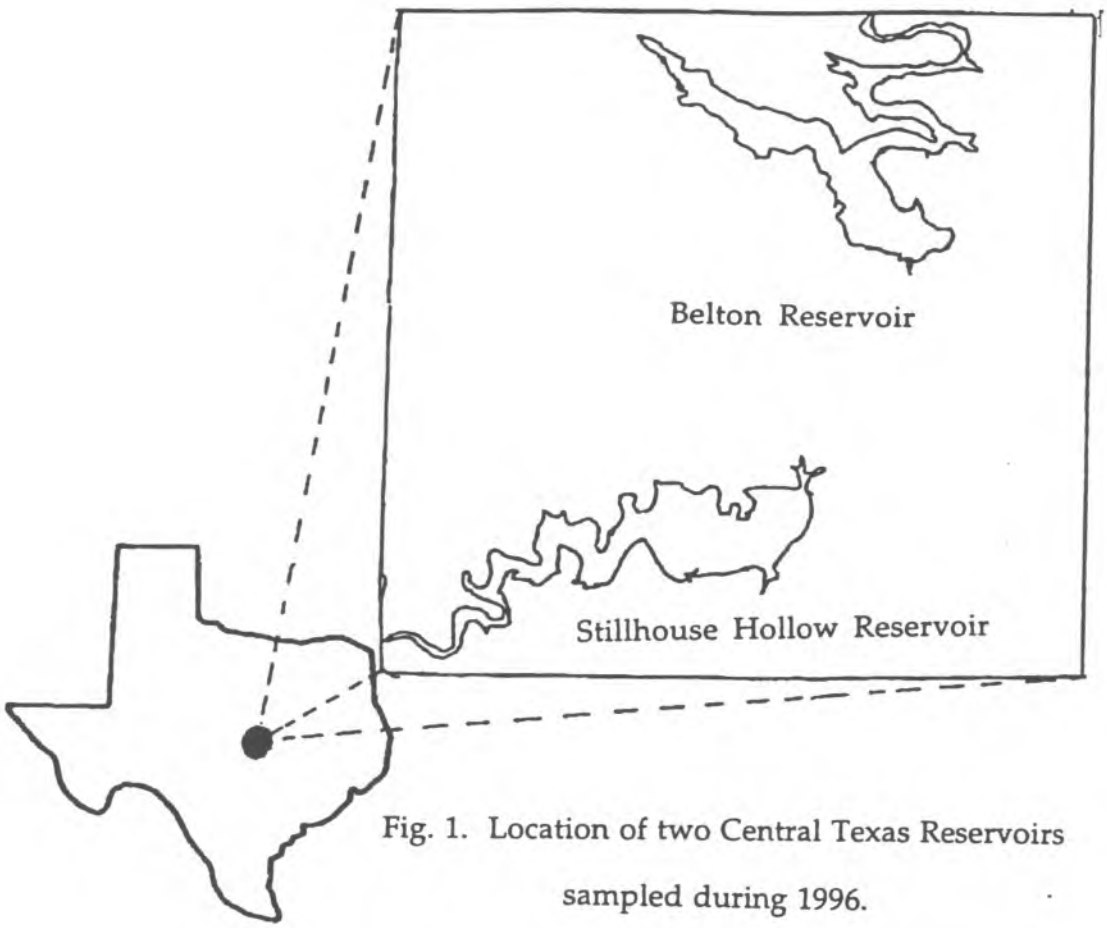


Fig. 1. Location of two Central Texas Reservoirs sampled during 1996.

The two sample reservoirs are trophically different (Table 1). Belton Reservoir is more productive and eutrophic than Stillhouse Hollow Reservoir.

Sample stations

Two stations were sampled on Belton Reservoir (Fig. 2). The first station was near the dam and the second station was up-reservoir. One station near the dam was sampled on Stillhouse Hollow Reservoir (Fig. 2).

A depth finder determined the river channel (deepest water), then a Magellan satellite positioning system determined the latitude and longitude of each station (Table 2).

Table 1. Trophic classification of sample reservoirs, using carlson's trophic state index (TSI).

| | Chlorophyll a | | Total Phosphorus | | Seechi Disk | |
|---|---|---|---|---|---|---|
| Reservoir | Mean mg / $m^3$ | Trophic State | Mean mg / $m^3$ | Trophic State | Mean m | Trophic State |
| Belton | 4.5 | Mesotrophic | 102.3 | Eutrophic | 2.2 | Eutrophic |
| Stillhouse Hollow | 1.7 | Oligotrophic | 14.6 | Mesotrophic | 2.3 | Mesotrophic |

TNRCC 1994

Table 2. Station locations.

| Reservoir | Station | Latitude and Longitude |
|---|---|---|
| Belton | 1 | $31^0$ 06'.57 N, $97^0$ 28'.64 W |
| | 2 | $31^0$ 08'.37 N, $97^0$ 28'.49 W |
| Stillhouse Hollow | 1 | $31^0$ 01'.24 N, $97^0$ 32'.29 W |

## Sampling

Sampling occurred twice a month in February, March and April, from each station on Belton Reservoir. Samples occurred in May every fifth day from each station on Belton and Stillhouse Hollow Reservoirs. Sampling occurred in the first week of June and a final sampling occurred in July (Table 3).

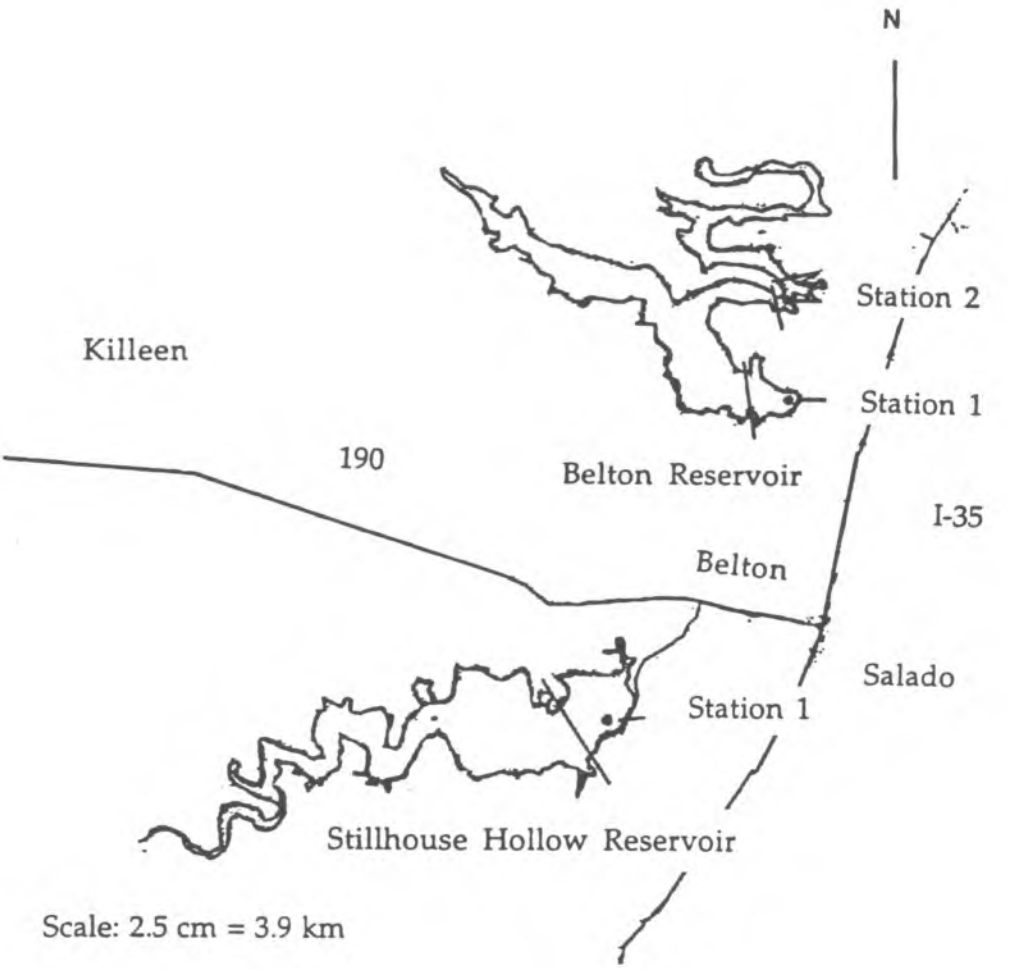


Fig. 2. Location of sample stations on two sampled reservoirs.

Table 3. Sampling trip numbers and dates in 1996.

| Sampling trip number | Date |
|---|---|
| 1 | February 24 |
| 2 | March 8 |
| 3 | March 29 |
| 4 | April 12 |
| 5 | April 26 |
| 6 | May 4 |
| 7 | May 9 |
| 8 | May 14 |
| 9 | May 18 |
| 10 | May 23 |
| 11 | May 28 |
| 12 | June 2 |
| 13 | June 7 |
| 14 | July 1 |

Field measurements

I measured wind velocity, Secchi depth, and air temperature at each station. I used a Wind Wizard® to measure the wind velocity in meters per second. I measured the Secchi depth at all stations by following the procedures outlined by Lind (1984).

Water. Temperature. I used a Hydrolab DataSonde to measure the air and water temperatures. I made measurements at one meter intervals between the surface and the bottom of the reservoir. For quality assurance, beginning on April 26, I made additional measurements from the bottom of the reservoir to the surface. Data analysis used the average of these two measurements.

Dissolved oxygen (milligrams / liter). I used the Hydrolab DataSonde, with stirring device, to measure the dissolved oxygen concentration, at one meter intervals between the surface and the bottom of each reservoir. For quality assurance, beginning on April 26, I made additional measurements from the bottom of the reservoir to the surface and averaged the two measurements. Pre- and post-calibrations determined the drift in the Hydrolab unit. I assumed linear drift and corrected the dissolved oxygen data if needed.

Water samples and preservation. I collected three different water samples: dissolved oxygen; bacteria and nanoflagellates; and ammonia nitrogen.

The dissolved oxygen sample was taken in addition to the Hydrolab measurements to confirm the Hydrolab dissolved oxygen measurements. For dissolved oxygen I transferred one 300-ml water sample from the Kemmerer metallic sampler into a BOD bottle.

For the bacteria and nanoflagellate water sample, I rinsed a 30-ml plastic syringe with water from the Kemmerer sampler, and then transferred 15-ml aliquots into two sample containers. The container was a clean numbered polyethylene container or glass test tube. I labeled and recorded the sample container number. I preserved the bacteria and nanoflagellate water immediately in two percent formalin (final concentration) (Sherr and Sherr 1993).

For the ammonia nitrogen sample I rinsed the glass bottle with water from the Kemmerer sampler, and then transferred a sample from the Kemmerer sampler into a labeled, acid rinsed 125-ml glass bottle with a screw-cap. I recorded the sample container number. To reduce biological activity, I put all samples inside a cooler with crushed ice (4 $^{0}$C).

Sample analysis

Careful washing of glassware prevented contamination. The automatic laboratory dishwasher washed the glassware with an acid-rinse cycle. I used low-phosphate or phosphate-free dish-washing detergent. I submerged the containers in ten percent muriatic acid bath. I then rinsed the containers once with tap water and once with distilled water.

Winkler titration. For the Hydrolab confirming samples, I used the azide modification of the Winkler method to determine dissolved oxygen (Lind 1984).

Ammonia nitrogen. I used the phenol-hypochlorite method to measure ammonia nitrogen (Lind 1984). For samples collected during February 24 through April 12, I spiked every fifth sample. A "spike" is a known amount of ammonia nitrogen added to a split sample to determine the percent of recovery. For better quality assurance, beginning with samples collected during April 26 through July 1, I spiked a sample from every station.

Bacterial analysis. I used the Acridine Orange Direct Count method (AODC) (Hobbie et al. 1977) to count and measure the size of bacterial cells. For each sampling depth I had two replicates, when possible I counted and

measured bacteria on a filter from each replicate. I filtered another aliquot from the first replicate if the second replicate was not collected or used. I adjusted the volume filtered so that the concentration of bacteria was between 20 and 40 cells per field and randomly selected and counted twenty fields. I counted more fields if necessary to attain ninety-five percent confidence level between the replicates' total cell concentrations.

I used a color image analysis system to measure the bacteria cells (Verity and Sieracki 1993; Psenner 1993). The color image analysis system measured all the samples at station 2 and samples collected during April 26 through May 23 ten of station 1. To verify the image analysis system's measurements, I measured 10% of the samples using an eyepiece micrometer. The image analysis software broke and I was unable to upgrade to the new image analysis software. After the image analysis software broke, I measured the bacteria from station 1 samples collected during May 28 through July 1, and all the samples from Stillhouse with an eyepiece micrometer. I randomly selected 40 coccus and 40 bacillus-shaped bacteria from each sample filter. I estimated the size of the bacteria to the nearest tenth of a micrometer. I calculated bacterial biovolumes according to Bratbak (1993). I assumed that bacillus-shaped bacteria were rods with half a sphere at each end. I assumed that the coccus-shaped bacteria were spheres. I measured more bacteria if necessary to attain ninety-five percent confidence between the replicates' average bacterial cell biovolumes.

Nanoflagellate analysis. I tried to differentiate the heterotrophic nanoflagellates from the autotrophic nanoflagellates, but the chlorophyll had leached out of the autotrophic nanoflagellates during sample storage. Because heterotrophic nanoflagellates could not be distinguished from autotrophic nanoflagellates, I determined the total nanoflagellate abundance. I used a modification of the Primulin method developed by Caron (1983) and Bloem et al. (1986) to enumerate nanoflagellates. I made the following modifications: I used a higher concentration of Primulin solution (945 mg / l), and a shorter staining time (two minutes).

For each sampling depth I had two replicates, and when possible I filtered and counted from each replicate. I filtered and counted another sample from the first replicate, if the second replicate was not collected or used. I randomly selected and counted eighty fields. I counted more fields if necessary to attain ninety-five percent confidence between the replicates' total nanoflagellate concentration. If the replicates did not meet the ninety-five percent confidence level I flagged those data. I used the flagged data in statistical tests. I calculated the nanoflagellate abundance according to Sherr et al. (1993).

Relative areal hypolimnetic oxygen deficit (RAHOD) method

I used the methods described in Lind and Dávalos-Lind (1993) to calculate the RAHOD of Belton and Stillhouse Hollow Reservoirs. To determine the top of the hypolimnion, I plotted temperature versus depth, and extrapolated lines from the greatest slope in the metalimnion and the

least or no slope in the hypolimnion. The intersection of these was the top of the hypolimnion (Appendix B) (Lind and Dávalos-Lind 1993).

Sectioned RAHOD

When determining the RAHOD for each station I sectioned each station's area using a bathymetric map. In sectioning each area, I tried to follow, when possible, the reservoir's natural barriers that helped separate each area (Fig. 2). I determined the volume and surface area of each 1 meter stratum using a bathymetric map and a polar planimeter (Appendix C). The contour map did not represent the greater depths, at the deep stations (Station 1 of Belton and Station 1 of Stillhouse Reservoir). For those depths I used the surface area data from the Volumetric Survey of Belton and Stillhouse Hollow Reservoirs (Texas Water Development Board 1994; Texas Water Development Board 1995).

I entered the following data: temperature, oxygen, stratum volume and surface area; into a Microsoft Excel spreadsheet (Lind and Dávalos Lind 1993). The spreadsheet calculated the areal oxygen content, volume-weighted mean hypolimnion temperature, hypolimnion mean depth, areal hypolimnetic oxygen deficit and RAHOD.

Whole lake RAHOD

When determining the RAHOD for the entire lake, for each 1 meter stratum I used the surface area and volume data from the Volumetric Survey of Belton and Stillhouse Hollow Reservoirs (Texas Water Development Board 1994; Texas Water Development Board 1995).

I entered the following data: average temperature of two stations, average oxygen of two stations, stratum volume and surface area; into a Microsoft Excel spreadsheet (Lind and Dávalos Lind 1993). The spreadsheet calculated the areal oxygen content, volume-weighted mean hypolimnion temperature, hypolimnion mean depth, areal hypolimnetic oxygen deficit and RAHOD.

## Statistical analysis

### Dissolved oxygen

To validate the dissolved oxygen data, I compared the dissolved oxygen values measured by the Hydrolab Datasonde and Winkler titration (Appendix A) using a Wilcoxon Sign Rank Test. There was no significant difference between the Hydrolab Datasonde and Winkler titration dissolved oxygen measurements ($P = 0.77$).

### Bacterial abundance

Bacterial abundance was analyzed using a Tukey's studentized range test for variance among the stations. Regression analysis compared bacterial abundance with time, dissolved oxygen, temperature, depth and nanoflagellate abundance. Using a t test, I compared the slope of the variable of interest against a slope of "0".

### Bacterial biovolume

To determine if there was a difference in measurement methods I compared the computer image analysis and ocular measurements using a t' statistical test (Table 4).

Table 4. Comparison of computer image analysis and ocular measurements of sample bacteria cell sizes.

| Sample | t' | nu' | P-value | Significance |
|---|---|---|---|---|
| 100&101 | -0.7016 | 277.02 | 0.48351769 | No |
| 125&128 | 1.782 | 307.98 | 0.07573753 | No |
| 230&251 | 0 | - | 1 | No |

The computer image analysis measurements were not significantly different from the ocular measurements (Table 4).

Bacterial biovolume was analyzed using a Tukey's studentized range test for variance among the stations. Regression analysis was used to compare bacterial biovolume with time, dissolved oxygen, temperature, depth and nanoflagellate abundance. Using a t test, I compared the slope of the variable of interest against a slope of "0".

Nanoflagellate abundance

Nanoflagellate abundance was analyzed using a Tukey's studentized range test for variance among the stations. Regression analysis was used to compare nanoflagellate abundance with dissolved oxygen. Using a t test, I compared the slope of the variable of interest against a slope of "0".

AHOD

To determine a difference of AHOD between the stations, I compared the 95% confidence intervals of the each station's AHOD, against the 95% confidence intervals of the other station's AHOD.

# CHAPTER THREE

## Results

### Stratification

Stations 1 and 2 on Belton Reservoir were stratified by April 26, 1996. Station 1 on Stillhouse Hollow Reservoir was stratified on May 18, 1996.

### Ammonia nitrogen

Eighteen ammonia nitrogen samples had acceptable spike recovery levels (Table 5, Table 6, and Table 7). Fourteen of the eighteen samples had ammonia concentrations that were below my detection limit. Ammonia nitrogen was not included in statistical analysis.

### Hypolimnetic bacterial abundance

Hypolimnetic bacterial abundance varied by stations (Table 5, Table 6, Table 7 and Table 8).

Table 8. Hypolimnetic bacterial seasonal abundance mean and standard deviation.

| Reservoir | Station | Range cells / liter $x10^9$ | Mean cells / liter $x10^9$ | Stan Dev cells / liter | N |
|---|---|---|---|---|---|
| Belton | 1 | 1.71 - 2.90 | 2.23 | $6.24 x10^7$ | 44 |
| | 2 | 2.19 - 4.19 | 3.23 | $1.62 x10^8$ | 42 |
| Stillhouse Hollow | 1 | 1.35 - 2.74 | 1.85 | $4.62 x10^7$ | 30 |

Table 5. Belton Reservoir, Station 2 - physical, chemical, bacterial and nanoflagellate sample data.

| | | | | | Bacteria Abun. cells / liter | | | | Bacteria Biovolume $\mu m^3$ | | | Nanoflagellate Abund. cells / liter | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample Date | Depth m | Temp °C | D.O. mg / l | $NH_4$ mg / l | Samp # | Mean x $10^9$ | Stan Dev | N | Mean | Stan Dev | N | Mean | Stan Dev | N |
| 4/26/96 | 15 | 14.6 | 6.9 | - | 56&56 | 4.19 | 3.08E+08 | 2 | 0.057 | 0.1 | 160 | 8.89E+05 | 2.47E+04 | 2 |
| 4/26/96 | 20 | 14.2 | 6.3 | - | 49&53 | 3.83 | 2.36E+08 | 2 | 0.067 | 0.09 | 160 | 6.40E+05 | 0.00E+00 | 2 |
| 5/4/96 | 18 | 15.8 | 4.5 | 0 | 107&117 | 3.10 | 1.30E+08 | 2 | 0.07 | 0.09 | 160 | 7.93E+05 | 1.09E+04 | 2 |
| 5/4/96 | 20 | 14.9 | 3.9 | 0 | 109&111 | 2.79 | 5.72E+07 | 2 | 0.066 | 0.08 | 160 | 8.37E+05 | 6.13E+04 | 2 |
| 5/9/96 | 18 | 15.5 | 4.0 | 0.008 | 125&128 | 2.62 | 5.47E+07 | 2 | 0.047 | 0.06 | 160 | 1.14E+06 | 3.26E+03 | 2 |
| 5/9/96 | 20 | 14.8 | 3.9 | 0 | 127&137 | 3.27 | 1.86E+08 | 2 | 0.051 | 0.07 | 160 | 1.78E+06 | 4.46E+04 | 2 |
| 5/14/96 | 18 | 15.4 | 3.6 | 0.09 | 143&148 | 2.81 | 6.07E+07 | 2 | 0.05 | 0.08 | 160 | 2.21E+06 | 8.92E+04 | 2 |
| 5/14/96 | 20 | 14.8 | 4.1 | 0 | 157&169 | 3.30 | 5.72E+07 | 2 | 0.043 | 0.05 | 160 | 1.67E+06 | 2.26E+05 | 2 |
| 5/18/96 | 18 | 15.7 | 2.7 | 0 | 203&206 | 3.51 | 2.29E+08 | 2 | 0.05 | 0.1 | 160 | 9.76E+05 | 5.70E+04 | 2 |
| 5/18/96 | 20 | 15.1 | 2.4 | 0 | 202&204 | 3.49 | 7.63E+08 | 2 | 0.028 | 0.04 | 160 | 2.47E+05 | 6.81E+03 | 2 |
| 5/23/96 | 17 | 16.6 | 3.2 | 0 | 236&243 | 3.89 | 0.00E+00 | 2 | 0.03 | 0.04 | 160 | 4.07E+05 | 1.11E+04 | 2 |
| 5/23/96 | 19 | 15.2 | 2.3 | 0 | 230&251 | 3.36 | 2.26E+08 | 2 | 0.021 | 0.03 | 160 | 2.01E+05 | 0.00E+00 | 2 |
| 5/23/96 | 21 | 15.0 | 2.0 | 0 | 229&245 | 3.90 | 2.86E+07 | 2 | 0.019 | 0.03 | 160 | 4.09E+05 | 1.43E+04 | 2 |
| 5/28/96 | 17 | 16.1 | - | - | 249&249 | 3.19 | 1.97E+08 | 2 | 0.018 | 0.03 | 160 | 4.22E+05 | 4.09E+04 | 2 |
| 5/28/96 | 19 | 15.1 | - | - | 272&273 | 2.93 | 9.53E+07 | 2 | 0.018 | 0.03 | 172 | 1.25E+05 | 5.02E+04 | 2 |
| 5/28/96 | 21 | 14.8 | - | - | 256&258 | 3.84 | 2.29E+08 | 2 | 0.013 | 0.02 | 160 | 6.77E+05 | 5.02E+04 | 2 |
| 6/2/96 | 17 | 16.5 | 1.4 | - | 270&314 | 2.90 | 3.81E+07 | 2 | 0.022 | 0.03 | 187 | 6.11E+05 | 1.74E+04 | 2 |
| 6/7/96 | 17 | 17.2 | 1.4 | - | 201&380 | 2.19 | 1.53E+08 | 2 | 0.016 | 0.02 | 162 | 2.01E+05 | 0.00E+00 | 2 |
| 6/7/96 | 19 | 16.2 | 0.6 | - | 341&368 | 2.27 | 1.44E+08 | 2 | 0.011 | 0.01 | 160 | 1.00E+05 | 0.00E+00 | 2 |
| 7/1/96 | 17 | 20.8 | 0.2 | 0.085 | 411&414 | 3.01 | 2.19E+08 | 2 | 0.016 | 0.03 | 172 | 9.09E+04 | 7.02E+03 | 2 |
| 7/1/96 | 19 | 19.0 | 0.2 | - | 296&328 | 3.40 | 2.63E+08 | 2 | 0.018 | 0.03 | 175 | 5.02E+04 | 0.00E+00 | 2 |

Table 6. Belton Reservoir, Station 1 - physical, chemical, bacterial and nanoflagellate sample data.

| | | | | | Bacteria Abun. cells / liter | | | | Bacteria Biovolume $\mu m^3$ | | | Nanoflagellate Abund. cells / liter | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample Date | Depth m | Temp °C | D.O. mg / l | $NH_4$ mg / l | Samp # | Mean x $10^9$ | Stan Dev | N | Mean | Stan Dev | N | Mean | Stan Dev | N |
| 4/26/96 | 20 | 14.0 | 7.7 | - | 42&42 | 1.99 | 1.13E+08 | 2 | 0.023 | 0.04 | 191 | 5.81E+05 | 2.29E+04 | 2 |
| 4/26/96 | 25 | 13.3 | 7.5 | - | 26&24 | 2.18 | 1.27E+08 | 2 | 0.02 | 0.04 | 177 | 1.14E+06 | 7.83E+04 | 2 |
| 5/4/96 | 20 | 14.4 | 6.1 | 0 | 102&103 | 2.50 | 1.91E+08 | 2 | 0.017 | 0.03 | 160 | 3.33E+05 | 1.65E+04 | 2 |
| 5/4/96 | 25 | 13.6 | 6.1 | 0.005 | 100&101 | 2.39 | 1.40E+08 | 2 | 0.013 | 0.02 | 160 | 7.16E+05 | 1.16E+04 | 2 |
| 5/9/96 | 20 | 14.6 | 5.7 | - | 59&121 | 2.62 | 1.12E+08 | 2 | 0.012 | 0.02 | 160 | 1.30E+06 | 5.96E+04 | 2 |
| 5/14/96 | 20 | 14.5 | 5.5 | - | 163&164 | 1.71 | 6.33E+07 | 2 | 0.013 | 0.02 | 160 | 2.21E+05 | 6.74E+03 | 2 |
| 5/14/96 | 22 | 14.2 | 6.4 | - | 149&151 | 2.64 | 1.84E+08 | 2 | 0.013 | 0.02 | 176 | 3.69E+05 | 2.16E+04 | 2 |
| 5/18/96 | 20 | 14.6 | 5.5 | 0 | 191&194 | 2.18 | 1.33E+08 | 2 | 0.011 | 0.02 | 160 | 2.56E+05 | 2.75E+04 | 2 |
| 5/18/96 | 22 | 14.3 | 5.5 | 0 | 177&195 | 2.68 | 2.00E+08 | 2 | 0.011 | 0.02 | 160 | 6.63E+04 | 1.79E+04 | 2 |
| 5/23/96 | 18 | 15.8 | 5.7 | - | 241&244 | 1.77 | 6.67E+07 | 2 | 0.007 | 0.01 | 179 | 5.76E+05 | 3.97E+04 | 2 |
| 5/23/96 | 20 | 15.1 | 5.9 | - | 248&250 | 2.77 | 1.47E+08 | 2 | 0.008 | 0.01 | 201 | 1.13E+06 | 7.72E+04 | 2 |
| 5/23/96 | 22 | 14.4 | 5.9 | - | 226&242 | 2.10 | 3.18E+07 | 2 | 0.008 | 0.01 | 210 | 7.22E+05 | 3.86E+04 | 2 |
| 5/28/96 | 20 | 15.0 | 4.4 | 0 | 257&260 | 2.34 | 1.62E+08 | 2 | 0.028 | 0.04 | 239 | 5.24E+05 | 3.34E+04 | 2 |
| 5/28/96 | 22 | 14.6 | 4.3 | 0 | 259&262 | 2.01 | 1.44E+08 | 2 | 0.028 | 0.04 | 236 | 3.60E+05 | 2.65E+04 | 2 |
| 6/2/96 | 18 | 16.9 | 4.2 | - | 280&281 | 2.34 | 6.67E+07 | 2 | 0.021 | 0.03 | 214 | 4.79E+05 | 6.58E+03 | 2 |
| 6/2/96 | 20 | 15.8 | 4.8 | - | 293&294 | 1.71 | 0.00E+00 | 2 | 0.023 | 0.03 | 221 | 5.77E+05 | 4.21E+04 | 2 |
| 6/2/96 | 24 | 14.6 | 3.7 | - | 279&303 | 2.20 | 1.05E+08 | 2 | 0.019 | 0.03 | 200 | 2.12E+05 | 0.00E+00 | 2 |
| 6/7/96 | 20 | 15.8 | 3.8 | - | 373&376 | 2.15 | 0.00E+00 | 2 | 0.016 | 0.03 | 303 | 1.93E+04 | 0.00E+00 | 2 |
| 6/7/96 | 25 | 14.9 | 3.0 | - | 349&351 | 1.85 | 9.53E+07 | 2 | 0.014 | 0.02 | 279 | 1.22E+05 | 9.80E+03 | 2 |
| 6/7/96 | 27 | 14.7 | 2.9 | - | 316&370 | 2.16 | 1.42E+08 | 2 | 0.014 | 0.02 | 290 | 2.15E+04 | 4.37E+03 | 2 |
| 7/1/96 | 19 | 19.8 | 1.8 | - | 312&323 | 1.80 | 4.77E+07 | 2 | 0.013 | 0.02 | 305 | 1.51E+05 | 2.39E+04 | 2 |
| 7/1/96 | 24 | 16.3 | 0.1 | - | 271&292 | 2.90 | 2.26E+08 | 2 | 0.024 | 0.04 | 233 | 2.33E+05 | 1.17E+04 | 2 |

Table 7. Stillhouse Hollow Reservoir, Station 1 - physical, chemical, bacterial and nanoflagellate sample data.

| | | | | | Bacteria Abun. cells / liter | | | | Bacteria Biovolume $\mu m^3$ | | | Nanoflagellate Abund. cells / liter | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample Date | Depth m | Temp $^0C$ | D.O. mg / l | $NH_4$ mg / l | Samp # | Mean x $10^9$ | Stan Dev | N | Mean | Stan Dev | N | Mean | Stan Dev | N |
| 5/18/96 | 16 | 14.6 | 5.7 | - | 174&185 | 2.15 | 1.20E+08 | 2 | 0.014 | 0.02 | 213 | 6.02E+05 | 0.00E+00 | 2 |
| 5/18/96 | 24 | 12.6 | 4.3 | - | 188&192 | 1.92 | 1.17E+08 | 2 | 0.013 | 0.02 | 176 | 5.52E+05 | 0.00E+00 | 2 |
| 5/18/96 | 28 | 12.4 | 3.2 | - | 179&197 | 1.80 | 1.39E+08 | 2 | 0.011 | 0.02 | 237 | 6.52E+05 | 0.00E+00 | 2 |
| 5/28/96 | 16 | 15.2 | 4.9 | - | 196&276 | 1.68 | 1.13E+08 | 2 | 0.009 | 0.02 | 221 | 1.69E+05 | 2.74E+04 | 2 |
| 5/28/96 | 22 | 12.7 | 4.5 | - | 180&187 | 2.28 | 1.57E+08 | 2 | 0.017 | 0.03 | 215 | 1.22E+06 | 6.04E+04 | 2 |
| 5/28/96 | 28 | 12.5 | 4.1 | - | 205&220 | 1.83 | 5.39E+07 | 2 | 0.015 | 0.03 | 214 | 3.63E+05 | 2.45E+04 | 2 |
| 6/2/96 | 20 | 13.2 | 4.6 | - | 334&330 | 1.36 | 8.85E+07 | 2 | 0.019 | 0.03 | 244 | 8.41E+05 | 5.21E+04 | 2 |
| 6/2/96 | 25 | 12.6 | 3.0 | - | 172&213 | 1.53 | 1.31E+07 | 2 | 0.01 | 0.02 | 247 | 5.38E+05 | 2.74E+04 | 2 |
| 6/2/96 | 28 | 12.6 | 2.5 | - | 320&322 | 1.35 | 1.01E+08 | 2 | 0.011 | 0.02 | 213 | 9.28E+05 | 5.02E+04 | 2 |
| 6/7/96 | 20 | 13.6 | 4.0 | - | 300&302 | 1.91 | 4.82E+07 | 2 | 0.014 | 0.03 | 252 | 1.13E+06 | 7.60E+04 | 2 |
| 6/7/96 | 25 | 12.9 | 3.0 | - | 266&289 | 2.74 | 1.99E+08 | 2 | 0.011 | 0.02 | 275 | 7.12E+05 | 3.66E+04 | 2 |
| 6/7/96 | 28 | 12.7 | 2.4 | - | 282&283 | 1.87 | 1.44E+08 | 2 | 0.01 | 0.02 | 234 | 8.78E+05 | 5.02E+04 | 2 |
| 7/1/96 | 15 | 22.3 | 2.5 | - | 339&363 | 1.87 | 1.02E+08 | 2 | 0.009 | 0.02 | 262 | 1.01E+06 | 9.12E+04 | 2 |
| 7/1/96 | 20 | 15.7 | 0.8 | - | 428&442 | 1.53 | 1.11E+08 | 2 | 0.013 | 0.03 | 229 | 8.37E+05 | 5.37E+04 | 2 |
| 7/1/96 | 24 | 13.4 | 0.5 | - | 345&365 | 1.90 | 1.36E+08 | 2 | 0.011 | 0.02 | 224 | 5.77E+05 | 5.02E+04 | 2 |

Hypolimnetic bacterial abundance was significantly different among the stations (Table 9). Station 2 of Belton Reservoir had more bacteria than Station 1 of Belton Reservoir and Station 1 of Stillhouse Hollow Reservoir. Station 1 of Belton Reservoir had more bacteria than Station 1 of Stillhouse Hollow Reservoir.

Table 9. Statistical comparison of hypolimnetic bacterial abundance by stations.

| Station Comparison | Simultaneous Lower Confidence Limit | Difference Between Means | Simultaneous Upper Confidence Limit | Significant Difference |
|---|---|---|---|---|
| 2 - 1 | 0.6854 | 1.0013 | 1.3171 | Yes |
| 2 - Stillhouse | 1.0301 | 1.3801 | 1.7301 | Yes |
| 1 - Stillhouse | 0.0322 | 0.3788 | 0.7255 | Yes |

Hypolimnetic bacterial abundance at any station was not significantly correlated with the following variables: date, dissolved oxygen, temperature, depth, and nanoflagellate abundance (Table 10). There was not significant difference between the slope of the variable of interest against a slope of "0", although, at Station 2 the slope of hypolimnetic bacterial abundance versus dissolved oxygen, was close to being significantly different. Dissolved oxygen only accounted for 21% of hypolimnetic bacterial abundance values at Station 2 (Table 10).

Table 10. Statistical comparison of hypolimnetic bacterial abundance versus independent variables by stations.

| Station | Independent variable | R-square value | P-value of t test | Significant |
|---|---|---|---|---|
| 1 | Date | 0.0061 | 0.7298 | No |
| | Dissolved Oxygen | 0.0002 | 0.9534 | No |
| | Temperature | 0.0437 | 0.3506 | No |
| | Depth | 0.0258 | 0.4751 | No |
| | Nanoflagellate Abund. | 0.0525 | 0.3051 | No |
| 2 | Date | 0.1029 | 0.1943 | No |
| | Dissolved Oxygen | 0.2106 | 0.0554 | No |
| | Temperature | 0.0765 | 0.2665 | No |
| | Depth | 0.0149 | 0.5974 | No |
| | Nanoflagellate Abund. | 0.0001 | 0.9720 | No |
| Stillhouse | Date | 0.0119 | 0.6993 | No |
| | Dissolved Oxygen | 0.0311 | 0.5298 | No |
| | Temperature | 0.0017 | 0.8854 | No |
| | Depth | 0.0011 | 0.9086 | No |
| | Nanoflagellate Abund. | 0.0109 | 0.7106 | No |

Hypolimnetic bacterial biovolume

Large hypolimnetic bacteria cell biovolumes were not found in Belton and Stillhouse Hollow Reservoir (Table 5, Table 6, Table 7, and Table 11).

Table 11. Comparison of hypolimnetic bacterial cell biovolumes by stations.

| Reservoir | Station | Range $\mu m^3$ | Mean $\mu m^3$ | Stan Dev $\mu m^3$ | N |
|---|---|---|---|---|---|
| Belton | 1 | 0.007 - 0.028 | 0.02 | 0.01 | 4614 |
| | 2 | 0.011 - 0.07 | 0.03 | 0.03 | 3428 |
| Stillhouse Hollow | 1 | 0.009 - 0.019 | 0.01 | 0.005 | 3456 |

Hypolimnetic bacterial cell biovolume was significantly different among some stations (Table 12). Station 2 of Belton Reservoir had larger

bacteria than Station 1 of Belton and Station 1 of Stillhouse Hollow Reservoir.

Table 12. Statistical comparison of hypolimnetic bacterial cell biovolume by stations.

| Station Comparison | Simultaneous Lower Confidence Limit | Difference Between Means | Simultaneous Upper Confidence Limit | Significant Difference |
|---|---|---|---|---|
| 2 - 1 | 0.009253 | 0.018580 | 0.027908 | Yes |
| 2 - Stillhouse | 0.011959 | 0.022295 | 0.032631 | Yes |
| 1 - Stillhouse | -0.006522 | 0.003715 | 0.013953 | No |

Hypolimnetic bacterial cell biovolume at Station 2 changed with date, dissolved oxygen, temperature and nanoflagellate abundance. The slope of hypolimnetic bacteria cell biovolume versus temperature and nanoflagellate abundance (Table 13) was significantly different from a slope of "0", temperature and nanoflagellate abundance accounted for 33% and 36% of variation by hypolimnetic bacterial cell biovolumes at Station 2 (Table 13).

Table 13. Hypolimnetic bacterial biovolume versus independent variables.

| Station | Independent variable | R-square value | P-value of t test | Significant |
|---|---|---|---|---|
| 1 | Date | 0.0167 | 0.5667 | No |
| | Dissolved Oxygen | 0.0522 | 0.3063 | No |
| | Temperature | 0.0003 | 0.9441 | No |
| | Depth | 0.0045 | 0.7670 | No |
| | Nanoflagellate Abund. | 0.0101 | 0.6565 | No |
| 2 | Date | 0.6955 | 0.0001 | Yes |
| | Dissolved Oxygen | 0.7359 | 0.0001 | Yes |
| | Temperature | 0.3263 | 0.0133 | Yes |
| | Depth | 0.0017 | 0.8710 | No |
| | Nanoflagellate Abund. | 0.3589 | 0.0086 | Yes |
| Stillhouse | Date | 0.0718 | 0.3344 | No |
| | Dissolved Oxygen | 0.1878 | 0.1066 | No |
| | Temperature | 0.1073 | 0.2332 | No |
| | Depth | 0.0042 | 0.8190 | No |
| | Nanoflagellate Abund. | 0.0936 | 0.2676 | No |

Hypolimnetic nanoflagellate abundance

Hypolimnetic nanoflagellate abundance did not vary by stations (Table 5, Table 6, Table 7, Table 14, and 15).

Table 14. Comparison of hypolimnetic nanoflagellate abundance by stations.

| Reservoir | Station | Range cells / liter | Mean cells / liter $x10^5$ | Stan Dev cells / liter $x10^4$ | N |
|---|---|---|---|---|---|
| Belton | 1 | $1.93 \times 10^4$ - $1.30 \times 10^6$ | 4.60 | 2.24 | 44 |
| | 2 | $5.02 \times 10^4$ - $2.21 \times 10^6$ | 6.89 | 5.09 | 42 |
| Stillhouse Hollow | 1 | $1.69 \times 10^5$ - $1.22 \times 10^6$ | 7.34 | 2.72 | 30 |

Table 15. Statistical comparison of nanoflagellate abundance by stations.

| Station Comparison | Simultaneous Lower Confidence Limit | Difference Between Means | Simultaneous Upper Confidence Limit | Significant Difference |
|---|---|---|---|---|
| 2 - 1 | -3.210 | 0.446 | 4.102 | No |
| 2 - Stillhouse | -0.877 | 2.744 | 6.366 | No |
| 1 - Stillhouse | -1.001 | 2.298 | 5.597 | No |

Nanoflagellate abundance did not change with dissolved oxygen at Station 1 of Stillhouse Hollow Reservoir. Nanoflagellate abundance did change with dissolved oxygen at Station 1 and 2 of Belton Reservoir. The slope of nanoflagellate abundance versus dissolved oxygen was significantly different from a slope of "0", dissolved oxygen accounted for 32% and 29% of variation in nanoflagellate abundance at Station 1 and 2 of Belton Reservoir (Table 16).

Table 16. Statistical comparison of nanoflagellate abundance versus dissolved oxygen.

| Station | R-square value | P-value of t test | Significant |
|---|---|---|---|
| 1 | 0.3158 | 0.0065 | Yes |
| 2 | 0.2897 | 0.0212 | Yes |
| Stillhouse | 0.0266 | 0.5616 | No |

Areal hypolimnetic oxygen deficit

I calculated the areal hypolimnetic oxygen content by day (Appendix D). The calculated regression of areal hypolimnetic oxygen content by day is the AHOD (Fig. 3). Station 1 of Belton Reservoir (by the dam) was more

eutrophic (i.e. had a steeper slope) than Station 2 of Belton Reservoir (up-reservoir) and Station 1 of Stillhouse Hollow Reservoir.

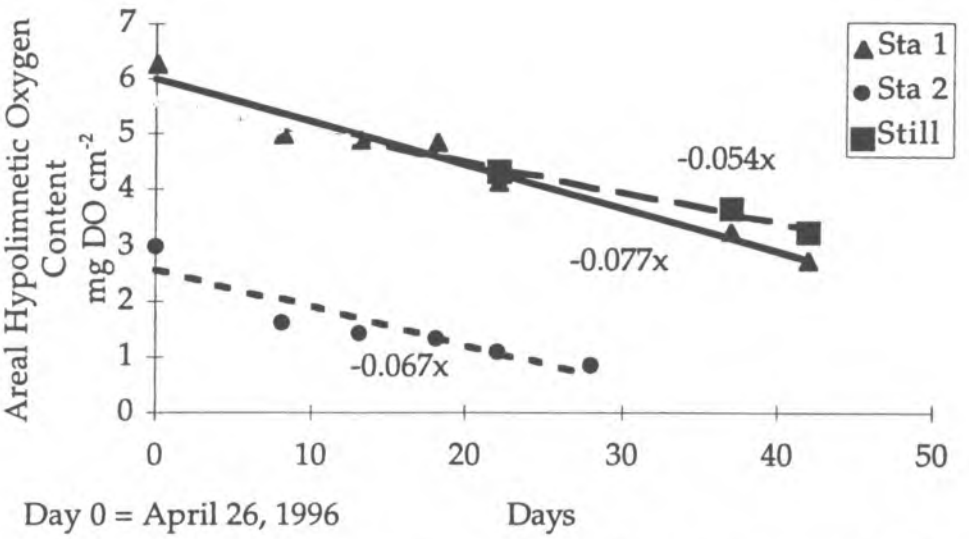


Fig. 3. Changes in areal hypolimnetic oxygen deficit

The slopes of the AHOD were statistically compared (Table 17). The 95% confidence intervals for each station overlapped, therefore I could not tell a statistical difference in their slopes (Table 17).

Table 17. Statistical comparison areal hypolimnetic oxygen deficits by stations.

| Reservoir | Station | 95% confidence interval of AHOD |
|---|---|---|
| Belton | 1 | $-0.095 \leq -0.075 \leq -0.060$ |
| | 2 | $-0.109 \leq -0.067 \leq -0.026$ |
| Stillhouse | 1 | $-0.154 \leq -0.054 \leq -0.046$ |

Relative areal hypolimnetic oxygen deficit

I converted the AHOD into the RAHOD (Table 18). There was no difference among the station's relative areal hypolimnetic oxygen deficit (Table 18).

Table 18. Station's relative areal hypolimnetic oxygen deficit

| Reservoir | Station | RAHOD (mg DO / $cm^2$ / d) |
|---|---|---|
| Belton | 1 | -0.037 |
| | 2 | -0.031 |
| Stillhouse | 1 | -0.035 |

# CHAPTER FOUR

## Discussion

### Hypolimnetic bacterial abundance

Reservoirs have heavier sediment and nutrient loads than natural, northern lakes. Reservoirs should have greater bacterial abundance than natural, northern lakes because bacterial abundance is proportional to a lake's trophic state (Silvey and Roach 1964; Straskrabova 1968; Kuznetsov 1970; Jones 1972, 1977; Faust and Correll 1976; Godlewska-Lipowa 1976, 1979; Ferguson and Palumbo 1979; Hobbie and Wright 1979; Rae et al. 1979; Fuhrman et al. 1980; Saunders 1980; Azam et al. 1983; Bird and Kalff 1984). Bacterial abundance in Belton and Stillhouse Hollow Reservoirs was not greater than in natural, northern lakes (Table 19).

Table 19. Comparison of previous researchers and this study's, ranges of hypolimnetic bacterial abundance.

| Researcher | Lake(s) | Total cells / liter |
|---|---|---|
| Cole et al. 1993 | 20 lakes in Wisconsin, Michigan, New York, New Hampshire | $1.1 \times 10^9$ - $2.2 \times 10^{10}$ |
| Ochs et al. 1995 | Mirror Lake, New Hampshire | $2.9 \times 10^9$ - $4.2 \times 10^9$ |
| This study | Belton Reservoir, Tx | $1.7 \times 10^9$ - $4.2 \times 10^9$ |
| | Stillhouse Hollow Reservoir, Tx | $1.4 \times 10^9$ - $2.7 \times 10^9$ |

In agreement with the bacterial abundance proportionality proposal, Belton Reservoir had more bacteria than Stillhouse Hollow Reservoir (Table 8). Stillhouse Hollow Reservoir is less eutrophic than Belton (Lind et al. 1993; TNRCC 1994) and should therefore have fewer bacteria.

Hypolimnetic bacterial abundance gradient

I observed a lengthwise pattern of bacterial abundance. Belton Reservoir had more bacteria up-reservoir (Station 2) than down-reservoir (Station 1) (Table 8). Taylor (1971) reported this horizontal bacterial abundance gradient in a Great Plains reservoir. Reservoirs are more eutrophic, have more nutrients and greater primary production up-reservoir than down-reservoir (Lind 1984; Wetzel 1990; Kennedy and Walker 1990; Kimmel et al. 1990; Lind et al. 1993).

Taylor (1942) states that areas that receive high concentrations of organic matter generally have more bacteria, and areas that receive lower concentrations of organic matter have fewer bacteria. Bacteria should be more abundant up-reservoir than down-reservoir. Probably the bacteria are more abundant up-reservoir because they are taking advantage of the greater availability of nutrients and dissolved organic molecules from primary production.

Another possibility is the relationship between bacteria and clay particles. Bacteria are more abundant attached to clay particles than in the open water (Lind and Dávalos-Lind 1991). Reservoirs have horizontal sedimentation gradients. Suspended sediments are more numerous

up-reservoir than down-reservoir (Thornton 1990). The more suspended sediments in an area the more bacteria present. Bacterial abundance may be lower down-reservoir because the bacterial cells could be sinking with their clay particles.

Bacterial abundance gradients could affect biological processes among the zones of a reservoir. Bacteria play three essential roles in biological processes: use dissolved organic matter and consume oxygen (Wright and Hobbie 1966; Wright 1975; Williams and Yentsch 1976); recycle nutrients (Hobbie and Crawford 1969; Hoppe 1976; Williams et al. 1976; Azam et al. 1983; Gude et al. 1985); convert dissolved substrates into particulate matter. Consumption of bacteria brings back particulate matter to the food web (Kuznetsov 1970; Williams 1983; Scavia D. and Laird G. 1987; Sherr and Sherr 1988). Bacteria are important to reservoir ecosystems (Chróst and Siuda 1977). Bacterial abundance gradients in reservoirs needs further study.

Hypolimnetic bacterial abundance and anoxia

Bacterial abundance did not increase with anoxia (Table 10). This agrees with Lind and Dávalos-Lind (personal communication) and in contrast to Jones (1978); Cole et al. (1993); and Ochs et al. (1995). Some possible explanations include the severe drought during sampling, and water residence time.

Severe drought. Texas had a severe drought during the spring of 1996 (Table 20). The drought could have reduced the phytoplankton production, which would affect the bacterial abundance (Fig. 4). Low nutrient values

have been associated with drought (Barnes 1990). Marshall (1988) reported that low inflows lead to low nutrient levels. Low chlorophyll a concentrations have occurred during droughts (Nichols 1985; Philips et. al 1995). Bacteria are affected by phosphorus and nitrogen levels (Chen 1968; Fouden 1969).

Table 20. Comparison of water inflow in normal (1995) and dry (1996) years by reservoirs.

| | Water Inflow average liters per day | | | | | |
|---|---|---|---|---|---|---|
| Reservoir | April 1995 | April 1996 | May 1995 | May 1996 | June 1995 | June 1996 |
| Belton | $8.85 \times 10^9$ | $3.80 \times 10^8$ | $5.36 \times 10^9$ | $2.89 \times 10^8$ | $3.62 \times 10^9$ | $8.29 \times 10^8$ |
| Stillhouse Hollow | $1.42 \times 10^9$ | $6.70 \times 10^7$ | $8.62 \times 10^8$ | $1.05 \times 10^8$ | $5.18 \times 10^8$ | $1.76 \times 10^8$ |

(BRA 1995, 1996)

drought ➡ low inflow ➡ low amounts of nutrients brought into the reservoir ➡ low phytoplankton production ➡ low dissolved organic carbon ➡ effect on bacteria

Fig. 4. Proposed drought effects on bacteria abundance.

Water residence time. Reservoirs generally have shorter water residence times than natural lakes. The water residence time is the amount of time required to fill up the reservoir volume. Reservoir's water residence times are short and vary from days to several weeks. In contrast to reservoirs, lake's water residence time are long and relatively constant from one to many

years (Wetzel 1990). During low inflow years the water residence time is longer than in normal flow years (Table 21). Reservoirs during low inflow years probably resemble lakes more than reservoirs. During low inflow years bacteria in a reservoir will spend more time in the reservoir. Hypolimnetic bacteria would have more time to react to low nutrient levels and anoxic conditions.

Table 21. Comparison of water residence time in normal and dry years by reservoir.

| Water residence time in months for sampling period (April - June) | | |
|---|---|---|
| Reservoir | 1995 | 1996 |
| Belton | 3 | 43 |
| Stillhouse Hollow | 12 | 94 |

Hypolimnetic bacterial abundance and temperature

Hypolimnetic bacterial abundance did not change with temperature in either reservoir (Table 10). This agrees with Lind and Dávalos-Lind (personal communication), and Cole et al. (1993).

Hypolimnetic bacterial abundance and total hypolimnetic nanoflagellates

Hypolimnetic bacterial abundance did not change with total hypolimnetic nanoflagellate abundance (Table 10). This is in contrast to other researchers (Haas and Webb 1979; Fenchel 1982; Azam et al. 1983; Linley et al. 1983; Sherr et al. 1984; Porter et al. 1985; Berman et al. 1987; de Giorgio et al. 1996).

The effect of nanoflagellates on bacteria is in dispute. Fukami et al. (1991) found that hypolimnetic nanoflagellate grazing rates and total bacterial consumption were significantly low. Other researchers state that ciliates are better bacteriovores (Sherr et al. 1989; Simek and Straskrabova 1992; Simek et al. 1995; Hwang and Heath 1997).

Usefulness of bacterial abundance counts to researchers

In contrast to Bird and Kalff's (1984) bacterial abundance proportionality proposal, Cole and Caraco (1993) found that bacterial abundance to be the least variable component of the plankton. Del Giorgio and Gasol (1995), Ducklow and Carlson (1992) found that large increases in phytoplankton biomass and production resulted in small increases in bacterial abundance. The underlying assumption of total bacterial abundance counts was that it represented the bacterial community activity. This assumption is invalid because not all bacterial cells are active (Stevenson 1979; Mason et al. 1986; del Giorgio and Scarborough 1995; Zweifel and Hagström 1995). Total bacterial abundance counts done with Acridine Orange may not be useful in assessing microbial communities because Acridine Orange does not differentiate between active and dormant cells.

Hypolimnetic bacterial biovolume

Reservoirs have heavier sediment and nutrient loads than natural, northern lakes, and therefore could have greater hypolimnetic bacterial biovolumes than natural, northern lakes. Heavier sediment and nutrient loads could stimulate phytoplankton production. Reservoirs are generally

more productive than natural lakes (Kimmel et al. 1990). Phytoplankton release dissolved organic carbon that bacteria utilize. Higher phytoplankton production in reservoirs results in higher dissolved organic carbon. Reservoirs with higher dissolved organic carbon could have greater hypolimnetic bacterial biovolumes than natural lakes. Heavier sediment loads in a reservoir could result in more particles for bacteria attachment. Lind and Dávalos-Lind (1991) found that bacteria attached to clay particles were larger than free floating bacteria. Reservoirs with higher sediment loads could have greater bacterial hypolimnetic biovolumes than natural lakes

Hypolimnetic bacterial biovolumes in Belton and Stillhouse Hollow Reservoir were not greater than in natural, northern lakes (Table 22 and 23).

Table 22. Comparison of ranges of hypolimnetic bacterial cell biovolumes between natural northern lakes and reservoirs.

| Researcher | Lake(s) | Hypolimnetic bacteria cell biovolume $\mu m^3$ |
|---|---|---|
| Cole et al. 1993 | 20 lakes in Wisconsin, Michigan, New York, New Hampshire | 0.01 - 0.2 |
| This study | Belton Reservoir, Tx | 0.0004 - 0.34 |
| | Stillhouse Hollow Reservoir, Tx | 0.0014 - 0.17 |

Table 23. Comparison of ranges of average hypolimnetic bacterial cell biovolumes between natural, northern lakes and reservoirs.

| Researcher | Lake(s) | Hypolimnetic average bacteria cell biovolume $\mu m^3$ |
|---|---|---|
| Ochs et al. 1995 | Mirror Lake, New Hampshire | 0.09 - 0.18 |
| Lind and Dávalos-Lind (personal communication) | Douglas Lake, Michigan | 0.03 - 0.2 |
| This study | Belton Reservoir, Tx | 0.007 - 0.07 |
| | Stillhouse Hollow Reservoir, Tx | 0.009 - 0.019 |

Average hypolimnetic bacterial cell biovolumes in my data set are lower than previous researchers (Table 24). Possible explanations why my bacterial biovolumes are low include methodology and drought.

Table 24. Comparison of previous researchers and this study's, average hypolimnetic bacterial cell biovolumes.

| Researcher | Lake(s) | Average hypolimnetic bacteria cell biovolume $\mu m^3$ | Stan Dev. |
|---|---|---|---|
| Cole et al. 1993 | 20 lakes in Wisconsin, Michigan, New York, New Hampshire | 0.03 | 0.028 |
| Ochs et al. 1995 | Mirror Lake, New Hampshire | 0.12 | 0.024 |
| Lind and Dávalos- Lind (personal communication) | Douglas Lake, Michigan | 0.08 | 0.049 |
| This study | Belton and Stillhouse Hollow Reservoir, Tx | 0.02 | 0.016 |

Methodology. Cole et al. (1993) said that the large variation of published cell sizes is the result of differences in cell size measurement methodologies. Differences in methodologies include equipment and cell size estimates.

Some researchers measure cells using computer image analysis software (Verity and Sieracki 1993), others take black and white photographs and measure the cells (Cole et al. 1993), while many researchers estimate bacteria using ocular micrometers (Lind and Dávalos-Lind 1991; Ochs et al. 1995; Lind and Dávalos-Lind personal communication). Bacteria have also been measured using scanning and transmission microscopy (Bratbak 1993).

Bacteria cell size estimates are varied. Some researchers measure to the nearest 0.1 µm (Cole et al. 1993) whereas others measure to the nearest 0.2 µm (Ochs et al. 1995). Cole et al. (1993) stated that estimates of absolute bacterial cell sizes are problematic and uncertain.

Drought. The drought could have also had an affect on the bacterial biovolumes. Texas had a severe drought during the spring of 1996 (Table 20). The drought could have affected the phytoplankton production, which could affect the bacterial biovolumes (Fig. 4).

Hypolimnetic bacterial biovolume gradient

I observed a bacterial biovolume horizontal pattern. Belton Reservoir had greater bacterial biovolumes up-reservoir (Station 2) than down-reservoir (Station 1) (Table 11). Although predicted from trophic states, this bacterial biovolume pattern has not been published.

A bacterial biovolume horizontal pattern is logical since reservoirs are more eutrophic, have more nutrients and greater primary production up-reservoir than down-reservoir (Lind 1984; Wetzel 1990; Kennedy and Walker 1990; Kimmel et al. 1990; Lind et al. 1993). The bacteria biovolumes may be greater up-reservoir because of the greater availability of nutrients and dissolved organic molecules from primary production.

Another possibility is the relationship between bacteria and clay particles. Bacteria attached to clay particles are larger than bacteria in open water (Simmon 1987; Lind and Dávalos-Lind 1991). Clay particles have high concentrations of nutrients (Arruda et al. 1983). Reservoirs have horizontal clay sedimentation gradients. Suspended sediments are more numerous up-reservoir than down-reservoir (Thornton 1990). The bacterial cells may be larger up-reservoir because of the clay particles. Average bacterial cells may be smaller down-reservoir because the larger bacterial cells could be sinking with their clay particles or the nutrients on the particles have been consumed. Bacterial cell biovolume gradients in reservoirs need further study.

Although Stillhouse Hollow Reservoir is less eutrophic than Belton (Lind et al. 1993; TNRCC 1994;) the bacterial biovolumes by the dams were not significantly different (Table 12). This suggests that bacterial biovolume may not be regulated by trophic state. The processes that regulate the bacterial biovolumes down-reservoir may be similar in both reservoirs.

Hypolimnetic bacterial biovolume and anoxia

Only at Station 2, was a relationship between hypolimnetic oxygen concentration and hypolimnetic bacterial cell biovolume significant (Table 13). Bacterial biovolumes decreased with anoxia (Fig. 5).

This is in contrast to Jones (1978); Cole et al. (1993); Ochs et al. (1995); and Lind and Dávalos-Lind (personal communication). Nevertheless, bacterial biovolumes have been found to decrease with anoxia in the Chesapeake Bay system (Cole personal communication). Some possible explanations include the severe drought during sampling, and shorter water residence time of reservoirs.

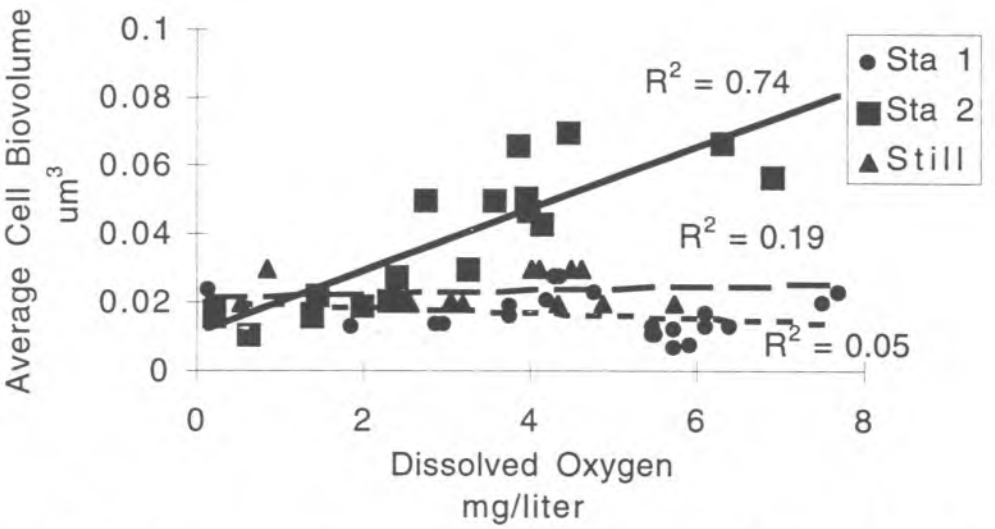


Fig. 5. Statistical correlation of average hypolimnetic bacterial biovolume and anoxia by stations.

Drought. As mentioned previously Texas had a severe drought during the spring of 1996 (Table 20). The drought could have affected the

phytoplankton production, which would affect the bacterial biovolume by decreasing the DOC levels (Fig. 4).

Water residence time. As mentioned previously reservoirs generally have shorter water residence times than natural lakes (Wetzel 1990). During the drought both reservoir's water residence time was longer than during regular inflow years (Table 21). During a drought, hypolimnetic bacteria would remain in the hypolimnion longer than during a normal inflow year. In a drought, the hypolimnetic bacteria would remain in the low nutrient and anoxic conditions longer. These conditions could have affected the hypolimnetic bacterial biovolumes.

Hypolimnetic bacterial biovolume and temperature

Only at Station 2 was there a relationship between hypolimnetic bacterial cell biovolume and temperature significant (Table 13). Temperature could only explain 33% of the data at Station 2. Bacterial biovolume did not change with temperature (Table 13). This agrees with Lind and Dávalos-Lind (personal communication), and Cole et al. (1993).

Hypolimnetic bacterial biovolume and nanoflagellates

Only at Station 2 was there a relationship between hypolimnetic bacterial cell biovolume and nanoflagellate abundance significant (Table 13). Nanoflagellate abundance explained 36% of the variation at Station 2. Bacterial biovolume did change with total nanoflagellate abundance (Table 13). This is in agreement with other researchers (Haas and Webb 1979;

Fenchel 1982; Azam et al. 1983; Linley et al. 1983; Sherr et al. 1984; Porter et al. 1985; Berman et al. 1987; de Giorgio et al. 1996).

Nanoflagellate abundance explained more hypolimnetic bacterial biovolume at Station 2 than the other stations (Table 13). Nanoflagellate grazing is size selective (Gonzalez et al. 1990; Sherr et al. 1992). The nanoflagellates may be more active at Station 2 than the other stations because Station 2 has larger bacteria cells (Table 12).

Usefulness of bacterial biovolumes to researchers

The underlying assumption of bacterial biovolume estimates was that they represented the bacterial community activity. This assumption is invalid because not all bacterial cells are active (Stevenson 1979; Mason et al. 1986; del Giorgio and Scarborough 1995; Zweifel and Hagström 1995). Bacterial biovolumes estimates done with Acridine Orange may not be useful in assessing microbial communities because Acridine Orange does not differentiate between active and dormant cell's sizes.

## Relative areal hypolimnetic oxygen demand

Comparing Belton and Stillhouse Hollow Reservoir's RAHOD

There was no difference in RAHOD rate between Belton and Stillhouse Hollow Reservoir. Belton Reservoir is typically more productive than Stillhouse Hollow (Table 1) so I had anticipated that Belton Reservoir's RAHOD would be higher than Stillhouse Hollow Reservoir's RAHOD.

Table 25. Comparison relative areal hypolimnetic oxygen deficit and trophic classification by station.

| Reservoir | Station | RAHOD (mg DO / $cm^2$ / d) | Mortimer (1941) | Hutchinson (1957) |
|---|---|---|---|---|
| Belton | 1 | -0.037 | Mesotrophic | Eutrophic |
| | 2 | -0.031 | Mesotrophic | Mesotrophic |
| Stillhouse Hollow | 1 | -0.035 | Mesotrophic | Eutrophic |

Comparing sample reservoir's RAHOD and natural northern lake's RAHOD

Belton and Stillhouse Hollow Reservoir had lower oxygen depletion rates than several natural, northern lakes (Table 25, Table 26). Typically reservoirs are more productive than natural lakes so I anticipated that reservoirs would have higher hypolimnetic oxygen depletion rates. During the sampling period Texas had a severe drought (Table 20). Drought could have affected primary production rates (Fig. 4). Low production rates would result in lower amounts of organic matter to be decomposed in the hypolimnion of the reservoir, which would affect oxygen deficits.

Table 26. Natural, northern lake RAHOD values (Charlton 1980b; Lind and Dávalos-Lind 1993).

| Lake | RAHOD (mg DO / $cm^2$ / d) | Mortimer (1941) | Hutchinson (1957) |
|---|---|---|---|
| Douglas Lake Depressions | | | |
| Fairy Island | -0.096 | Eutrophic | Eutrophic |
| Grapevine Point | -0.058 | Eutrophic | Eutrophic |
| South Fishtail Bay | -0.113 | Eutrophic | Eutrophic |
| Lake Superior | -0.039 | Mesotrophic | Eutrophic |
| Lake Michigan | -0.069 | Eutrophic | Eutrophic |
| Lake Ontario | -0.120 | Eutrophic | Eutrophic |
| Georgian Bay | -0.031 | Mesotrophic | Mesotrophic |
| Lake East Erie | -0.054 | Mesotrophic | Eutrophic |

RAHOD intra-reservoir comparisons

The RAHOD method did not detect a hypolimnetic oxygen depletion difference between Belton Reservoir Stations 1 and 2. Station 2 (up-reservoir) was less "eutrophic" than Station 1 (by the dam). This was unexpected. Typically up-reservoir stations are more eutrophic than down-reservoir stations (Cole and Hannan 1990; Lind et al. 1993). The RAHOD method may be unable to determine intra-reservoir differences because of lake morphology, water residence time, and advection of organic carbon.

Lake morphology and RAHOD. Lake morphology can affect RAHOD values. I calculated the RAHOD for the entire hypolimnion of Belton and Stillhouse Hollow Reservoirs (Table 27).

Table 27. Whole lake relative areal hypolimnetic oxygen deficit for sample reservoirs

| Reservoir | RAHOD (mg DO / $cm^2$ / d) | Mortimer (1941) | Hutchinson (1957) |
|---|---|---|---|
| Belton | -0.016 | Oligotrophic | Oligotrophic |
| Stillhouse Hollow | -0.025 | Oligotrophic | Mesotrophic |

The RAHOD for the entire hypolimnion of Belton and Stillhouse Hollow Reservoirs were lower than the RAHOD for the segmented hypolimnia (Table 27, Table 25). Belton Reservoir, based on the RAHOD for the entire hypolimnion, is classified as oligotrophic (Table 27) instead of mesotrophic (Table 25). This difference can be attributed to the differences between the two stations that were averaged. Station 2 (up-reservoir) went

anoxic earlier than Station 1 (down-reservoir). When calculating the average oxygen level, Station 2 data lowered the average oxygen level.

Stillhouse Hollow Reservoir's RAHOD was not very different from the segmented RAHOD (Table 27, Table 25). I did not have an up-reservoir station on Stillhouse Hollow Reservoir to include in the RAHOD. More research needs to be done on intra-reservoir comparisons using the RAHOD method. Future research could study whether a segmented hypolimnion RAHOD or hypolimnion RAHOD better represents the trophic status of the reservoir.

Water residence time, advection of organic carbon and RAHOD. Many reservoirs have short water residence times. The RAHOD method, developed on natural lakes, does not account for water flowing into and out of a hypolimnion. How does one account for the advection of organic carbon in a reservoir? Further research could be done to study the possible effects of short water residence times on RAHOD values.

Should the RAHOD method be used to assess eutrophication?

The RAHOD method can be used on reservoirs to assess eutrophication because it accounts for temperature and depth differences (Table 28).

Table 28. Comparison of hypolimnion temperature and volume between sample reservoirs.

| Reservoir | Station | Mean Volume Weighted Hypolimnion Temperature $^0$ C | Hypolimnion Volume $m^3$ |
|---|---|---|---|
| Belton | 1 | 14.2 | $1.6 \times 10^6$ |
| | 2 | 15.0 | $2.8 \times 10^5$ |
| Stillhouse Hollow | 1 | 10.5 | $3.2 \times 10^6$ |

Belton Reservoir Station 2 (up-reservoir) had higher temperature and less volume than the Station 1 (down-reservoir). Stillhouse Hollow Reservoir had lower temperature and more volume than the Belton stations. The AHOD method cannot account for this temperature and volume differences (table 28). The sample station's AHOD (Table 29) show differences in trophic states.

Table 29. Comparison of areal hypolimnetic oxygen deficit by stations.

| Reservoir | Station | AHOD (mg DO / $cm^2$ / d) | Mortimer (1941) | Hutchinson (1957) |
|---|---|---|---|---|
| Belton | 1 | -0.075 | Eutrophic | Eutrophic |
| | 2 | -0.067 | Eutrophic | Eutrophic |
| Stillhouse Hollow | 1 | -0.054 | Mesotrophic | Eutrophic |

The RAHOD method accounts for temperature and depth differences (Charlton 1980b). The sample station's RAHOD values (Table 25) do not show trophic state difference. The RAHOD method can be used on reservoirs. Further research needs to be done on determining how RAHOD values

represent eutrophication or primary production. This could be studied by comparing reservoir primary production rates to reservoir RAHOD values.

Future RAHOD research

Other research questions that should be investigated include: Can you divide a single hypolimnion? How much epilimnetic area is represented by the hypolimnion in a reservoir (high flushing rates) as compared to a natural lake (low flushing rates)? Do hypolimnetic processes in a section of the hypolimnion contribute to the overall hypolimnetic processes? More research needs to be done on using the RAHOD method for intra-reservoir comparisons. If intra-reservoir comparisons are possible using the RAHOD method, it would allow lake managers to evaluate lake management policies between zones in their reservoir.

## Conclusions

### Hypolimnetic bacteria

Hypolimnetic bacterial abundance. Hypolimnetic bacterial abundance in Belton and Stillhouse Hollow Reservoir was not greater than bacterial abundance in natural, northern lakes. Hypolimnetic bacterial abundance's were not correlated with time, dissolved oxygen, temperature, and nanoflagellate abundance.

Hypolimnetic bacterial cell size. Hypolimnetic bacteria cell sizes in Belton and Stillhouse Hollow Reservoir were smaller than bacterial cell sizes in natural, northern lakes. Hypolimnetic bacteria cell sizes at Station 2 of Belton Reservoir were correlated with time, and dissolved oxygen.

Hypolimnetic bacteria cell sizes at Station 1 of Belton and Stillhouse Hollow Reservoir was not correlated with time, and dissolved oxygen. Hypolimnetic bacteria cell sizes at all stations were not correlated with temperature or nanoflagellate abundance.

RAHOD

The RAHOD of Belton and Stillhouse Hollow Reservoir were lower than the RAHODs of natural northern lakes. The RAHOD method did not detect a trophic state difference between Belton and Stillhouse Hollow Reservoirs. The RAHOD method did not detect a trophic state difference between the stations of Belton Reservoir.

# APPENDICES

Appendix A. Comparisons of Hydrolab DataSonde and Winkler Titration Dissolved Oxygen Measurements mg $l^{-1}$

| Trip # | Date | Station | Depth (m) | Hydrolab DataSonde | Winkler titration |
|---|---|---|---|---|---|
| 12 | June 2, 1996 | 1 | 05 | 7.67 | 8 |
| 12 | June 2, 1996 | 1 | 10 | 7.82 | 8.1 |
| 12 | June 2, 1996 | 1 | 15 | 4.69 | 4.7 |
| 12 | June 2, 1996 | 1 | 20 | 4.75 | 5.1 |
| 12 | June 2, 1996 | 1 | 24 | 3.74 | 3.9 |
| 12 | June 2, 1996 | 2 | 05 | 7.81 | 8.7 |
| 12 | June 2, 1996 | 2 | 10 | 6.24 | 6.4 |
| 12 | June 2, 1996 | 2 | 15 | 3.41 | 2.8 |
| 12 | June 2, 1996 | 3 | 05 | 7.53 | 7.6 |
| 12 | June 2, 1996 | 3 | 10 | 3.4 | 3.5 |
| 12 | June 2, 1996 | 4 | 05 | 6.89 | 6.7 |
| 12 | June 2, 1996 | 4 | 08 | 4.98 | 1.8 |
| 12 | June 2, 1996 | 5 | 04 | 6.58 | 6.2 |
| 12 | June 2, 1996 | Stillhouse | 10 | 7.22 | 6.9 |
| 12 | June 2, 1996 | Stillhouse | 15 | 5.28 | 4.6 |
| 12 | June 2, 1996 | Stillhouse | 20 | 4.6 | 4.1 |
| 12 | June 2, 1996 | Stillhouse | 25 | 3.03 | 2.7 |
| 13 | June 7, 1996 | 1 | 15 | 4.66 | 6.2 |
| 13 | June 7, 1996 | 1 | 25 | 2.96 | 3.2 |
| 13 | June 7, 1996 | 2 | 15 | 2.75 | 2.4 |
| 13 | June 7, 1996 | 2 | 19 | 0.61 | 0.5 |
| 13 | June 7, 1996 | 3 | 12 | 1.02 | 1.4 |
| 13 | June 7, 1996 | 4 | 10 | 5.64 | 4.6 |
| 13 | June 7, 1996 | Stillhouse | 25 | 3.04 | 3.2 |
| 14 | July 1, 1996 | 1 | 15 | 1.42 | 1.4 |
| 14 | June 1, 1996 | 1 | 20 | 1.86 | 2.1 |
| 14 | July 1, 1996 | 1 | 24 | 0.14 | 0.5 |
| 14 | July 1, 1996 | 2 | 10 | 1.63 | 1.2 |
| 14 | July 1, 1996 | 2 | 15 | 0.21 | 0.3 |
| 14 | July 1, 1996 | 2 | 19 | 0.18 | 0.1 |
| 14 | July 1, 1996 | 3 | 10 | 0.24 | 0.6 |
| 14 | July 1, 1996 | 4 | 08 | 1.37 | 2.7 |
| 14 | July 1, 1996 | Stillhouse | 15 | 2.52 | 2.2 |
| 14 | July 1, 1996 | Stillhouse | 20 | 0.83 | 0.4 |
| 14 | July 1, 1996 | Stillhouse | 24 | 0.51 | 0.5 |

Appendix B. Comparison of depth to the top of the Hypolimnia by trip number and stations.

| Trip # | Reservoir | Station | Top of the Hypolimnion (m) |
|---|---|---|---|
| 5 | Belton | 1 | 18 |
| | | 2 | 15 |
| 6 | Belton | 1 | 18 |
| | | 2 | 17 |
| 7 | Belton | 1 | 19 |
| | | 2 | 17 |
| 8 | Belton | 1 | 18 |
| | | 2 | 17 |
| 9 | Belton | 1 | 18 |
| | | 2 | 16 |
| | Stillhouse | 1 | 16 |
| 10 | Belton | 1 | 18 |
| | | 2 | 17 |
| 11 | Belton | 1 | 18 |
| | | 2 | 17 |
| | Stillhouse | 1 | 15 |
| 12 | Belton | 1 | 18 |
| | | 2 | 17 |
| | Stillhouse | 1 | 15 |
| 13 | Belton | 1 | 18 |
| | | 2 | 17 |
| | Stillhouse | 1 | 15 |
| 14 | Belton | 1 | 18 |
| | | 2 | 17 |
| | Stillhouse | 1 | 15 |

Appendix C. Comparison of surface area and volume of each hypolimnetic stratum by station.

| Reservoir | Station | Depth m | Surface area of stratum ($m^2$) | Volume of stratum ($m^3$) |
|---|---|---|---|---|
| Belton | 1 | 18 | 1713180 | |
| | | 19 | 1661371 | 1687275 |
| | | 20 | 16140406 | 1637705 |
| | | 21 | 1566859 | 1590450 |
| | | 22 | 1518618 | 1542739 |
| | | 23 | 1438586 | 1478602 |
| | | 24 | 1358553 | 1398569 |
| | | 25 | 1278520 | 1318537 |
| | | 26 | 1008685 | 1143602 |
| | | 27 | 738106 | 873395 |
| | | 28 | 467528 | 602817 |
| | | 29 | 338834 | 403181 |
| | | 30 | 217642 | 278238 |
| | | 31 | 96450 | 157046 |
| | | 32 | 24282 | 60366 |
| | | 33 | 4047 | 14165 |
| Belton | 2 | 15 | 308743 | |
| | | 16 | 270019 | 289381 |
| | | 17 | 232747 | 251383 |
| | | 18 | 195474 | 214111 |
| | | 19 | 182048 | 188761 |
| | | 20 | 142208 | 162128 |
| | | 21 | 101732 | 121970 |
| | | 22 | 61261 | 81496 |
| | | 23 | 35204 | 48232 |
| | | 24 | 21484 | 28344 |
| | | 25 | 7764 | 14624 |

Appendix C. Continued, comparison of surface area and volume of each hypolimnetic stratum by station.

| Reservoir | Station | Depth m | Surface area of stratum ($m^2$) | Volume of stratum ($m^3$) |
|---|---|---|---|---|
| Stillhouse | 1 | 15 | 3305826 | |
| | | 16 | 3159044 | 3232435 |
| | | 17 | 3003845 | 3081445 |
| | | 18 | 2848646 | 2926246 |
| | | 19 | 2703605 | 2776126 |
| | | 20 | 2582848 | 2643227 |
| | | 21 | 2462090 | 2522469 |
| | | 22 | 2204953 | 2333521 |
| | | 23 | 1936012 | 2070482 |
| | | 24 | 1667325 | 1801669 |
| | | 25 | 1484315 | 1575820 |
| | | 26 | 1287021 | 1385668 |
| | | 27 | 1089727 | 1188374 |
| | | 28 | 808415 | 949071 |
| | | 29 | 535223 | 671819 |
| | | 30 | 262030 | 398626 |
| | | 31 | 161880 | 211955 |
| | | 32 | 36423 | 99152 |
| | | 33 | 16188 | 26306 |
| | | 34 | 8094 | 12141 |
| Stillhouse | 1 | 35 | 0 | 4047 |
| | | 36 | 0 | 0 |

Appendix D. Comparison of areal hypolimnetic oxygen content by day by station.

| Areal Hypolimnetic Oxygen Content by Day (mg $O_2$ $cm^{-2}$) | | | |
|---|---|---|---|
| Sample Day | Belton, Station 1 | Belton, Station 2 | Stillhouse, Station 1 |
| 0 | 6.28 | 2.96 | - |
| 8 | 5.02 | 1.59 | - |
| 13 | 4.90 | 1.43 | - |
| 18 | 4.87 | 1.31 | - |
| 22 | 4.14 | 1.10 | 4.37 |
| 28 | - | 0.86 | - |
| 37 | 3.24 | - | 3.67 |
| 42 | 2.75 | - | 3.24 |

Appendix E. Comparison between image analysis and ocular method's bacterial cell biovolumes.

| | Computer | | | Ocular | | |
|---|---|---|---|---|---|---|
| Sample | Biovolume | Stan Dev. | N | Biovolume | Stan Dev. | N |
| 100&101 | 0.013 | 0.02 | 160 | 0.015 | 0.03 | 160 |
| 230&251 | 0.021 | 0.03 | 160 | 0.021 | 0.03 | 160 |
| 125&128 | 0.047 | 0.06 | 160 | 0.036 | 0.05 | 160 |

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