SURVIVAL OF FECAL INDICATOR BACTERIA, BACTERIOPHAGE AND PROTOZOA IN FLORIDA'S SURFACE AND GROUND WATERS

Potential Implications for Aquifer Storage and Recovery

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EXECUTIVE SUMMARY

One technology of rising importance, which could allow for better management of water resources in Florida, involves injection of surface water (generally post-treatment) to aquifers for storage and later recovery for use, termed Aquifer Storage and Recovery (ASR). The Fate of Microorganisms in Aquifers Study has been jointly funded by the South Florida Water Management District and the Southwest Florida Water Management District to investigate survival of several groups of water-quality-related microorganisms used as fecal indicators and public health risks in situations that may be encountered in the Floridan aquifer system, particularly in the course of injection of surface water into the subsurface. The study objectives included:

- A literature review summarizing current knowledge on factors affecting survival and decline of important microbes in ground water
- Bench-scale (laboratory) survival studies of several groups of microorganisms to define the relative impacts of temperature and total dissolved solids under controlled conditions
- Bench-scale survival studies of these microorganisms in representative surface water and ground water sources with potential for, or in active utilization of, ASR systems, focusing on relative impacts of temperature and background microbial community on survival of seeded indicator microbes, and temperature on parasites
- Description of predicted survival periods and inactivation rates of indicator bacteria and viruses, as well as parasites, in Florida surface and ground waters potentially involved in ASR projects at temperatures typical of the Florida subsurface environment

Specifically, two main branches of laboratory investigations were performed for this project: survival studies in controlled conditions to isolate the effects of temperature and total dissolved solids (TDS), and studies in both raw and pasteurized samples of representative aquifer and reservoir water to characterize survival in these types of water, with additional interest in effects of temperature and reduced background microbial levels (via pasteurization). Microorganisms evaluated included two groups of fecal indicator bacteria (fecal coliform and enterococci), three groups of fecal indicator bacteriophage (DNA coliphage, F+ RNA coliphage, and PRD-1, all of which are viruses that infect bacteria with the coliphage being suggested as a

option under the Groundwater rule and PRD-1 used primarily as a tracer due to it's known stability) and two pathogenic parasites, *Cryptosporidium parvum* and *Giardia lamblia*. The parasites were evaluated in raw (unpasteurized) water only.

Bench-scale microcosm experiments were performed to evaluate inactivation over time of the various organisms in water samples at three temperatures: 5° C, 22° C, and 30° C. To make comparisons of the relative effects of factors such as TDS and temperature, or in environmental water trials water type and pasteurization treatment effects, a single comparative statistic was necessary for analysis of variance (ANOVA) tests. In the scientific literature there are typically two ways of analyzing data on survival. The first is the plotting of the decrease in numbers of microorganisms over time and examining the slope of a regression curve, often a first-order regression model (k values which are inactivation rates, expressed as a decrease in $\log_{10}$/day). The second method is to examine a level of decrease in numbers in percentages, for example in the EPA *Surface Water Treatment Rule* and the development of the new *Long-Term Enhanced Surface Water Treatment Rule*, one goal is 99.9% decrease in surface water concentrations of *Giardia* for finished drinking water.

Kinetics of decline in microcosms often deviated from first-order. Additionally, differences in inactivation kinetics between conditions and organisms, and the limitation of being able to measure only about 4-5 $\log_{10}$ total decline precluded the use of total inactivation over a period of time as a comparative statistic. Thus, for comparisons of the relative impact of factors that were examined for the bacteria and two types of coliphage (along with *Cryptosporidium* in TDS-temperature experiments), observed inactivation kinetics were fit with one of two regression equations and the resulting number of days for 99% decline was used as a comparative statistic. However, for PRD-1, *Cryptosporidium parvum* in environmental waters, and *Giardia lamblia*, first-order inactivation rate constants were employed as this was a superior comparative statistic for these organisms. The periods or rates from each independent trial data set were used to compare variability between conditions with ANOVA tests, which revealed factors that were determined to be statistically significant in affecting relative survival capacity.

Artificial Waters

Results of survival studies in controlled water conditions revealed that statistically significant effects on inactivation were observed for temperature for all organisms except for

PRD-1 (Table E1). Variation of TDS over the range from 200 - 1000 mg/L did not affect inactivation for any of the organisms. When TDS concentrations up to 3,000 mg/L were considered, statistically significantly higher rates of inactivation, due to TDS concentrations above 3000 mg/L, were observed for enterococci and F+ RNA coliphage. However, this effect was also clouded by a temperature correlation for F+ RNA coliphage, such that survival was longer at higher TDS only at 5º C. Also, for enterococci the TDS effect was not clear due to inconsistent trends, meaning inactivation on average was most rapid at 1000 mg/L and least rapid at the next higher TDS of 3000 mg/L. Although temperature significantly affected inactivation when 5° C was considered, mean days for 99% decline in culturable concentrations were frequently grouped close together for 22° and 30° C, suggesting that temperature differences over this range may not definitively affect inactivation of these organisms.

Table E1. Statistical significance of TDS and temperature for indicator organisms and *Cryptosporidium*

Organism	TDS range (mg/L)	Significant factors (95% unless noted)
fecal coliform	200 - 3000	**temp**
	200 - 1000	**temp**
enterococci	200 - 3000	**temp, TDS (90%)**
	200 - 1000	**temp**
F+ RNA coliphage	200 - 3000	**temp, TDS, interact**
	200 - 1000	**temp**
DNA coliphage	200 - 3000	**temp (90%)**
	200 - 1000	**temp (90%)**
PRD-1	200 - 3000	**none**
	200 - 1000	**none**
Cryptosporidium	200 - 1000	**temp**

Natural Waters

Representative water from two locations in Florida was utilized for natural water survival studies. This included samples from Avon Park aquifer and Bill Evers reservoir as well as Lake

Lytal Park aquifer and Clear Lake reservoir. The main objectives of this phase of the investigation were twofold. Firstly, a comparison of the relative impacts of temperature, water type, and background microbial community was made using predicted 99% inactivation periods or first-order rate constants (for PRD-1 and the parasites) as a proxy for survival, and as a statistic for ANOVA tests. Pasteurization treatment to evaluate the effect of background microorganism effects was done only for the indicator organisms. Second and most importantly, a determination of survival in temperature conditions typical of the Florida subsurface was desired. To describe this, the predicted number of days for 2-log (99%) inactivation in only the raw (unpasteurized) water from the 4 sites, at temperatures of 22º and 30º C, were examined separately from the pasteurized or colder temperature conditions. The determination of first-order rate constants was also made for all organism groups. This was enabled because in the raw water trials at these temperatures, inactivation kinetics were generally first-order and could be accurately fit by such a regression curve, unlike the artificial water trials, pasteurized samples, and some 5º C conditions, where kinetics frequently necessitated the use of alternative regression models.

Based on comparative analyses of observed inactivation behavior, several overall trends were statistically significant. Primarily, inactivation increased steadily with increasing temperature; this was true in both combined raw and pasteurized comparisons and in raw water comparisons. Also, inactivation was typically more rapid in surface water under raw conditions. One exception is the F+ RNA phage, which were quite fragile and survived poorly under both conditions. The other exception is *Giardia lamblia*. It is not clear why data for *Giardia* showed an opposite trend. It may be the method used for measuring viability was affected by the TDS concentrations or another chemical component of the groundwater. The viability dyes, used for assessing cyst viability, are based on the permeability of the membrane. It is conceivable the nature of the water affects this permeability. It is known that the dye test is not completely reliable for assessing parasite viability. The viability of *Cryptosporidium* oocysts, on the other hand, is measured via cell culture and is a true measure of infectivity.

Heat pasteurization to reduce native organism populations also had a statistically significant effect of reducing inactivation of seeded indicator organisms in many cases, and its effect was sometimes more significant in surface water than ground water (for enterococci and DNA coliphage this was the case). Another point of interaction was the relatively greater

increase of inactivation of fecal coliform and PRD-1 with increasing temperature in surface water over ground water.

Predicted survival times and inactivation rates for the different types of microorganisms were determined in environmental water sources (raw), specifically at temperatures typical of the Floridan aquifer system (22° to 30° C). Regarding the indicator organism populations, for fecal coliform, 2-log inactivation was predicted over periods on the order of 1 - 6 weeks in ground water, depending on site and temperature, and 1 - 2 weeks in surface water. Enterococci predictions ranged slightly less at around 1 - 5 weeks in ground water, also depending on temperature and site, and about 1 week in surface water sources. A comparison of the two types of coliphage revealed that DNA coliphage was much hardier in the conditions evaluated. F+ RNA coliphage had the shortest periods for 2-log decline, thus the most rapid inactivation in these experiments. At subtropical temperatures, 2-log inactivation in these water sources (both surface and ground water) would be predicted in 1 week or less for F+ RNA. DNA coliphage results, on the other hand, indicated 2-log inactivation over periods on the order of 2 - 6 weeks in both water types. As expected, PRD-1 in these conditions was the most stable of the indicator organisms evaluated. For instance, periods for 2-log decline in Avon Park well water were predicted at around 6 months at 22° or 30° C based on observed inactivation rates over 28 days. Predicted durations were shorter in other water sources, and were within experimental durations for the surface water at 2 -3 weeks.

In comparing indicator inactivation to the parasites, *Cryptosporidium* inactivation was much slower than the bacterial indicators in the Avon Park samples at 22° C with little observed decline over the course of the experiment, but was more similar (although slightly less) to those of the two bacterial indicators in Lake Lytal Park well water at 22° C and in both ground water sources at 30° C. 99% declines for *Cryptosporidium* were predicted to take about 7 weeks at 22º C in Lake Lytal Park water and 2-3 weeks at 30º C in both ground water sources. In surface water, 2-log inactivation of *Cryptosporidium* at 22º C was predicted over 4-7 weeks and at 30º C in 1-2 weeks. *Giardia* inactivation rates were down to ½ those of bacterial indicators in ground water at 22° and 30° C with 2-log declines predicted in 7-9 weeks at 22º and 2-3 weeks at 30º C. There was negligible measured inactivation observed for *Giardia* in surface water at 22º C, while 2-log declines at 30º would be predicted in 3-4 weeks. Once again, the method of analysis for *Giardia* may have influenced the apparent lack of decline, since the assay for its viability relies

only on cyst membrane integrity and not actual viability or infectivity. With the exception of Avon Park water at 22° C, inactivation of the two parasites was fairly similar to each other in ground water at these temperatures.

The following tables show computed first-order inactivation rates from combined data sets of each organism in water from each of the four sites. Rates from survival studies at 22º and 30º C are shown, as these are the temperatures most representative of the Floridan aquifer system.

Table E3. First-order inactivation rates of all organism groups in ground water at 22° and 30° C

Avon Park 22° C		Lake Lytal Park 22° C		Avon Park 30° C		Lake Lytal Park 30° C	
C. parvum	-0.0010	PRD-1	-0.027	PRD-1	-0.015	PRD-1	-0.045
PRD-1	-0.017	*G. lamblia*	-0.030	*G. lamblia*	-0.098	*G. lamblia*	-0.11
G. lamblia	-0.040	*C. parvum*	-0.042	*C. parvum*	-0.11	*C. parvum*	-0.12
DNA coliphage	-0.064	enterococci	-0.062	DNA coliphage	-0.13	enterococci	-0.13
fecal coli	-0.10	fecal coli	-0.065	fecal coli	-0.17	fecal coli	-0.15
enterococci	-0.16	DNA coliphage	-0.072	enterococci	-0.25	DNA coliphage	-0.15
F+ RNA phage	-0.51	F+ RNA phage	-0.45	F+ RNA phage	-1.6	F+ RNA phage	-2.4

Table E4. First-order inactivation rates of all organism groups in surface water at 22° and 30° C

Bill Evers 22° C		Clear Lake 22° C		Bill Evers 30° C		Clear Lake 30° C	
G. lamblia	-0.0050	*G. lamblia*	-0.0042	*G. lamblia*	-0.081	*G. lamblia*	-0.076
C. parvum	-0.045	C. parvum	-0.066	*PRD-1*	-0.15	*PRD-1*	-0.12
PRD-1	-0.10	*PRD-1*	-0.084	DNA coliphage	-0.17	DNA coliphage	-0.16
DNA coliphage	-0.12	DNA coliphage	-0.092	C. parvum	-0.20	C. parvum	-0.18
F+ RNA phage	-0.25	fecal coli	-0.17	F+ RNA phage	-0.63	fecal coli	-0.30
enterococci	-0.38	enterococci	-0.27	enterococci	-0.77	enterococci	-0.50
fecal coli	-0.42	F+ RNA phage	-0.94	fecal coli	-1.0	F+ RNA phage	-2.0

Conclusions:

- Among the lower TDS concentrations evaluated, ranging from 200 - 1000 mg/L, TDS was not a significant variable for survival of any organism group. TDS had a statistically significant effect on enterococci and F+ RNA, but only at the highest TDS concentration of 3000 mg/L, which increased the survival of the bacteria and phage on average. However, closer inspection of these results revealed that this trend may not be consistent.

- Temperature in TDS-temperature experiments had a statistically significant effect on all microbial inactivation except for PRD-1, with greater declines at faster rates as temperature increased. PRD-1 is not commonly taken as an indicator of pathogenic virus survival.
- Fecal coliform, enterococci, DNA coliphage, PRD-1 and *Cryptosporidium* all experienced greater inactivation in surface water than in ground water. In contrast, RNA coliphage and *Giardia* experienced greater inactivation in groundwater than surface water.
- In the raw water experiments, water type (surface versus ground water) was statistically significant for the enterococci, F+ RNA coliphage, PRD-1, *Cryptosporidium*, and *Giardia*.
- For fecal coliform, 2-log inactivation was predicted over periods on the order of 2 - 6 weeks in ground water and 1 - 2 weeks in surface water; enterococci predictions ranged slightly less at around 1 - 5 weeks in ground water and about 1 week in surface water sources and F+ RNA coliphage less than a week.
- The parasites and the DNA coliphage were much more resistant than the fecal coliform or enterococci and as much as 7 months would be predicted to achieve 99% reduction of *Giardia* cysts in surface waters, while 1 to 4 months would be required for DNA phage and *Cryptosporidium* oocysts.
- The fragility of the F+ RNA coliphage suggests that if these are found in ground water very recent contamination (1 to 6 days) has occurred. However these are not adequate indicators of the attenuation of microorganisms of fecal origin under storage conditions.
- No bacterial or coliphage indicators are adequate indicators of the risks potentially associated with the increased survival of the enteric protozoa.
- Site-specific monitoring of any test injection sites using water containing these organisms should be required until survival behavior in field conditions can be better defined.

The Fate of Microorganisms in Aquifers Study has sought to fill data gaps in the published literature on quantitative analysis of survival and inactivation of a number of organism

groups in conditions particular to what might be encountered in the context of ASR impacts on the Floridan Aquifer system and other Florida ground water environments. The bench-scale experiments provided a controlled set of situations to carefully examine the behavior of these organism groups, with the intended purpose of both establishing actual inactivation kinetics in water sources and conditions representative of the Florida environment, and to elucidate some of the factors which may affect survival of organisms in question. Results from these experiments were analyzed to describe some important factors influencing the variability of inactivation via statistical tests, and information on observed inactivation was used to estimate some possible persistence times under the conditions found in Florida ground or surface water that may be injected underground. The results of this report will hopefully constitute a stepping stone that will help make informed decisions about the future direction of studies on public-health microorganism survival in subtropical ground water environments and ultimately help to clarify some of the unknown potential impacts of surface water injection to aquifers.

INTRODUCTION AND PURPOSE OF THE STUDY

The management of water resources is important in the State of Florida for maintaining healthy ecosystems (such as the Florida Everglades), supplying growing communities with potable water and providing industries and agriculture with adequate water for their needs. One technology of rising importance that could allow for better management involves injection of surface water to aquifers for storage and later recovery for use, termed Aquifer Storage and Recovery (ASR). ASR is the practice of injecting surface water, which has been treated to drinking water standards, into suitable aquifers during times of excess supply, in order to store large quantities of water for later withdrawal during times of reduced supply (either seasonal or long-term drought) or for other instances of need. In addition to water quantity, quality of the injected water and the ground water remains an area of interest including the physical, chemical and biological nature of the water. In order to address public health risks it is the microbiological nature of the water that is very often evaluated.

Microbiological water quality using both indicator bacteria, indicator viruses (e.g., the coliphage) and direct monitoring for pathogens (e.g., the enteric protozoa, *Cryptosporidium* spp. and *Giardia* spp.) has now been addressed under new rules for ground and surface water within the *Safe Drinking Water Act*. Waterborne microorganisms of public-health concern and the indicator microorganisms used to judge the safety of water are found in surface waters but are generally considered to be absent from ground water. Microorganisms enter aquifers via several sources and mechanisms, including percolation from surface water, sinkholes, septic systems, leaky sewer lines or direct injection of inadequately treated wastewater effluent or stormwater.

In Florida, fresh water may be injected into aquifers of varying physical and chemical conditions, including brackish salinity. In cases of higher total dissolved solids (TDS) aquifers, the stored water would form a freshwater layer of low-TDS water in a zone of discharge around the ASR well, to be later drawn up for treatment and distribution. The main intended users of ASR systems are municipal and regional water supply management agencies, for storage of water to ultimately be utilized for potable (drinking) water distribution and other non-potable uses such as agriculture or wetlands restoration. A major component of the Comprehensive Everglades Restoration Plan (CERP) involves the use of ASR wells to pump excess surface water during the summer rainy season into the Upper Floridan Aquifer for recovery during drier

months for stabilization of water flows through the Everglades ecosystem. The scale of this proposed use for ASR is unprecedented.

Under current regulations, ASR wells are classified by the U.S. EPA as Class V Underground Injection Control (UIC) wells, and thus subject to regulation under the U.S. Safe Drinking Water Act. Development of controls on and oversight of Class V UIC wells, and generally other classes of UIC wells, falls primarily on state environmental protection agencies. As such, the Florida Department of Environmental Protection (FDEP) has set rules regulating ASR systems utilizing aquifer regions classified as Underground Sources of Drinking Water (USDW), which encompasses aquifers of under 10,000 mg/L TDS including the Upper Floridan Aquifer. A key component of these regulations is that ASR wells may not inject water which violates the Total Coliform Rule of the U.S. Safe Drinking Water Act, which specifies that potable water must have no total coliform bacteria per 100 ml. Since surface water in Florida would most always violate this rule, water for injection in ASR systems must be disinfected prior to aquifer recharge to reduce total coliform concentrations below detection in 100 ml and, if the water is to be utilized for potable water, treated again after withdrawal. The need for this is to avoid introducing harmful microbial organisms such as bacteria, protozoa, and viruses possibly present in surface water, into relatively pristine ground water.

In the future, the ability to reduce pre-treatment of surface water to be stored in ASR aquifers may represent considerable cost savings for implementation of these systems. If natural conditions within the subsurface zone of discharge are shown to provide adequate attenuation of potentially harmful microbes (e.g., microorganisms are inactivated) and the systems are run with proper monitoring and safeguards, the need for pre-treatment of storage water may be reduced. However, there is a paucity of information on the fate of coliform bacteria and other indicator and pathogenic organisms under the subsurface temperature, salinity, pressure, and other native conditions present in the Upper Floridan Aquifer.

The overall purpose of the Fate of Microorganisms in Aquifers Study has been to better define the potential for survival of some public-health-related microorganisms under conditions as found in surface waters and ground waters of Florida that will potentially be used for ASR. The study objectives included:

- A literature review summarizing current knowledge on factors affecting survival and decline of important microbes in ground water

- Bench-scale (laboratory) survival studies of several groups of microorganisms to define the impacts of temperature and total dissolved solids under controlled conditions

- Bench-scale survival studies of these microorganisms in representative surface water and ground water sources with potential for, or in active utilization of, ASR systems, focusing on relative effects of temperature and background microbial community on survival

- Determination and comparison of predicted inactivation periods and inactivation rates for various microorganisms in Florida surface and ground waters at temperatures typical of the Floridan aquifer

The key groups of organisms evaluated in the study were fecal coliform bacteria, enterococci bacteria, male-specific (F+) RNA coliphage, DNA coliphage, the intestinal parasites *Giardia lamblia* and *Cryptosporidium parvum*, and the *Salmonella* bacteriophage PRD-1. The groups of bacteria and coliphage were composite populations of isolates obtained from surface water or secondary wastewater effluent (the RNA coliphage), thus representing organisms found in the natural surface water environment rather than pure laboratory strains. Coliphage and PRD-1 are viruses that infect only bacterial cells, but they are used as models for the behavior of viruses significant to human health such as enteroviruses. The DNA and F+ RNA coliphage differ in their genetic material, the basis of their reproductive ability. Pathogenic viruses may be either DNA or RNA viruses, depending on the strain. Generally, bacteriophage MS-2, which is an F+ RNA coliphage, has been considered to be indicative of transport of enteroviruses. However, the use of environmental isolates for survival studies is not well described, and no studies were found which directly compared the survival of environmental isolates of the two types of phage. Thus, both were used for this study to gain additional information on their relative survival potential and possible use as indicators. Fecal coliform and enterococci are generally non-pathogenic enteric bacteria used to indicate the potential fecal contamination of water and possible presence of pathogenic bacteria such as *Salmonella* or pathogenic *E. coli*. The two parasites are pathogens, capable of causing illness if ingested.

SUMMARY OF THE LITERATURE REVIEW

The literature review of survival studies on microorganisms in ground water was conducted to organize the base of knowledge on this subject. A total of 19 studies that evaluated survival of microorganisms in ground water, and some involving surface water, were reviewed and inactivation rates for the various organisms examined were obtained or extrapolated from reported data. For purposes of this report, a brief summary of results from this data analysis are presented. The completed literature review is also available.

Part of the purpose for the review was to analyze the body of published data to elucidate possible trends in inactivation rates in response to environmental variables. Some individual studies evaluated the impact of various parameters within more or less controlled conditions and the findings of these types of studies reveal possible trends. Temperature has always been known to act as one of the key variables affecting microbial survival. Several investigators observed that virus inactivation increased with increasing temperature, but similar trends for bacteria were observed less consistently (8, 9, 16, 18-20). Other studies also described an increase in inactivation rates in non-sterile vs. sterile water sources; however, the opposite was also observed in some cases (1, 7, 8, 16). In others still, no effect of sterilizing the environmental water source was observed. Regarding the effect of TDS, no reviewed studies demonstrated an impact of TDS ranging from near zero to around 1100 mg/L. However, only one study directly evaluated TDS effects (19). Unfortunately, a large proportion of reviewed studies did not include TDS as a reported parameter, which made analysis of TDS effects using data from many studies difficult.

Data on inactivation from the various studies were also extracted and combined to analyze trends and central tendencies, revealing some interesting observations. The inactivation rate is obtained by plotting of the decrease in numbers of microorganisms over time and examining the slope of line (k values which are inactivation rates, expressed as a decrease in $\log_{10}$ number/day [log/d]). These rates were taken from the publication or were developed from the original data in the paper. In addition many survival studies use the number of days for 90% reduction or 99% reduction, instead of using k values (similar to using the half lives of chemicals).

The median value for inactivation rates across all temperatures for coliphage (0.079 log/d, n=72), poliovirus (0.081 log/d, n=41), echovirus (0.079, n=15), and coliform bacteria (0.071 log/d, n=22) were almost identical. In addition, the Q1 values (lower boundary of the middle 50% of data points) of data sets for coliform bacteria, enterococci, *Salmonella* spp., coliphage, poliovirus, and echovirus were all similar, ranging from 0.032 to 0.057 $\log_{10}$ /day inactivation (1, 3, 9, 16, 19, 20). These rates in the literature correspond to days for 90% reduction in a range between 17.5 to 31.3 days. Inactivation data obtained from multiple studies were summarized in a more useful format by grouping the compiled inactivation rates for several organisms into temperature ranges up to greater than 20° C. Inactivation rates were not sub-grouped above 20º C, even though this may have been even more descriptive of ground water temperatures in Florida, due to lack of data from reviewed studies at temperatures above 25º C. Only environmental water sources were considered, but data from both sterilized and non-sterilized natural water experiments were grouped together. For polioviruses, hepatitis A, echoviruses, and coliphage, mean inactivation rates were most rapid at temperatures above 20º C (9, 16, 19, 20). Also, inactivation rates from hepatitis A virus were somewhat longer than the others (16). Thus, perhaps the adequacy of viral indicators like coliphage for predicting hepatitis A presence in ground water needs further study. However, another study indicated that PRD-1 may be a better model for hepatitis A survival (3). For coliform, enterococci, *Salmonella*, and *Shigella* bacterial groups, the slowest inactivation rates were at 15 - 20º C and increased somewhat for temperatures over 20° C. No studies on *Cryptosporidium* or *Giardia* survival in ground water were located, but studies which quantify the decline of these organisms in fresh surface water have indicated that temperature negatively affects survival, and perhaps more so in raw water than in sterilized surface water samples (8).

Other specific questions about the potential effects of nutrient variation and incidence of re-growth of fecal organisms in ground water studies have been raised. No studies were reviewed which specifically identified the incidence of re-growth of fecal bacteria in ground water, although this was not specifically evaluated per say nor were studies sought which did evaluate this phenomenon. Re-growth of fecal coliform has been suspected in tropical surface water environments, particularly in stream sediments (17), and certainly these organisms can grow outside the intestinal tract given the proper nutrient and environmental conditions. However, the incidence and factors that control re-growth have not been extensively reported

and papers on this topic were not reviewed since the review focused on ground water. Nutrients themselves, specifically bioavailable nitrogen, phosphorous, and carbon compounds, can have significant impacts on the ecology of any water system and thus would be expected to impact survival of enteric organisms. However, in ground water, concentrations of these compounds are typically much less and in a much more narrow range than in surface water. Possible correlations of background nitrate concentrations in ground water to survival of several seeded virus types did not reveal correlation to inactivation rates (19, 20). Studies that evaluated correlations of nutrient concentrations to survival of enteric microorganisms were not reviewed. More information on such impacts may possibly be found in literature more specific to surface waters.

STUDY METHODS

Organism Populations

Seven types of microbes were employed in survival studies in both the TDS-temperature experiments and the field samples of aquifer and reservoir water. They were composite populations of fecal coliform and enterococci bacteria, composite populations of DNA coliphage (combined somatic and male-specific) and RNA coliphage (male-specific only), the *Salmonella* bacteriophage PRD-1 (pure strain), and the intestinal parasites *Cryptosporidium parvum* and *Giardia lamblia*.

Bacterial isolates were obtained from water samples of Bullfrog Creek, Hillsborough County and from storm water collected from Lake Jackson, Hillsborough County. Samples were initially assayed on mFC (Standard Methods for Evaluation of Water and Wastewater) or mEI agar plates (US EPA Method 1600) using membrane filtration to select for fecal coliform or enterococci colonies, respectively. Typical fecal coliform (blue on mFC) or enterococci (gray with blue halo on mEI) colonies were picked and streaked to isolate on non-selective tryptic soy agar (TSA) plates, which were incubated at 37° C for 24 hours. Isolates were then identified using the API identification system and 10 fecal coliform and 9 enterococci isolates were selected to comprise the populations for each bacterial type. The species as identified by API were, for fecal coliform, 8 *Escherichia coli*, and 2 *Klebsiella pneumoniae*, and for enterococci, 7 *Enterococcus faecalis*, 1 *Enterococcus faecium*, and 1 *Enterococcus durans*. Isolates were then propagated in tryptic soy broth (TSB) and frozen as a 50% mixture with dimethylsulfoxide for storage at –70 C.

Male-specific (F+) RNA coliphage isolates (infect host cells via pilli structures) were obtained from secondary wastewater effluent at the Albert Whitted wastewater treatment facility in St. Petersburg. The effluent sample was taken by grab sampling directly from the secondary clarifier basin and aliquots of this sample were immediately plated by the double agar overlay method (described in EPA method 1602) on *E. coli* Famp strain (designation HS[pFamp]R) host cells to select for male-specific phage. Plaques were picked and isolated by additional plating and plaque selection using additional agar overlay procedures with Famp, then isolates were typed using the method described by Hsu involving use of ribonuclease A (RNAse) to determine

which were RNA phage (5). Ten isolates were chosen from these for the male-specific phage population, which were then propagated in TSB, filtered to remove host cells, and stored as multiple aliquots of each isolate at -70° C.

The DNA coliphage population was created from phage isolated from a water sample collected from Bullfrog Cr., Hillsborough County. Water sample aliquots were plated using the double agar overlay technique with *E. coli* strain C-3000 which selects for both male-specific and somatic (infect via cell-wall receptors) coliphage. Resulting plaques were then purified in the same way as for the RNA coliphage. An individual plaque from each isolate was again propagated in *E. coli* C-3000, filtered, and 10 isolates were stored as multiple aliquots at -70° C. These phage isolates were also typed by the RNAse method, and determined to be all DNA phage. Of the 10 isolates, 2 were male-specific and 8 were somatic phage, based on inability to grow on e. *E. coli* Famp cells.

A pure stock of PRD-1 was obtained from the laboratory of Mark D. Sobsey (University of North Carolina, Chapel Hill, NC). This phage isolate was diluted and plated in an agar overlay with *Salmonella typhimurium* (strain designation LT2) to create plaques, then a single plaque was chosen to propagate in broth culture to further ensure purity. After filtration to remove host cells, aliquots of the suspension were stored frozen at -70° C.

Cryptosporidium parvum oocysts, obtained from the Sterling Parasitology Laboratory at the University of Arizona, were collected from a calf infected with the Iowa isolate of *Cryptosporidium parvum* (National Animal Disease Center, Ames, Iowa). The oocysts were purified using discontinuous sucrose and cesium chloride centrifugation gradients and stored at 5° C in an antibiotic solution containing 0.01% Tween 20, 100ug of penicillin, and 100ug of gentamicin per mL (from University of Arizona, Department of Veterinary Science and Microbiology, Tucson, AZ). The oocysts were used in the experiment within 60 days of being shed. Fresh *Giardia lamblia* cysts (10,000,000 cysts/ mL) were obtained from Waterborne (Waterborne, Inc.) one day prior to initiating the experiments.

Organism preparation for survival experiments

Prior to each experiment, each bacterial isolate was propagated separately from frozen cultures to 5 ml TSB, incubated overnight at 37° C. The following day, 0.1 ml of each isolate

culture was seeded into 50 ml of TSB, with fecal coliform and enterococci being grown as separate populations. These cultures were again incubated with shaking overnight at 37° C. Cells were washed in phosphate buffered saline solution (PBS) three times by successive centrifugation and re-suspension in PBS. Centrifugation parameters were 7 minutes at 2000 x g. The resulting viable cell concentrations (titer) after these procedures were consistent at 8×10^8 cfu/ml for fecal coliform and 5×10^8 cfu/ml for enterococci. These rinsed cells were used for seeding into experimental vessels at the initiation of each trial.

Coliphage isolates were grown individually from freezer stocks prior to each trial in cultures of the respective bacterial strains (*E. coli* Famp for F+ RNA phage and *E.* coli C-3000 for DNA phage), then purified by centrifugation and filtration through 0.22 μm membrane filters to remove host cells and debris. The titer of each isolate was determined independently, and for each trial since propagation results were not sufficiently consistent. Isolates for the respective populations of DNA or RNA coliphage were then combined in equal proportions into sterile reagent-grade water for seeding into survival experiment vessels. Likewise, PRD-1 was grown from freezer stock and titered for each trial. In all phage experiments, the DNA phage population and PRD-1 were combined together in each experimental bottle, and F+ RNA phage were tested in separate experiments. Also, fecal coliform and enterococci populations were evaluated together in the same experimental vessels, since selective media was used to determine survival results. However, phage and bacteria were always evaluated separately. Thus, the experimental groups for indicator organisms could be summarized as bacteria, DNA coliphage and PRD-1, and F+ RNA coliphage, with the members of each group being combined in respective experimental bottles. Parasite experiments were performed with the stock suspensions of cysts/oocysts as received from suppliers without further processing.

TDS-temperature trials

Solutions for evaluation of the effects of TDS and temperature on microbe survival were created using Instant Ocean artificial sea salt (Aquarium Systems, Mentor, Ohio). The artificial seawater was utilized to provide a solution containing a mixture of ions as opposed to a solution composed only of sodium chloride. Table 1 contains the composition of Instant Ocean solutions

at 34 ppt (seawater salinity) as given by the manufacturer and the concentrations of these ions if the solution is at a concentration of 1000 mg/L (ppm).

Table 1. Composition of mixed ion solution for TDS-temperature trials (Instant Ocean sea salt)

Major components	Concentration at 34 ppt (mg/l)	Concentration at 1000 ppm (mg/l)
Chloride	19251	566.206
Sodium	10757	316.382
Sulfate	2659	78.206
Magnesium	1317	38.735
Potassium	402	11.824
Calcium	398	11.706
Carbonate/bicarb	192	5.647
Strontium	8.6	0.253
Boron	5.6	0.165
Bromide	65	1.912
Fluoride	1	0.029
Iodide	0.22	0.006
Lithium	0.18	0.005

Trace components at 34 ppt			
Copper	Trace (< 0.03)	Arsenic	Trace (< 0.0002)
Iron	Trace (< 0.03)	Cadmium	Trace (< 0.02)
Nickel	Trace (< 0.04)	Chromium	Trace (< 0.0006)
Zinc	Trace (< 0.02)	Aluminum	Trace (< 0.04)
Manganese	Trace (< 0.01)	Tin	Trace
Molybdenum	Trace (< 0.01)	Antimony	Trace
Cobalt	Trace (< 0.05)	Rubidium	Trace
Vanadium	Trace (< 0.04)	Barium	Trace (< 0.05)
Selenium	Trace	Mercury	None
Lead	Trace (< 0.005)	Nitrate	None
		Phosphate	None

For the bacteria and viruses (phage), experiments were conducted using re-usable polypropylene bottles as reaction vessels, either 250 ml or 100 ml in size. Prior to each trial run, each bottle was acid-washed with 10% hydrochloric acid (HCl), rinsed with water and thoroughly washed with detergent, then triple-rinsed with tap water followed by de-ionized water. Bottles were air-dried then sterilized by autoclaving. For parasite analysis, experimental microcosms were prepared in sterile polypropylene centrifuge tubes, with 50-ml tubes for *Cryptosporidium* and 2-ml polypropylene microcentrifuge tubes for *Giardia*. Tubes were new

prior to use. Test solutions of the varying TDS levels were made by dissolving carefully weighed amounts of Instant Ocean in reagent-grade de-ionized water in glass bottles. Experiments were performed using TDS concentrations of 200, 500, 1000, and 3000 mg/L for the indicator bacteria and viruses, and at the three lower concentrations for parasites. The reason for a more-focused study on the lower TDS concentrations was due to the opinions of the advisory committee that lower concentrations under 1000 mg/L are more indicative of what would be observed in injected surface water. Due to the greater cost of performing parasite studies, the scope was reduced to reflect only concentrations up to 1000 mg/L. The pH of artificial water solutions was neutralized to between pH 6.5 – 7.5 using HCl or sodium hydroxide (NaOH), and the actual conductivity/TDS was recorded. Solutions were then sterilized by autoclaving in the glass bottles. The bottles used had pressure-resistant closures, such that the caps were fastened tightly to prevent any concentration of TDS by evaporation. When pH was re-checked after autoclaving for initial representative solutions, it did not change due to autoclaving so in general pH was measured and adjusted prior to autoclaving only. Phosphate-buffered saline (PBS) was made according to EPA Method 1623 and sterilized by autoclaving, also in glass bottles. Immediately prior to each experimental trial, test solutions were distributed to the sterilized bottles and solutions were placed at the respective temperature levels. For the 5° C temperature, bottles were placed in a foil-covered basket to exclude light and placed in a 5° C walk-in refrigerator or sliding-glass door refrigerator. Bottles at 22° C were placed in a water bath at room temperature and covered to exclude light. The 30° C temperature was maintained in a water bath set at 30° C. The building climate control for indicator experiments (at USF in St. Petersburg) was very stable such that the room temperature water bath remained constant at 22° C without adjustment. However, for parasite experiments (at MSU in East Lansing, MI), ice packs were placed in the water bath during times over which the building climate control resulted in higher temperatures than 22° C. In these instances, the temperature of the water remained between 22º - 25º C, although these data were not recorded.

For the indicator bacteria/viruses, each survival experiment was initiated by seeding respective bottles of test solutions with organisms, using a quantity to achieve an approximate concentration of 2-3 x 10^5 cfu or pfu/ml. Enterococci and fecal coliform were seeded to the same bottles, and were not used in the same bottle as bacteriophage. This combination was used since the two types of bacteria are not able to grow on the other's respective selective media used

for analysis. The time 0 sample was taken from each bottle immediately after seeding. Subsequent samples were taken on or about days 1, 2, 4, 7, 10, 14, 21, and 28. For *Giardia lamblia* experiments, aliquots of 20 µl from the stock cyst preparation (10,000 cysts/ml) were seeded into 30 µl of each water type and the microcentrifuge tubes were placed at the different temperature settings. A separate tube was used for each sample point, at days 0, 1, 3, 6, 10, 14, and 20. To initiate each *Cryptosporidium parvum* experiment, 500ul of oocyst were seeded into 20mL of each TDS mixture (200 mg/L, 500 mg/L, 1000 mg/L) in 50 mL polypropylene centrifuge tubes to achieve an oocyst concentration of approximately 10^6 oocysts/mL. For these TDS-temperature experiments, duplicate oocyst preps were created for each TDS concentration at each temperature. Also, a water sample at each TDS was placed in each incubator without oocysts to serve as a negative control and as a reservoir for testing pH and TDS changes throughout the experiment. For infectivity analysis over each experiment, 1-ml samples were removed from the artificial water tubes on days 0, 1, 3, 6, 10, 15 and 20 and processed as described below.

Survival of seeded bacteria and viruses was determined by evaluation of the concentration of culturable organisms at each time point. Standard membrane filtration methods were employed for culturable counts for bacteria, using 0.45 µm pore-size membrane filters to capture bacteria. Fecal coliform were assayed using mFC agar, and enterococci were assayed using mE agar. Dilutions of water samples were done using sterile PBS. Plates for fecal coliform were incubated at 44.5º for 24 hours, enterococci at 41.5º C for 48 hours. Bacteriophage counts were done using double agar overlay methods adapted from EPA method 1602 (Single Agar Layer Method, also describes double agar layer method). Phage were assayed for routine sample measurements using the same bacterial hosts as used for isolation of the population components. DNA coliphage were assayed using the *E. coli* C-3000 host (ATCC #15597), F+ RNA coliphage using the *E. coli* Famp host (ATCC #700891), and PRD-1 using *Salmonella typhimurium* LT2 (ATCC #19585). Growth media for host was TSB, overlay agar was TSB with 1% agar, and bottom agar was TSA. Plates were incubated 24 hrs at 37º C. For all bacteria and virus analyses, duplicate plate counts were performed for each dilution, and the average of the two was taken as the viable number. At each time point, typically 1-2 dilutions were used, depending on the observed rates of decline and previous sample point's counts. Positive controls for bacteria were *E. coli* for the fecal coliform, *Enterococcus faecalis* for

enterococci, and MS-2 for the DNA and F+ RNA coliphage. PRD-1 stocks were used for positive controls on the PRD-1 assay. Negative controls were used for bacteriophage hosts, to ensure host cells were not contaminated by phage. Negative controls for host involved plating 1 ml of host culture. For the bacteria, rinse buffer and dilution buffer were checked to ensure that they were not contaminated with the target types of bacteria.

Giardia cyst viability was determined using the inclusion or exclusion of the fluorogenic dyes 4'-6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI), which evaluates potential membrane integrity as an indirect indication of cyst viability. This assay has been previously used as a marker of intact membranes in waterborne *Cryptosporidium* and *Giardia* (4, 12). PI is capable of passing through only damaged cell membranes, and intercalates with the nucleic acids of injured and dead cells to form a bright red fluorescence complex (11). DAPI is a DNA stain that can pass through both intact and damaged cyst membranes, thus staining all cysts with DNA present (4). Therefore, viability of stained cysts in a suspension is inferred by examining the number of total cysts stained by DAPI, and the number of presumably non-viable cysts which are stained by PI. The interpretation of viability from fluorogenic dye inclusion or exclusion, however, must be undertaken cautiously since the dye tests are known to overestimate viability and the staining can be variable with a portion of the oocysts not staining with either dye (4, 6, 10). For viability analysis, the entire aliquot was stained with 15 µl of each fluorochrome along with a monoclonal fluorescent antibody stain to confirm the identity of *Giardia* cysts (FITC-mAb [Giardi-a-Glo, Waterborne, Inc], DAPI, and PI) and incubated at room temperature for 15 minutes. Following the 15 minutes incubation period, samples (entire aliquot) were transferred to glass slides and the cysts enumerated using epifluorescence microscopy. A Zeiss Axioscope 2 Plus microscope equipped with a UV filter block (350-nm excitation) for DAPI, blue filter block (450-490 nm excitation) for FITC and green filter block (546-nm excitation) for PI was used. Slides were scanned with the 20X and 40X Zeiss Plan Neofluar UV objective lens.

For *Cryptosporidium* infectivity analysis, a previously described cell culture system was used in this study, with some changes (13). In brief, human ileocecal adenocarcinoma cells (HCT-8 cells, CCL-224 from ATCC, Manassas VA) were obtained from and maintained in 75 cm^2 tissue culture flasks with passage every 2-3 days, ending with the 15^{th} passage. HCT-8 maintenance medium was used in maintenance of the cell line and in preparation of the

chambered well slides. Maintenance medium consisted of RPMI 1640 supplemented with 5% fetal bovine serum, 2% 1 M hepes buffer, 10% opti-mem reduced serum, 1% sodium pyruvate, and 1% antibiotic mixture. To prepare well slides for infection, HCT-8 cells were seeded into 8-chambered well slides and incubated at 37° C with 5%CO_2 for 48 hours until a cell monolayer confluency of approximately 80% was achieved. The cells were then infected with oocyst suspensions of differing concentrations as described below.

The 1mL samples at each sampling period were treated for 8 minutes at room temperature with 10.5% vol/vol sodium hypochlorite (Sigma) in PBS, then centrifuged for 4 minutes at 12,000rpm and the supernatant was discarded. 1.0 mL of PBS was added, samples were vortexed and centrifuged again and were finally resuspended in 1mL growth medium (maintenance medium with 10% fetal bovine serum). Replicate hemacytometer counts were used to determine concentration of oocysts and 10-fold serial dilutions were prepared in sterile polypropylene 1.5mL microcentrifuge tubes containing growth medium. 150ul of the oocyst suspensions were then aliquoted into each of 6 wells within the chambered slide. The two remaining wells on each slide were inoculated with growth medium to serve as negative controls and to assure that leakage between wells did not occur during incubation. After inoculation, the well slides were incubated at 37° C with 5% CO_2 for 90 minutes, then approximately 800ul of growth of medium was added. The slides were incubated for 48 hours.

After 48 hours the slides were fixed with 100% methanol for 10 minutes and an indirect antibody staining procedure was used to label the reproductive stages of the infectious oocysts on the monolayer. The slides were labeled using an FDM procedure described previously (15). Slides were flooded with a 0.002% primary polyclonal rat anti-sporozoite antiserum (Waterborne Inc., New Orleans, LA) in PBS, washed with PBS, and then flooded with a secondary goat anti-rat antibody conjugated to fluorescein isothiocyanate (Sigma). The cells were viewed using epifluorescent microscopy at 200X using an Axioscope 2 plus microscope (Carl Zeiss Microimaging Inc., Thornwood, NY). Each well within the slide was screened and then scored as either positive or negative for oocyst infection. The infected cells were distinguished by the apple-green fluorescence of a focus of infection against a dark background of uninfected cells under the IFA filter. Foci of infection were distinguished by the presence of a cluster of sporozoites that had invaded the cells and at least 3 life stages had to be present within a cluster for the well to be scored as positive. The number of positive wells per dilution was entered into

the EPA most probable number (MPN) program for viruses (www.epa.gov/nerlcwww/mpn/exe). This program allowed for the inclusion of slides derived from oocyst suspensions of varying concentrations by relying on user specified volumes, numbers of replicates and numbers of dilutions under the non-standard dilution setting. The software program delivered an MPN value, with confidence limits, representing the number of infectious oocysts per mL. The MPN value was then converted to percent infectivity via the following formula: percent infectivity = (MPN per mL/hemacytometer oocyst count per mL of the stock) X 100.

Representative Aquifer and Surface Reservoir Trials

Two sample sites were utilized to provide water sources for representative aquifer and surface reservoir waters that may be involved in ASR projects. The two sites were near the municipalities of Bradenton and West Palm Beach. The Bradenton site involved the Bill Evers reservoir that provides raw water to the City of Bradenton drinking water treatment facility. Aquifer water was drawn from the Avon Park formation of the Upper Floridan Aquifer using the ROMP TR4-7 well. West Palm Beach surface reservoir source water was the Clear Lake reservoir, which supplies raw water to the adjacent City of West Palm Beach drinking water treatment facility. Ground water samples were taken from the Lake Lytal Park wellsite using the PBF-3 well which taps the Upper Floridan Aquifer. Samples from all sites were collected in 1-L polypropylene bottles (pre-sterilized). Avon Park samples were collected after purging of 3 well volumes via a gasoline centrifugal pump, followed by pumping using an electric peristaltic pump to withdraw sample water. Lake Lytal Park well purging was performed by opening this artesian well 24 hours prior to sampling to allow development. Both reservoir site samples were taken directly from the surface. Temperature, conductivity, and pH of the water were measured on site when possible. Temperature data was not recorded on site for the first set of West Palm Beach samples. After the initial measurements of the background physical and chemical parameters, these were not checked again during the experiment.

Water samples were transported or shipped on ice to the laboratory for use in survival studies. For studies with bacteria and viruses, the water samples were divided and some of the water was subjected to heat treatment to reduce background microbial populations prior to seeding with test organisms. The heat pasteurization procedure involved raising the temperature

of water samples while still in collection bottles to 70° C in a hot water bath, and holding the temperature at 70° C for 30 minutes. This step was performed to gain some comparative assessment of the impact of intact native microbial populations on survival of seeded non-native water quality indicator microorganisms. Pasteurized samples were used for bacterial and viral experiments only; parasite experiments were performed in only raw water.

Some background microbial parameters were also determined from raw and pasteurized water samples. These parameters were fecal coliform bacteria, enterococci bacteria, combined somatic and male-specific coliphage (DNA and/or RNA), heterotrophic plate count (HPC) bacteria, and in samples for PRD-1 survival study, any background PRD-1. The membrane filtration method described above was employed for fecal coliform and enterococci bacteria, using mFC media for fecal coliform (2), and mEI media for enterococci (as described in EPA method 1600). Coliphage were determined by assaying 10 ml in 2-ml aliquots by the double agar overlay procedure, using *E. coli* C-3000 host. HPC bacteria numbers were determined by the spread-plate method for raw water and membrane filtration for pasteurized samples, using R2A agar incubated at 22° C (room temperature) for 1-4 days. PRD-1, when measured, was performed using 10 ml of water with host and procedures described previously.

For initiating survival trials, water samples were distributed to test bottles/tubes which were prepared as described for TDS-temperature trials. Bacteria and virus survival trials were performed in the aforementioned polypropylene bottles, prepared as described for TDS-temperature experiments. Parasite trials were performed in similar 50-ml or 2-ml centrifuge tubes. Water samples were set to the experimental temperatures of 5°, 22° and 30° C for temperature equilibration for a short time, then test organisms were seeded. Bacteria and viruses were seeded to achieve a concentration of approximately 2-3 x 10^4 organisms per ml. Time 0 samples were taken immediately. Subsequent time-point samples were taken on similar days as for TDS-temperature trials, out to approximately 28 days for bacteria and viruses, 20 days for *Giardia* lamblia, and on days 0, 1, 3, 6, 10, 20 and 25 for *Cryptosporidium parvum*. Sample analysis for culturable/viable organisms at each time point was performed the same as described for TDS-temperature trials.

Data Analysis

Two different methods for analyzing data from survival experiments were employed with two separate intended purposes. Firstly, a comparison of the relative impacts on survival of temperature and TDS in the first set of experiments (Artificial water experiments), or temperature, water type, and background microbial community in the natural water experiments, was made using predicted 99% inactivation periods or first-order rate constants (for PRD-1 and the parasites) as a proxy for survival, and as a statistic for analysis of variance tests. Pasteurization treatment to evaluate the effect of background microorganism effects was done only for the indicator organisms.

Second and most importantly, a specific determination of survival in conditions typical of the Florida subsurface was desired. To describe this, the predicted number of days for 2-log (99%) inactivation in only the raw (unpasteurized) water from the 4 sites, at temperatures of 22º and 30º C, were examined separately from the pasteurized or colder temperature conditions. The determination of first-order rate constants was also made for all organism groups. This was enabled because in the raw water trials at these temperatures, inactivation kinetics were generally first-order and could be fit by such a regression curve, unlike the artificial water trials, pasteurized samples, and some 5º C conditions, where kinetics frequently necessitated the use of alternative regression models.

Comparative Analyses of Factors Affecting Survival

To make comparisons of the relative effects of factors such as TDS and temperature, or in environmental water trials water type and pasteurization treatment effects, a single comparative statistic was necessary for analysis of variance (ANOVA) tests. In the scientific literature there are typically two ways of analyzing data on survival. The first is the plotting of the decrease in numbers of microorganisms over time and examining the slope of a regression curve, often a first-order regression model (k values which are inactivation rates, expressed as a decrease in $\log_{10}$/day: See equation below). The second method is to examine a level of decrease in numbers in percentages, for example in the EPA *Surface Water Treatment Rule* and the development of the new *Long-Term Enhanced Surface Water Treatment Rule*, one goal is 99.9% decrease in surface water concentrations of *Giardia* for finished drinking water. In survival

studies one can use the number of days for 90% reduction or 99% reduction, (similar to using the half lives of chemicals) instead of using k values.

In these experiments, kinetics of decline in microcosms often deviated from first-order, particularly in the TDS-temperature trials, which employed sterile, laboratory grade water; in pasteurized natural water trials; and at the 5º C temperature. In addition, differences in inactivation kinetics between conditions, and the limitation of being able to measure only about 4-5 $\log_{10}$ total decline precluded the use of total inactivation over a period of time as a comparative statistic. Thus, for comparisons of the relative impact of factors that were examined for the bacteria and two types of coliphage (along with *Cryptosporidium* in TDS-temperature experiments), observed inactivation kinetics were fit with one of two regression equations and the resulting number of days for 99% decline predicted from the best-fit equation for each independent trial/data set was used as a comparative statistic. In effect, this statistic is only a proxy for the observed inactivation kinetics, but a predicted inactivation period was employed due to the fact that kinetics varied and a single rate constant could not be employed as a statistic for ANOVA tests. However, for PRD-1, *Cryptosporidium parvum* in environmental water, and *Giardia lamblia*, first-order inactivation rate constants were employed as this was a superior comparative statistic for these organisms, These periods or rates were used to compare variability between conditions with ANOVA tests, which revealed factors that were determined to be statistically significant in affecting relative survival capacity.

The scope of work for this study had originally assumed a 180 days maximum duration for the survival studies. Subsequently, this was not determined to be feasible. First, in the microcosm studies we determined that the water held on the bench for more than 40 to 60 days changed in the numbers of heterotrophic bacteria growing on the sides of the beakers and test tubes. This was particularly a problem for natural waters. Thus the biological nature of the water being tested is not stable under this time frame. Second, the long holding times in a static system in the laboratory were not reflective of the true environment. Finally, the long holding times were not needed to define the inactivation rates. The 180 day duration was also discussed as a time point that could be used for storage prior to withdrawal of the water, and it was of interest to estimate whether once the water was withdrawn after this time frame, one might expect to find the indicator organisms.

A specific description of the process for determining the statistic for comparative analyses of factors affecting survival follows. The series of data points comprised of $\log_{10} N/N_0$ ratios for each time point measured for each individual data set (data sets being each experimental trial for a given set of conditions and each microorganism) were analyzed independently. For the bacteria and coliphage (and *Cryptosporidium* in artificial water experiments), the purpose of fitting data to model equations was to enable prediction of a number of days before 2 $\log_{10}$ (99%) declines in culturable/infective/viable concentrations would be observed. The number of days for 2-log decline was further used as a statistic for comparative analysis of various factors on observed inactivation. To fit data to predictive models, actual data was matched with an inactivation equation. The general equation used is shown here as Equation 1.

$$\text{Log}_{10} N/N_0 = -k * \text{time}^m \quad (1)$$

This model incorporates an inactivation rate constant which describes the overall slope of the curve (k) and an exponent m which enables a better matching of inactivation kinetics. If $m = 1$, the curve is a straight line, indicating first-order kinetics, if $m < 1$, tailing is indicated, and if $m > 1$, a shouldering effect is indicated. The use of such a parameter was necessary since many of the data sets deviated from first-order kinetics, and a better fit to observed data was desired. All time values were in days. Observed data was fit to model equations by the use of least squares analysis to adjust the values of k and m to minimize the error sum of squares between observed log N/N_0 and predicted based on model equations. Microsoft Excel was used to perform the iterative problem solving. In many cases, inactivation over the duration of a trial was too slight to allow the use of the additional exponent. The variability between time points overshadowed the general trend of decline and the model in Equation 1 was not able to fit observed data. Thus, in general, in data sets for which total decline was less than 1 log (90%), the exponent m was set to 1 and the model became first-order.

In several cases, kinetics of change over the course of the experiment were such that an initial increase in viable counts or infectivity (of *Cryptosporidium*) was observed, usually followed by a decrease after some time. A model such as Equation 1 would not fit such a situation, and in these cases an alternative model was used in order to obtain a prediction of days before 2-log decline for further comparison. Equation 2 is a polynomial curve which when fit to data in these cases, has the shape of a shallow inverted parabola.

$$\text{Log } N/N_0 = -k_1 * \text{time}^2 + k_2 * \text{time} \quad (2)$$

However, this type of curve often extrapolated from observed data an assumed kinetic behavior which could not be observed (since actual time points only extended to approximately 28 days). The use of this curve was only to enable a prediction for comparative analysis in situations where it was necessary, and was only necessary in conditions which were for comparison of artificial to natural environmental factors, primarily TDS-temperature experiments for *Cryptosporidium* and pasteurized natural water samples for fecal coliform bacteria and in one case coliphage. Kinetics were modeled exclusively with Equation 1 in situations of raw aquifer and reservoir water sources.

The predictive model for each data set was then used to estimate a number of days for 2-log decline with Microsoft Excel. Predicted days for 2-log decline from each independent condition set and trial were used as a statistic for analyses of variance (ANOVA) with Minitab rel.12. However, for PRD-1, *Cryptosporidium* in natural water experiments, and *Giardia*, inactivation over the 20- to 28-day experiments were slow to the point that very large numbers of days were predicted. Since these periods were well outside the experimental durations, and kinetics could be more accurately modeled with first-order models in these particular cases, statistics used for analysis were the first-order inactivation rate constants (k in Eq. 1, with m at 1). ANOVA were used for each organism group to determine statistically significant factors accounting for variability in observed data. For TDS-temperature trials ANOVA, factors compared for each organism were the TDS concentration and temperature. Additional analyses were performed which examined only TDS concentrations equal to and less than 1000 mg/L. In natural water source trials, numerous comparisons were performed, as detailed in the RESULTS section. The general factors which were matched for comparative analysis for each organism were temperature, water type (surface water or ground water), and treatment (raw or pasteurized).

Description of Inactivation Rates in Environmental Water Samples

Data points from duplicate trials of the bacteria and viruses in raw water from reservoir and aquifer samples were combined and an average regression equation was formulated. Combined data for this analysis were used since the trials in raw water produced generally good agreement between the duplicate trials. For parasite trials, only one trial was performed in the

environmental water sources so these data were used. Since the kinetics of decline in these experiments were generally first-order, a linear regression model was used on the log-transformed data to determine inactivation rate constants. Specific attention should be paid to those data that correspond to temperatures of 22º and 30º C, as these are the temperature ranges found in the Floridan Aquifer.

RESULTS

Effect of Total Dissolved Solids and Temperature

Part One of the research involved the evaluation of impacts on survival due to variation in total dissolved solids (TDS) and temperature in sterilized reagent-grade water. Thus, the objective was to create a relatively controlled environment for the seeded organisms in order to isolate the effects of these parameters. The artificial seawater salts were mixed to provide concentrations of 200, 500, 1000, and 3000 mg/L while providing a mixture of ions equal to proportions found in seawater. For the indicator bacteria and viruses, each concentration was evaluated at temperatures of 5°, 22° (room temperature) and 30° C. These temperatures correspond to 41°, 72°, and 86° F. For the intestinal parasites *Giardia lamblia* and *Cryptosporidium parvum*, survival was evaluated in TDS concentrations of 200, 500, and 1000 mg/L at the three temperatures. Each of the following sub-chapters presents results obtained from the various organism populations.

Fecal coliform bacteria

Due to the large number of figures, plots of these data are shown in the appendices for visual reference of the actual changes in concentrations observed over time. Although there were two species of bacteria composing the fecal coliform population (*E. coli* and *K. pneumoniae*), the results shown in these figures are the counts from both bacteria types combined, as a single population.

Data points were generally fit to the basic equation described in the STUDYMETHODS section as Equation 1. Both k and m were varied to fit observed data, unless the total decline over the experiment was 1 log or less, in which case generally the curve was linearized with m=1. Occasionally, kinetics of culturable counts variation over time in the experimental microcosms necessitated the use of a different model in order to obtain a value for predicted days to 2-log decline for comparison. An alternate model was used if there was an initial increase (either apparent or actual) in culturable concentration of bacteria, Equation 2. If there was an observed decrease after an initial increase in concentration, the curve fit this trend. However, if no decrease in concentration was observed up to the terminus of the trial run, this curve predicts an

assumed decrease at some future point following kinetics that mirror the initial increase and flattening of the observed data trend. Examples of this type of curve can be seen in the data for fecal coliform bacteria in 3000 mg/L TDS at 22° and 30° C in Appendix 1-A. This type of model was used only in a very few cases with the TDS-temperature trials (2) in order to estimate a value of days to 2-log decline as a proxy for kinetic behavior to enable comparison to other conditions. Tables in Appendix 5-A show the predicted days for 2-log inactivation along with means and standard deviations for each set of conditions. These model predictions were determined independently for each trial.

In some cases, inactivation was so slight during the period of the experiment that the model for that data set predicted a very large number of days before a decline of 2 log. Since such long periods were well outside experimental durations, and it is realistic to assume a change in kinetics such that inactivation rates would increase after an extended period, any predicted period greater than 200 days is shown as " > 200" and 200 was used for the value in statistical analyses of trends. This method is a compromise to capture the difference in order of magnitude between kinetics observed under various conditions while avoiding overly unrealistic values, some in excess of a year's time based on 4 weeks of observed data. Likewise, the methods used could not accurately determine periods of time less than 1 day, so if a best-fit model equation predicted less than 1 day for 2-log inactivation, the value was rounded up to 1.

Using the predicted number of days until a 2-log decline as a statistic, the various artificial water conditions were compared via analyses of variance to evaluate any statistically significant effects on observed inactivation. ANOVA results for fecal coliform bacteria at all 4 TDS concentrations are shown in Appendix 6-A. Since only two trials were performed at 3000 mg/L (vs. three trials for other concentrations), a general linear model function was used to analyze the unbalanced data from all TDS concentrations. These results indicate a statistically significant effect of temperature on the predicted number of days until 2 log decline ($p < 0.01$). The effect described was that of more rapid inactivation with higher temperature. However, statistically significant differences between TDS concentrations (for all temperature levels combined) were not observed, nor was there a statistically significant interaction of TDS concentration and temperature. Also, three outlying observations for fecal coliform inactivation were observed, including the two that were > 200. All outlying observations were at 5° C, one at 200 mg/L TDS, one at 1000 mg/L, and one at 3000 mg/L.

To further confirm the relationship of TDS and temperature on fecal coliform inactivation, a regression model was used. The results of this are shown in Appendix 6-A. Once again, the predictable effect of temperature was statistically significant when taken across all TDS levels, but TDS was not a statistically significant predictor of fecal coliform inactivation time. The regression equation was determined to be:

```
FC days = 106 - 3.35 Temp  + 0.00688 TDS
```

Regressions of this type allowed assignment of significance levels to the respective predictors, such that the constant and temperature variable were statistically significant in the regression at the 99% level, and the overall regression equation allows a statistically significant prediction of variability in inactivation times ($p < 0.01$). However, TDS was not a statistically significant predictor. Also, the r^2 value of 40.6% shows that this predictive equation for decline over time does not explain a major part of observed variability. This may be due to large differences in inactivation between trials at the same conditions in some cases. This regression analysis also highlighted 4 unusual observations as seen at the bottom of the table in Appendix 6-A.

Since prior discussions have highlighted the importance of lower TDS values, below 1000 mg/L as would be found in injected surface water, fecal coliform results were also analyzed while considering only TDS concentrations of 200, 500, and 1000 mg/L. Since three trials were performed at these concentrations, the balanced ANOVA design that was employed provided a depiction of means and confidence intervals for these means (Appendix 6-A). Statistically significant differences in inactivation times are attributed to temperature ($p < 0.01$), but not due to TDS variation. The means across all temperatures do increase with increasing TDS, from 34 days at 200 mg/L to 64 days at 1000 mg/L. However, variability between replicates does not allow the demonstration of statistical significance for this trend.

The observed results from TDS-temperature trials for fecal coliform indicate that temperature resulted in a statistically significant increase in observed inactivation, resulting in lower numbers of days until 2-log declines were observed. However, variations in TDS concentrations did not produce statistically significant differences or trends, even when considering the interaction of temperature and TDS. Also, large differences were sometimes observed between replicate trials testing the same conditions, as can be seen by looking at a table of model prediction results of independent data sets in Appendix 5-A. These trials were different

both in terms of different batches of the fecal coliform population and in the batches of prepared experimental solutions.

Enterococci bacteria

Results for enterococci bacteria in TDS-temperature trials were determined and analyzed in the same manner as described for fecal coliform. Individual plots of measured culturable concentration, expressed as the log of ratios at time points to starting concentrations, are included in Appendix 1. Predicted days until 2-log declines were determined from model equations fit to data from each trial and are shown in Appendix 5-A. Unlike fecal coliform bacteria, only the general model equation (Equation 1) was used to fit enterococci inactivation data, since in no case was an initial increase in concentration observed.

Appendix 6-B shows results of statistical analyses on predicted days for 2-log declines. As with fecal coliform, having only two trials at 3000 mg/L rather than three as for the other concentrations necessitated the use of a general linear model to produce an ANOVA from an unbalanced design when considering TDS concentrations from 200 - 3000 mg/L. Temperature produced strongly statistically significant effects on inactivation of enterococci ($p < 0.01$), while the effect of TDS was moderately significant, to the 90% level but not the 95% level ($p < 0.1$). The significant effects were an increase of inactivation at higher temperatures and an overall decrease of inactivation at the highest TDS concentration of 3,000 mg/L. The interaction of TDS and temperature was not statistically significant. Along with the description of statistical significance due to the two parameters, two values were determined to be outliers, the value of 114 days in 200 mg/l and 5°, and the value of 110 days in 500 mg/l and 5°.

To further examine the relationship of temperature and TDS to enterococci inactivation, a regression was performed as for fecal coliform. The regression equation determined for the relationship of temperature and TDS to enterococci decline is:

```
Ent Days = 65.7 - 2.44 Temp + 0.00825 TDS
```

As indicated by regression p values, both temperature and TDS concentration are statistically significant components of the prediction model at the 95% level, and with an r^2 value of .587, the model fits data for predicted number of days for 2 log decline marginally better than the fecal coliform regression. Also, the same 2 observations were deemed unusual, being much larger

than the regression model predicted. Besides these two condition sets, there were large differences between trials at 22° and 500 mg/l and 30° and 3000 mg/l.

Enterococci data were also compared for the three lower TDS concentrations (200, 500 and 1000mg/L). This analysis showed that TDS was not a statistically significant factor in enterococci inactivation while temperature was significant (Appendix 6-B). In taking a closer look at the values used for comparative analysis here (i.e. Appendix 5-A), there was an inconsistent trend in terms of increasing TDS concentration. The most rapid inactivation, averaged by TDS concentration, was at 1000 mg/L, while the least rapid was at 3000 mg/L. Thus, the increase in mean days for 2 log inactivation at the three temperatures at 3000 mg/l was great enough to offset the observed decline in the mean at 1000 mg/l and produce a significant effect due to TDS. Also, results of replicate trials in some cases had quite large differences in behavior, particularly trials at 500 mg/l and 22°, and 3000 mg/l and 30°. In fact, the predicted days for 2-log decline at 30° C in 3000 mg/l (Appendix 5-A) show that in one trial, the value was very close to those observed at 30° for the lower TDS concentrations, while the other trial showed a much longer period. Therefore, longer inactivation periods at higher TDS may be an artifact at this temperature at least.

RNA and DNA coliphage

For results of TDS-temperature studies with the two types of coliphage, graphs with observed data and model curves fit to these data are in Appendix 1-C and 1-D. Two trials were performed for all conditions with these organisms. For DNA coliphage, each separate trial was conducted using separate batches of phage and ionic solutions. However, for the RNA coliphage, trials for 200 and 1000 mg/L were performed simultaneously as were both trials for 500 and 3000 mg/L, but the two sets were separate from each other.

Predicted periods for 2-log decline are presented in Appendix 5. In examining the mean values for each condition between the two types of phage, inactivation for RNA phage was generally more rapid than for DNA phage. This was apparent qualitatively at all condition sets except one. Also, the variability between trials was less for RNA phage than for DNA phage, indicating that unknown differences between batches of phage or in preparation of the test solutions could be resulting in noticeable variation in inactivation kinetics. Inactivation in one

trial for DNA coliphage resulted in a predicted number of days to 2-log decline that was greater than 200 days.

The results of ANOVA for RNA coliphage are presented in Appendix 6-C. This analysis indicated that both TDS concentration and temperature had statistically significant effects on RNA coliphage inactivation, and a statistically significant interaction of the two factors also existed ($p < 0.05$). Thus the relative impact of TDS was greater at greater temperatures. This can be shown quantitatively by a regression of the two factors. The regression equation for RNA coliphage is:

```
RNA days = 78.7 + 0.0110 TDS - 2.47 temp
```

All components of the regression equation were significant to the 95% level. However, the r^2 value was .579, which is still fairly low and indicates a number of points were not modeled well by this equation.

These statistical analyses quantitatively describe a trend that temperature increases inactivation of RNA coliphage, and greater TDS reduces inactivation, thereby resulting in greater days until 2-log decline. An ANOVA for only the three lower TDS concentrations was also done (Appendix 6-C) and this analysis revealed that TDS concentration in this lower range was not a statistically significant factor (to the 90% or 95% level), while temperature still was statistically significant ($p < 0.01$). Furthermore, in looking at the values estimated for 2-log decline in Appendix 5-A, it is apparent that the source of differences between TDS concentrations is due to greater number of days (mean 148) at 5° C in 3000 mg/l TDS, and this large value is of course a result of extrapolation of observed inactivation kinetics, which were slow during the 28-day experiment. The means at 5° at the other TDS concentrations were 45, 61, and 57 days.

Averages by temperature and TDS in this table indicate that at ambient temperatures of the Floridan aquifer system, there was little to no difference between the TDS concentrations, with inactivation being most rapid actually at 30° C at 3000 mg/l. This explains the statistically significant interaction of temperature and TDS that was described by a 2-way ANOVA. The means by TDS are quite similar at 22° C and are actually somewhat less at 3000 mg/l at 30° C than the other TDS concentrations. Due to the variation with temperature, and the fact that the TDS effect actually reverses at higher temperatures, it may be that this apparent effect is more

due to experimental variability or even the result of extrapolation of inactivation kinetics beyond the measured periods (28 days).

Statistical analyses results for DNA coliphage are given in Appendix 6-D. These analyses are equivalent to those described for RNA coliphage. In both ANOVA tests, encompassing all TDS concentrations and just the lower three concentrations, TDS was not a statistically significant factor for DNA coliphage inactivation. Temperature was only significant at the 90% level, and not at the 95% level, with more rapid inactivation at higher temperature. Regression results also indicate that temperature and not TDS was a statistically significant component of a model equation to fit the observed data. However, with an r^2 value of 0.218, the regression was not an accurate model for predicting the number of days for 2-log decline. This is possibly due to the variability present between replicates at the same set of conditions.

PRD-1 bacteriophage

Results for survival experiments with the *Salmonella* bacteriophage PRD-1 indicated that it was considerably hardier under the examined conditions. For this reason, and because many of the predicted number of days until 2-log inactivation were greater than 200, the statistic used for analysis of PRD-1 decline was the linear inactivation rate. Besides the extended periods for decline predicted from observed data values, most all of the PRD-1 data sets showed less than 1-log decline and were analyzed with a first-order model. Thus, since this statistic was used, the remaining trials for which a non-linear model was employed were replaced with a linear one. Appendix 5 contains the inactivation rates in log N/N_0 change per day from TDS-temperature trials. It is important to note that as these values are negative rate constants, the greater magnitude (more negative) numbers indicate a greater rate of inactivation.

These inactivation rates were used to perform statistical analyses of the relative impacts of temperature and TDS concentration in the same fashion as for the other phage. Results of ANOVA using all TDS concentrations, regression results for the effect of the two predictors, and ANOVA results from only the three lower TDS concentrations are in Appendix 6-E. In short, neither ANOVA set nor the regression model indicated a statistically significant impact of TDS or temperature with the conditions evaluated. However, the mean inactivation rate for PRD-1 did display an upward trend at 30° C.

Cryptosporidium parvum

The impact of TDS and temperature on *C. parvum* infectivity over time was evaluated in duplicate for each condition set, using three lower TDS concentrations of 200, 500, and 1000 mg/L at the same temperature regime evaluated for the indicators. Replicate trials were performed simultaneously with the same batch of oocysts. Charts of observed infectivity data from cell culture analyses and corresponding model curves are shown in Appendix 1-F. Both model equations were employed for *C. parvum*, with the polynomial Equation 2 being used in a number of cases. Apparently, it was common for oocyst infectivity to increase after seeding in the TDS-temperature microcosms. While the actual number of oocysts present did not increase, the percentage of seeded oocysts that were able to establish infection in cell culture did. Thus a number of trials showed kinetics in which the N/N_0 ratio of infective oocysts rose above 1 initially, then began to decline. Therefore, the number of days predicted for 2-log decline in infectivity in these trials is a result of the observed rate of decline in infectivity after the initial peak. The assumption in modeling with the type of parabolic function used is that the declining infectivity of oocysts in these trials followed the kinetics that were observed as the infectivity first began to decline; in many cases, the ratio of infective oocysts at time *t* to initial concentrations did not fall back below 1 before the end of the experiment. The number of days predicted for 2-log decline used for comparative analysis in TDS-temperature trials for *C. parvum* are shown in Appendix 5-G.

The predicted days for 2-log decline in infective concentrations of *C. parvum* were used as a statistic for ANOVA to gauge the relative impacts of each factor on infectivity changes. The statistical test is shown in Appendix 6-K. This ANOVA showed that temperature was statistically significant as a factor ($p < 0.01$) in decline of *C. parvum*, but TDS was not. The largest difference in days for inactivation due to temperature was between 5° C and the two higher temperatures representative of ground water conditions in Florida (22° and 30°). However, the trend was still consistent between the two higher temperatures, with inactivation being fastest at 30° C. The predicted days for 2-log decline in infectivity were used in a regression equation for the effect of temperature. The complete statistical test for the regression is in Appendix 6-K also. This analysis revealed that the regression could successfully be used to fit the predicted days based on temperature; temperature was a statistically significant component of the regression with $p < 0.01$, but TDS was not, and the regression itself provided an r^2 value of

0.813. Thus, the regression equation shown below is a fairly good predictor of the days for 2-log decline that were estimated from observed results, based on temperature. The regression equation is:

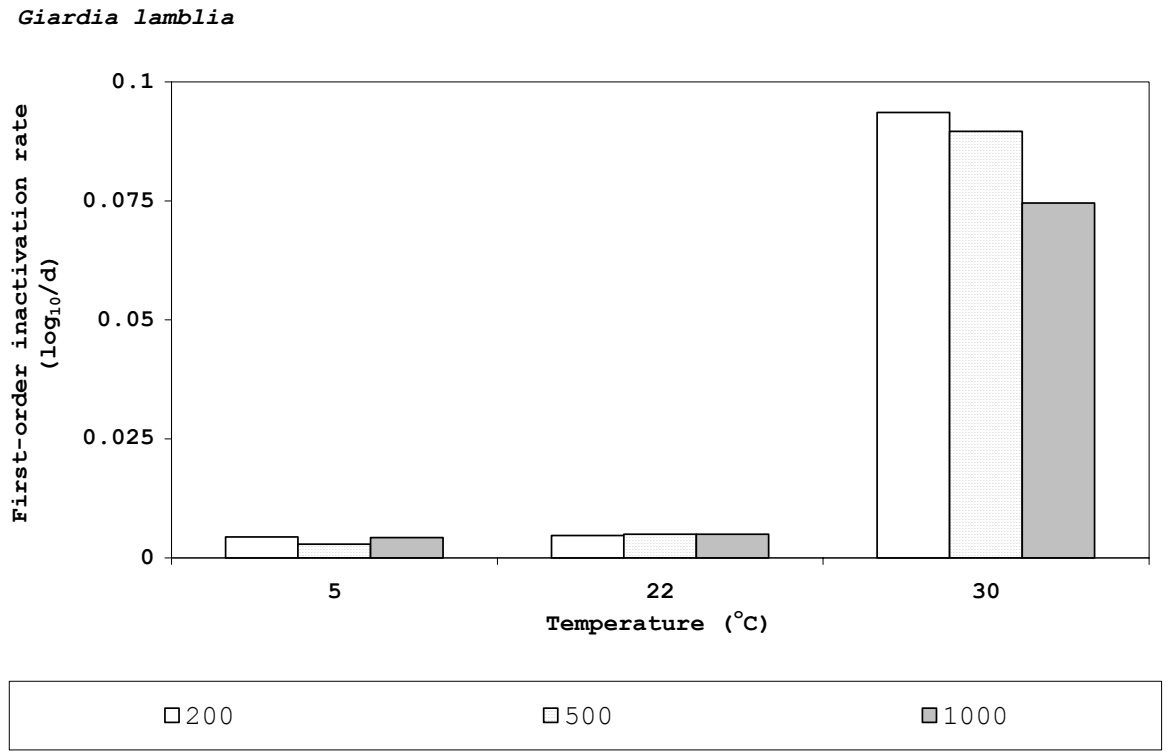
```
crypto days = 75.4 - 2.31 temp + 0.0118 TDS
```

Giardia lamblia

Effects on *Giardia* cyst viability due to changes in TDS and temperature and TDS were also evaluated at the three lower TDS concentrations of 200, 500, and 1000 mg/L at three temperatures. As explained in the STUDY METHODS section, cyst viability was determined by evaluation of staining properties, such that cysts that did not take up the dye propidium iodide were considered structurally compromised and thus non-viable. This is an indirect method for viability that does not directly evaluate infectivity, since a cell culture assay for *Giardia* is not available.

TDS-temperature effects for *Giardia* were performed in one trial for each condition set. Charts of observed viability data and model curves fit to these data are shown in Appendix 1-G. All *G. lamblia* data sets were fit with first-order model equations. However, little to no decline in viability was detected in these experiments up to 20 days at the two lower temperatures of 5° and 22°. As with PRD-1, observed changes in viable cysts were expressed as first-order inactivation rate constants, and these values are shown in tabular form in Appendix 5-A. Since only one replicate was performed for *G. lamblia*, ANOVA or regression were not performed on these data. However, the trends related to temperature and TDS are clear from the observed inactivation rates. These rates are shown graphically here in Figure 1.

Figure 1. First-order inactivation rates based on changes in *Giardia lamblia* cyst viability in TDS-temperature experiments



From this figure it is evident that inactivation was much more rapid at 30° than at 5° or 22° C. Also, there was no difference in inactivation rate due to TDS at the two lower temperatures, but a trend may be present at 30° C, such that the inactivation rate declined slightly with increasing TDS up to 1000 mg/L.

Fate in Aquifer and Surface Reservoir Water

Representative water samples from two locations in Florida were utilized for natural water source survival studies. The main objectives of this phase of the investigation were to compare survival in natural surface waters with natural ground waters and to study the effect of the native microbial flora in the waters on the survival of fecal indicators and protozoa, examined by eliminating the microorganisms via pasteurization as a controlled comparison.

Water from each site was characterized for each trial run in terms of several microbial and basic chemical parameters. In performing survival experiments with these water samples, water from the first two sample events was used for the first and second replicates on fecal coliform, enterococci, DNA coliphage, and PRD-1. Water from the third sample event was used for both replicates with F+ RNA coliphage, performed simultaneously. Water from the fourth sample event was used for intestinal parasite experiments (*C. parvum* and *G. lamblia*). One replicate was used for each parasite survival experiments. All sample events occurred in the summer or fall. The results of basic characterization analyses for the raw (unpasteurized) water samples from each of these sites are given in Table 2 (chemical parameters and temperature) and Table 3 (microbial parameters).

Table 2. Physiochemical measurements of raw water from the two sample sites.

		Avon Park Well	Bill Evers Reservoir	Lake Lytal Park Well	Clear Lake Reservoir
T (°C)	a	30	32	22	26
	b	30	29	23	27
	c	30	27	23	30
	d	30	30	22	29
pH	a	7.1	6.6	7.0	7.0
	b	7.2	7.2	7.3	8.1
	c	7.1	6.9	7.5	8.4
	d	7.1	7.2	7.6	8.1
TOC (mg/l)	a	2.13	18	0.73	13.2
	b	1.14	16.1	< 0.5	11.8
	c	ND	ND	ND	ND
	d	ND	ND	ND	ND
Conductivity	a	2.98 mS/cm^2	384 µS/cm^2	8.15 mS/cm^2	417 µS/cm^2
	b	2.94 mS/cm^2	353 µS/cm^2	6.23 mS/cm^2	317 µS/cm^2
	c	3.08 mS/cm^2	224 µS/cm^2	8.16 mS/cm^2	450 µS/cm^2
	d	3.00 mS/cm^2	347 µS/cm^2	6.45 mS/cm^2	323 µS/cm^2
Approx TDS (mg/l)	a	1500	200	4,000	200
	b	1500	175	3,000	150
	c	1500	112	4,000	225
	d	1500	174	3,000	162

One notable observation from these measurements was the expected differences in conductivity and thereby TDS between the ground water and surface water sources, such that the surface water TDS concentrations were much lower than ground water source values. Also, TDS in the Lake Lytal Park aquifer (West Palm Beach area) was 3,000-4,000 mg/L, while that of the Avon Park aquifer was only 1500 mg/L. For total organic carbon, when measured, concentrations were greater in the surface reservoirs than from the aquifer samples. Representative data were only obtained from the first two sample events due to difficulties with

out-sourcing the analyses. Temperature was consistently in the range of 22° to 30° C, with the two aquifers having stable temperature measurements over the three sample events. As expected, the surface water reservoirs were more variable. The pH of all ground water samples was near neutral, while the pH of Bill Evers reservoir water was slightly below neutral and that of Clear Lake reservoir was generally higher, around 8.

Table 3. Microbial background measurements from raw water at the two sample sites.

		Avon Park Aquifer	Bill Evers Reservoir	Lake Lytal Park Aquifer	Clear Lake Reservoir
HPC (cfu /100 ml)	a	1.0×10^7	7.3×10^5	1.1×10^5	2.0×10^6
	b	2.0×10^5	3.3×10^6	8.7×10^5	4.0×10^5
	c	6.1×10^4	2.7×10^6	1.1×10^5	1.7×10^5
	d	4.5×10^3	5.7×10^5	1.9×10^4	3.5×10^5
Fecal coliform (cfu /100 ml)	a	< 0.5	26	< 0.5	849
	b	< 1	75	< 1	408
	c	< 1	85	< 1	995
	d	< 1	70	< 1	870
Enterococci (cfu /100 ml)	a	< 0.5	23	< 0.5	689
	b	15	52	< 1	977
	c	< 1	197	< 1	230
	d	< 1	15	4	850
Coliphage (pfu / 100 ml)	a	< 10	430	< 10	50
	b	< 10	< 10	< 10	50
	c	< 10	24	< 10	< 10
	d	< 10	< 10	< 10	< 10
PRD-1 (pfu / 100 ml)	a	< 10	< 10	< 10	10
	b	< 10	< 10	< 10	< 10
	c	ND	ND	ND	ND
	d	ND	ND	ND	ND

The data in Table 3 show background microbial concentrations of heterotrophic plate count bacteria (HPC), fecal coliform, enterococci, combined somatic and male-specific coliphage (DNA and RNA) and analysis for background PRD-1. HPC analysis showed a range of over 2 orders of magnitude between the three Avon Park samples. HPC bacteria in the first Avon Park sample were dominated by a single type of small, clear colony and it is unknown why

these were present at the initial sampling and absent for subsequent ones. The other aquifer samples and surface water samples had HPC counts in the range of 10^5 to 10^6 CFU/ 100 ml. Water quality indicator organisms were found in the raw surface water samples and in one instance in a ground water sample (enterococci in the second Avon Park sample). The colonies were confirmed as enterococci by esculin iron reduction, but it is not known if the presence of these organisms was due to contamination of the well, or of the sample, or from the presence of enterococci-like organisms in the aquifer. Water quality indicator bacteria concentrations were much higher in Clear Lake than Bill Evers reservoir, although coliphage do not show the same trend. *Salmonella typhimurium* phage such as PRD-1 were generally not present in these samples.

Table 4 shows the microbial counts in natural water samples after pasteurization treatment. Large reductions in HPC counts were achieved, and all water quality indicator organism counts were reduced to below detection limits.

Table 4. Background microbial concentrations after pasteurization of aquifer and reservoir water.

		Avon Park Aquifer	Bill Evers Reservoir	Lake Lytal Park Aquifer	Clear Lake Reservoir
HPC	a	18	800	96	< 1
(cfu /100 ml)	b	2	3900	3	677
	c	50.5	760	11.5	2400
Fecal coliform	a	< 0.5	< 0.5	< 1	< 1
(cfu /100 ml)	b	< 1	< 1	< 1	< 1
	c	< 1	< 1	< 1	< 1
Enterococci	a	< 0.5	< 0.5	< 1	< 1
(cfu /100 ml)	b	< 1	< 1	< 1	< 1
	c	< 1	< 1	< 1	< 1
Coliphage	a	< 10	< 10	< 10	< 10
(pfu / 100 ml)	b	< 10	< 10	< 10	< 10
	c	< 10	< 10	< 10	< 10
PRD-1	a	< 10	< 10	< 10	< 10
(pfu / 100 ml)	b	< 10	< 10	< 10	< 10
	c	ND	ND	ND	ND

Summary of the Natural Water Experiments

The following section summarizes the results of the natural water experiments. This is followed by sections with a more detailed explanation and analysis of each bacteria, phage and protozoan. It is important to note that the physical, chemical and biological nature of any water source may impact the survival of introduced microorganisms. To evaluate survival of seeded test microorganisms, estimated days for 2 log (99%) inactivation were used as the value for comparison of the bacteria and phage, and protozoa (*Cryptosporidium* and *Giardia*) to each other in raw surface and ground waters at two temperatures. For PRD-1 and the protozoa, for which inactivation rates were used to compare the effects of various conditions on each organism, the rates were converted to 99% inactivation times to enable comparisons to the indicator bacteria and coliphage. At the lower temperature of 22^{o} C, most microorganisms survived longer in ground water than in surface water. One exception is the F+ RNA phage, which were quite fragile and survived poorly under both conditions. The other exception is *Giardia lamblia.* It is not clear why data for *Giardia* showed an opposite trend from the other organisms. It may be the method used for measuring viability was affected by the TDS concentrations or another chemical component of the ground water. Viability dyes were used for assessing cyst viability; this analysis is based on permeability of the cyst membrane. It is conceivable that the nature of the water affected permeability. It is known that the dye test is not completely reliable for assessing parasite viability. The viability of *Cryptosporidium* oocysts, on the other hand is measured via cell culture and is a true measure of infectivity (14).

The parasites and DNA coliphage were much more resistant than fecal coliform or enterococci and as much as 7 months would be predicted for achieving 99% reduction of *Giardia* cysts in surface waters, while 1 to 6 weeks would be required for DNA phage and *Cryptosporidium* oocysts in most cases. However, several caveats surround this prediction. One, as stated above, the test used for *Giardia* cyst survival in these experiments does not truly reflect its viability and may thus not be a very accurate determination of its actual longevity. Also regarding *Giardia*, due to the need for staining cysts in suspension and the need for very high concentrations of cysts to enable microscopic viewing, small volumes of water (< 1 ml) were mixed with *Giardia* cyst suspensions. Thus, these small volumes may not have been representative of the conditions in bulk water volumes. Also, for several predicted survival

periods, the length of time predicted was an extrapolation based on shorter test periods. Thus, future changes in kinetics could result in different actual periods for 2-log inactivation.

Table 5. Ranges of days for 2-log$_{10}$ (99%) decline of bacteria, protozoa and phage in natural water trials at two temperatures.

		Ranges of estimated days for 2-log$_{10}$ (99%) decline in natural water samples						
Sample Site	Temp. °C	Fecal coliform	Enterococci	F+ RNA coliphage	DNA coliphage	PRD-1	*Cryptosporidium*	*Giardia*
Avon Park well	22	17-22	5-17	1-3	28-45	89-186	> 200	51
	30	8-12	2-7	1	10-16	109-192	18	20
L. Lytal Park well	22	35-45	32-35	4	24-41	52-132	48	66
	30	11-12	13-16	1	10-14	39-53	17	19
Bill Evers res.	22	4-6	3-4	7	11-19	10-31	45	> 200
	30	1-2	1-2	2-3	5-11	8-15	10	25
Clear Lake res.	22	6-10	6-7	2	19-28	23-25	30	> 200
	30	4-5	4	1	6-13	14-23	11	26

At higher temperatures of 30°C, the time needed for 99% reductions was dramatically reduced. The parasites and the DNA coliphage remained the most robust (excluding PRD-1, as it is not commonly taken as an indicator of pathogenic virus survival); however, less than a month was needed to achieve the 2-log$_{10}$ reductions. At 30 ° C, there were much smaller differences between survival in the two types of water (aquifer vs. reservoir samples), thus suggesting that temperature is an over-riding factor compared to chemical or biological impacts in the water.

Enterococci were just as adequate as indicators of fecal pollution compared to the fecal coliform, at least based on survival, but neither were adequate indicators of the risks potentially associated with the increased survival of the enteric protozoa. The fragility of the F+ RNA coliphage suggests that if these are found in ground water, recent contamination (1 to 6 days) has occurred. However, these do not appear to be adequate indicators of the attenuation of microorganisms of fecal origin under storage conditions.

Subsamples of each water sample for indicator trials (excludes parasites) were pasteurized to reduce the native microbial populations in the surface and ground waters, to determine if the background biological constituents of selected water samples may play a role in survival of non-native (seeded) water quality indicator bacteria and viruses. In the eight comparisons of survival between pasteurized and non-treated waters, six demonstrated that the treatment had a statistically significant impact on the rate of decline of introduced organisms in

both surface waters and ground waters. The decrease in survival in the raw surface water trials was generally greater than that seen in ground waters. The difference in survival for the DNA coliphage and enterococci was much greater between the treated and raw surface water trials in contrast to the treated and raw ground water trials. In treated and raw ground water the enterococci showed no statistical difference based on the presence or absence of the biological components of the aquifer. For the fragile F+RNA coliphage there was no statistically significant difference between treated and untreated surface waters, and a slight decrease in survival in the raw groundwater trials.

Fecal coliform

Survival studies in natural water sources with fecal coliform were performed twice, with water collected at separate times and organisms grown in separate batches. Results of cultivable fecal coliform counts at each time point were analyzed in the same way as for the TDS-temperature trials. The resulting statistic obtained for comparison of conditions affecting inactivation was the number of days until 2-log decline. This statistic was predicted based on model curve equations fit to observed data points (Appendix 2-A, 2-C for data points and model curves, Appendix 5-B for 2-log-inactivation days). The predicted days for 2-log inactivation were used for comparison of temperature, pasteurization, and water type (reservoir or aquifer) effects. Model curves were developed using the same two equations as for artificial water trials described previously, depending on observed kinetics of fecal coliform survival and decline. More experiments resulted in initial growth of the fecal coliform populations than for artificial water trials, but exclusively in the pasteurized sub-samples, and these necessitated the use of the polynomial (parabolic) model (Equation 2).

ANOVA were performed on these modeling results in order to compare the effects of various factors in natural water experiments. Predicted 2-log inactivation days from each independent trial were used for comparison of conditions, rather than means from duplicate trials. ANOVA tests were done using varying levels of categorization of the conditions evaluated, so as to clarify differences in trends between surface and ground water, or in raw water only. Appendix 6-F contains results of fecal coliform statistical tests for the aquifer and reservoir water studies.

In a comparison of temperature and pasteurization effects in all natural water sources combined, both temperature and treatment were statistically significant, to the 95% and even 99% level. The effect of temperature was a decrease in the number of days and thus a decrease in survival at higher temperatures. Also, the raw water resulted in much more rapid decline than treated water when considering all temperature levels at once. There was not a statistically significant interaction between temperature and pasteurization, however, indicating that the impact of treatment was not greater at higher temperatures, nor was the impact of temperature more pronounced in raw water rather than treated. This analysis design combined the results from both surface and ground water, only considering temperature and treatment. Inclusion of water type as a potential factor in a 3-way ANOVA was also performed (Appendix 6-F). When water type, pasteurization, temperature, and their interactions were considered, all three independent factors were statistically significant, but the interactions were not ($p < 0.05$). Thus, inactivation was greater in surface water than in ground water.

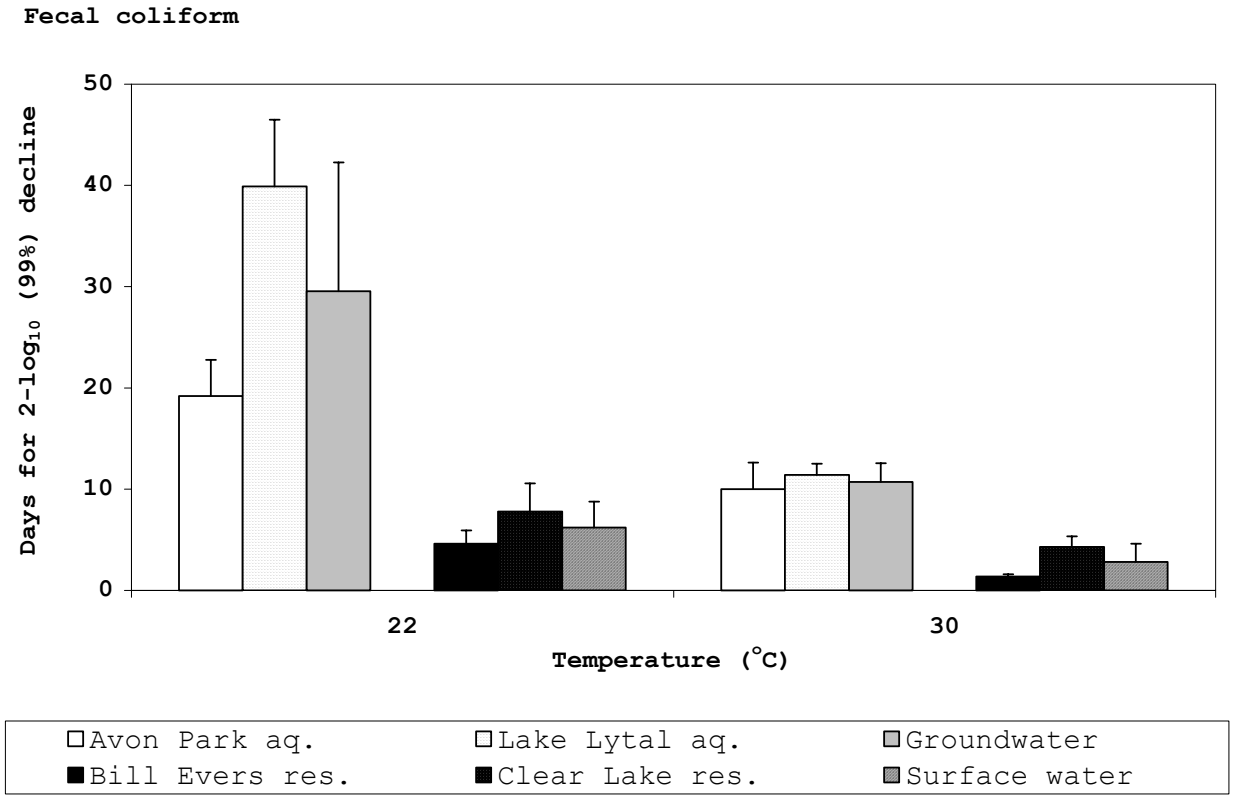
The pasteurization treatment applied to subsamples of each water source was conducted to evaluate the effect of a reduction in native microbial populations on survival of introduced organisms. For evaluating behavior in real situations, the raw water trials are much more important. Thus, analysis of temperature and water type as important factors in fecal coliform survival in only the raw water sources was also performed. In this comparison, only temperature was statistically significant ($p < 0.01$). Inactivation was less rapid on average in ground water than surface water, as was found when considering both pasteurized and raw water sources. Means were 15 days for surface water and 28 days for ground water (average of predicted days from all temperatures). However, this difference was not statistically significant.

The intricacies of introduced fecal coliform survival in these water sources can be further described by considering the behavior in surface water and ground water separately. This is appropriate since analysis of variance on combined results (both raw and pasteurized) determined a statistically significant difference in survival between these water types. Tables of the results from these ANOVA are in Appendix 6-F as well. When considering both treated and raw surface water, pasteurization and temperature significantly affected survival, and in raw surface water, the temperature effect was still significant ($p < 0.05$ in all cases), with pasteurization increasing survival and higher temperature reducing survival. Regarding factors affecting survival just in ground water, pasteurization produced a statistically significant impact,

with longer survival in pasteurized samples. However, temperature was not statistically significant to the 95% or 90% level in combined raw and pasteurized or raw-only ground water comparisons. The mean values for number of days to 2-log inactivation did show the typical temperature- related trend, with shorter periods for inactivation at higher temperature (Values may be seen in Appendix 5-B or 6-F). In these experiments, however, the variability between trials in ground water and a less pronounced temperature effect resulted in the lack of statistical significance.

For purposes of gauging actual survival potential of these bacteria in ASR-related water sources in Florida, behavior in the raw water samples (unpasteurized) at the two higher temperatures of 22° and 30° are more of a concern in regards to survival and inactivation of contaminant microorganisms. To graphically summarize inactivation trends in the four natural water sources (raw), Figure 2 displays the mean number of days for 2-log inactivation as listed in Appendix 5-B (combining the results of each of the 2 trials). Error bars represent the standard deviation of each mean value. It is important to recall that as the bars represent number of days, the smallest bars signify most rapid inactivation. Figure 2 graphically shows the trends described above, for raw water only, wherein it is apparent that inactivation was more rapid in surface water at both temperatures than in ground water, and for both types of water, inactivation increased on average at 30° over 22° C. Although not shown, inactivation at 5º C was considerably less rapid. Also, the combined average of each of the two water types is shown. Table 6 shows the actual ranges of these values from the two trials in each water source at the two higher temperatures.

Figure 2. Chart of mean days for 2-log inactivation for fecal coliform in raw aquifer and surface water at ambient temperatures for Florida



Note: The ground water and surface water bars are the combined data from Avon Park and Lake Lytal aquifers AND Bill Evers and Clear Lake reservoirs, respectively.

Table 6. Table of days for 2-log inactivation for fecal coliform in raw aquifer and surface water at ambient temperatures for Florida

Sample Site	Temp. °C	Range of days for 99% decline
Avon Park aq.	22	17-22
	30	8-12
L. Lytal Park aq.	22	35-45
	30	11-12
Bill Evers res.	22	4-6
	30	1-2
Clear Lake res.	22	6-10
	30	4-5

Due to the importance of inactivation rates under the raw water conditions as shown above, in contrast to the artificial situations created for comparative analysis of effects on survival, first-order inactivation rates were also determined for the combined data from each site. Charts of the raw water data points combined for each aquifer and reservoir source are given in Appendices 2-B and 2-D. Linear regression analysis of the combined points at all three temperatures are shown on these charts, and these rates are shown here in Table 7. Although not quite as accurate a depiction of actual inactivation kinetics as the more-precise models employed for predicted days for 2-log decline, these rates are helpful for comparison to other studies as well, as first-order inactivation rates are often used to express results from studies such as these. Since data points from both trials in each water source were modeled for the regression together, the rate expressed here is essentially an average of data from the two trials. As can be seen from the graphs on which these regressions were formulated, fairly good agreement between the two trials was observed for these conditions (raw water, temperatures of 22º - 30º C).

Table 7. First-order inactivation rates of fecal coliform in raw aquifer and surface water sources

Sample Site	Temp. °C	Linear inact. rate (log_{10}/d)
Avon Park aq.	5	-0.021
	22	-0.101
	30	-0.166
L. Lytal Park aq.	5	-0.141
	22	-0.065
	30	-0.149
Bill Evers res.	5	-0.052
	22	-0.420
	30	-1.012
Clear Lake res.	5	-0.066
	22	-0.173
	30	-0.296

Enterococci bacteria

Enterococci survival studies in the aquifer and surface water sources were performed alongside fecal coliform studies. The data obtained from enterococci experiments were analyzed in the same way as the fecal coliform, and results were compiled in the same types of tables and figures. Figures of sample points and model curves appear in Appendices 2-E and 2-G, Appendix 5-C displays the predicted days until 2-log decline for enterococci in ground water and surface water in both raw and pasteurized conditions created for comparative analyses on factors affecting inactivation kinetics.

The same types of analyses of variance used for the fecal coliform data were performed for the enterococci data. Appendix 6-G displays the results of these analyses, first of a two-way test for water temperature and pasteurization treatment effects in all natural water sources (both surface and ground water samples), a three-way test for these two factors and water type, and then a two-way test for the significance of temperature and water type in only raw (unpasteurized) waters. These test results revealed some relationships and interactions regarding enterococci inactivation. Temperature was strongly significant ($p < 0.01$) in all these statistical tests, such that inactivation was more rapid with increasing temperature.

Regarding the effect of pasteurization, in a two-way ANOVA with temperature and pasteurization as the factors, pasteurization was not significant at the 90% level, although it was nearly so ($p = 0.108$). However, when more factors and interactions were introduced to explain variability in a 3-way ANOVA, pasteurization (averaged across all temperature levels) was statistically significant at the 90% level. Inactivation was more rapid in raw water than in pasteurized. In addition, the interactions of water type and treatment, and the interaction of water type, treatment, and temperature were both statistically significant at the 95% level. However, water type as an independent factor was not statistically significant. Pasteurization reduced inactivation in surface water to a much greater degree than in ground water. The temperature interaction with pasteurization effects and water type was interesting in that relative differences between raw and pasteurized conditions increased with increasing temperature in surface water, but not in ground water. This was determined from analysis of inactivation periods from each condition set as computed from values in Appendix 5-C.

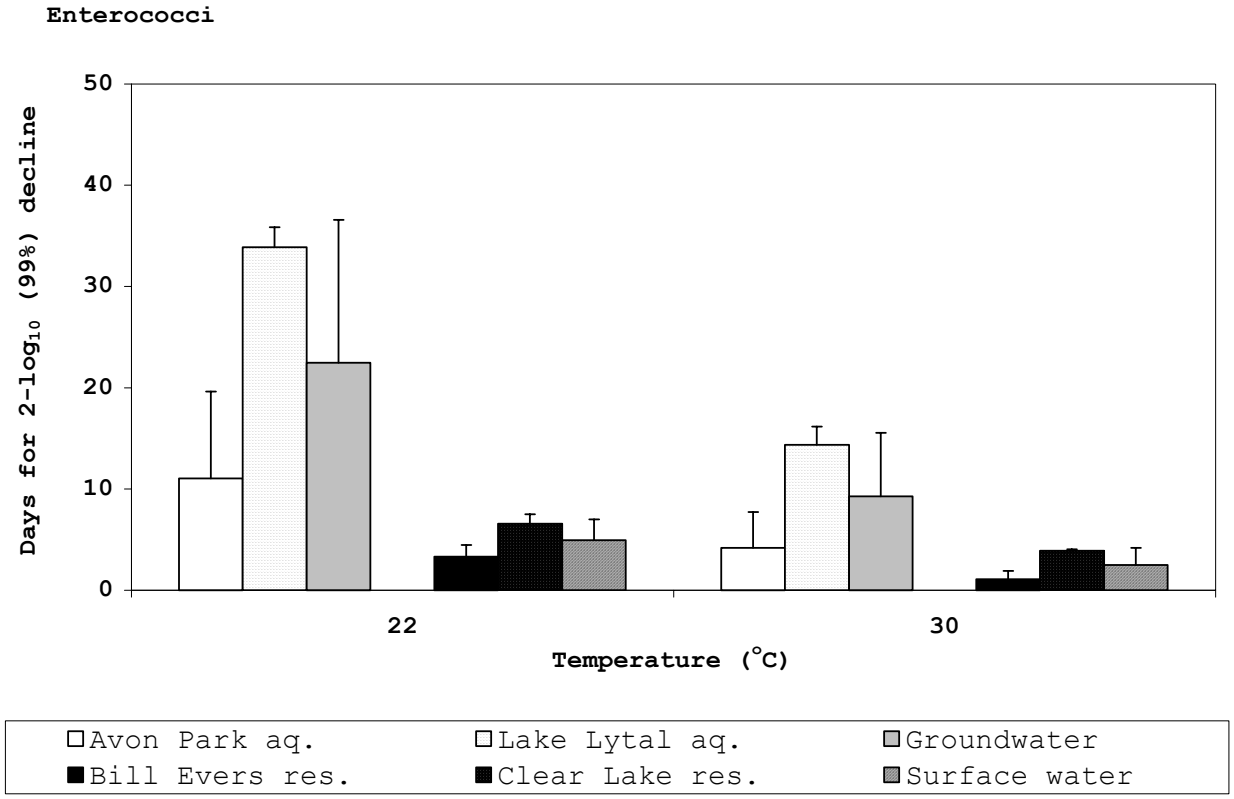
When considering raw water only, both temperature and water type were significant to the 95% level, and the interaction of the two factors was significant at the 90% level.

Inactivation was more rapid in surface water, and this difference was more pronounced at 5° C than at the higher temperatures (hence the statistically-significant interaction of water type and temperature). Thus, this interaction may be of limited importance when considering temperatures found in the Floridan aquifer system.

Results of ANOVA performed independently on the two types of water are given in Appendix 6-G also. As with fecal coliform, both types were analyzed independently, while also considering both combined raw and pasteurized and raw only conditions. In surface water, temperature, treatment, and the interaction of the two factors were all statistically significant ($p < 0.01$), and as expected so was temperature when considering only raw surface water ($p < 0.01$). However, in ground water, only temperature was statistically significant ($p < 0.01$), while pasteurization did not produce a statistically significant effect. The difference between the ground water and surface water in significance of treatment and the interaction of temperature and treatment reveals the source of the interactive dependence on water type demonstrated when considering the 3-way ANOVA comparison. It is evident that reduction of the native microbial populations had more of an influence on enterococci survival in surface water than in the ground water sources.

Once again, behavior in the raw water samples (unpasteurized) at the two higher temperatures of 22° and 30° C is more of a concern in regards to survival and inactivation of contaminant microorganisms. Figure 3 graphically displays the means of days for 2-log decline for enterococci in raw water sources. The averages at both temperatures were greater in Lake Lytal Park water than in Avon Park water. This site-related difference was also the case between Clear Lake reservoir and Bill Evers reservoir. The column chart also demonstrates the more rapid inactivation in surface water on average compared to ground water at the respective temperature levels, and the more rapid decline at 30° C than 22° C for each water type. Table 8 shows the actual ranges of these values from the two trials in each water source at the two higher temperatures.

Figure 3. Chart of mean days for 2-log inactivation for enterococci in raw aquifer and surface water at ambient temperatures for Florida



Note: The ground water and surface water bars are the combined data from Avon Park and Lake Lytal aquifers AND Bill Evers and Clear Lake reservoirs, respectively.

Table 8. Table of days for 2-log inactivation for enterococci in raw aquifer and surface water at ambient temperatures for Florida

Sample Site	Temp. °C	Range of days for 99% decline
Avon Park aq.	22	5-17
	30	2-7
L. Lytal Park aq.	22	32-35
	30	13-16
Bill Evers res.	22	3-4
	30	1-2
Clear Lake res.	22	6-7
	30	4

Charts of combined data points from experiments in each water source, from which average first-order inactivation rates were determined by regression analysis, are shown in Appendices 2-F and 2-H. These rates are shown here in Table 9.

Table 9. First-order inactivation rates of enterococci in raw aquifer and surface water sources

Water source	Temperature (C)	Linear inact. rate (log_{10}/d)
Avon Park aq.	5	-0.060
	22	-0.162
	30	-0.253
Lake Lytal aq.	5	-0.011
	22	-0.062
	30	-0.132
Bill Evers res.	5	-0.049
	22	-0.377
	30	-0.774
Clear Lake res.	5	-0.054
	22	-0.265
	30	-0.501

F+ RNA coliphage

Plots of log-transformed survival ratios for F+ RNA coliphage and resulting best-fit model curves for natural water trials are in Appendix 3-A and 3-C. Predicted days for 2-log decline are given in Appendix 5-D. Unlike the experiments for the other four types of organisms, duplicate trials for the RNA coliphage were performed simultaneously. Therefore, the variability that would be associated with obtaining water samples at different times and from using different batches of cultivated test organisms was not present in the results. Thus, not unexpectedly, the overall variability between trials shown in the results given in these tables was generally much less than was present with the other sets of organisms. The same types of ANOVA comparisons as were done for the bacteria were performed for F+ RNA coliphage 2-log days and tables showing these are given in Appendix 6-H.

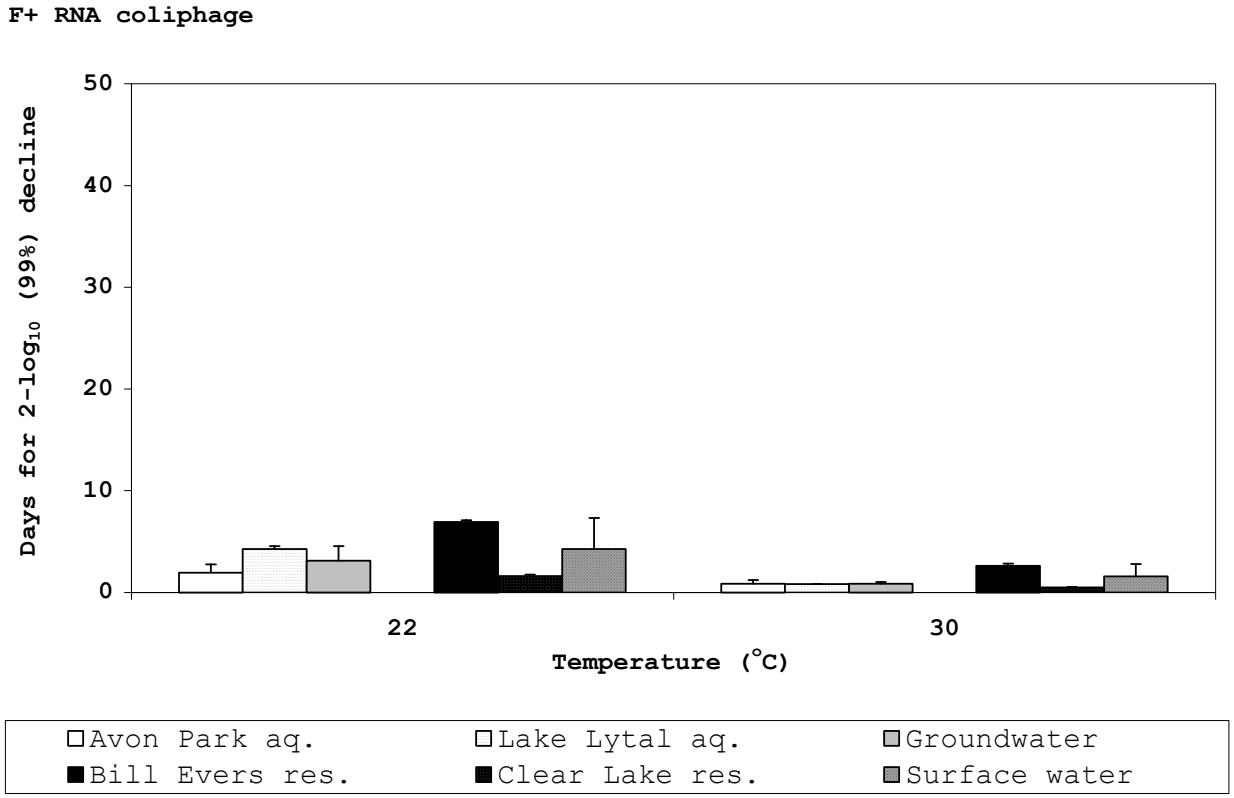
When both pasteurized and raw water of both types (surface and ground water) were considered, temperature was statistically significant in a two-way test comparing temperature and treatment, while treatment and the interaction of the two did not result in any statistically significant effects. If water type was also considered in a three-way test, temperature was still determined to be significant ($P < 0.05$), while water type was significant to the 90% level. Also, the three-way interaction of temperature, water type, and treatment was significant to the 90% level. Higher temperature consistently reduced survival of the F+ RNA coliphage. But differences due to water type and treatment were primarily due to the differences at 5° C, and a consistent trend was not present. Pasteurization increased survival at 5° C in ground water and decreased survival at 5° C in surface water. Thus, while pasteurization produced a statistically-significant reduction in inactivation in ground water and did not in surface water, this trend was due to variability at low temperature and is of limited importance when considering the subsurface environment of Florida.

In looking at only raw (unpasteurized) water of both types, temperature was once again a statistically significant factor, and water type and the interaction of water type and temperature were statistically significant to the 90% level. In contrast to both types of bacteria (fecal coliform and enterococci), the relationship of water type to survival was reversed. But while survival was less on average in ground water compared to surface water in both raw and combined raw and pasteurized analyses, this effect was again due to differences at 5° C more so than at the higher temperatures of concern to the Florida subsurface. This may be inferred from examining 2-log inactivation days from Appendix 5-D.

Comparisons of factors affecting inactivation were also broken down into the separate water types. The tables of these ANOVA tests are also in Appendix 6-H. In surface water, temperature was statistically significant ($p < 0.01$), but pasteurization was not. In ground water, both when combining treated and raw, and in raw only, the temperature effect was the same and was statistically significant, although only at the 90% level in raw ground water. As the ANOVA diagram in Appendix 6-H shows, there was a large difference among the means between 5° and the two higher temperatures, but the large standard deviation at 5° in raw ground water reduced the significance level assigned to this effect. Also, pasteurization created a statistically significant impact on inactivation in ground water ($p < 0.05$), and the effect was interrelated with temperature (interaction was significant with $p < 0.05$). Once again this

interaction is due to a larger difference between pasteurized and raw water survival at 5° compared with the effect at environmental temperatures (22° and 30°). It becomes apparent that at the two higher temperatures of 22º and 30º C, inactivation in the two water types was both similar and rapid, regardless of treatment.

Figure 4. Chart of mean days for 2-log inactivation for F+ RNA coliphage in raw aquifer and surface water at ambient temperatures for Florida



Note: The ground water and surface water bars are the combined data from Avon Park and Lake Lytal aquifers AND Bill Evers and Clear Lake reservoirs, respectively.

Inactivation of F+ RNA coliphage in raw water at temperatures typical of Florida is shown in Figure 4. The mean values displayed in this column chart underscore the more rapid inactivation observed with F+ RNA coliphage than for fecal coliform or enterococci overall, particularly in ground water. All values under these conditions were less than 10 days. The ranges of days for 2-log inactivation in these conditions, which are important as they are more indicative of actual conditions, are shown in Table 10.

Table 10. Table of days for 2-log inactivation for F+ RNA coliphage in raw aquifer and surface water at ambient temperatures for Florida

Sample Site	Temp. °C	Range of days for 99% decline
Avon Park aq.	22	1-3
	30	1
L. Lytal Park aq.	22	4
	30	1
Bill Evers res.	22	7
	30	2-3
Clear Lake res.	22	2
	30	1

Data from duplicates for each condition set were combined and linear regression rates were obtained, and these charts are displayed in Appendices 3-B and 3-D. The first-order rates are shown in Table 11.

Table 11. First-order inactivation rates of F+ RNA coliphage in raw aquifer and surface water sources

Water source	Temperature (C)	Linear inact. rate (log_{10}/d)
Avon Park aq.	5	-0.271
	22	-0.509
	30	-1.594
Lake Lytal aq.	5	-0.064
	22	-0.448
	30	-2.427
Bill Evers res.	5	-0.048
	22	-0.249
	30	-0.633
Clear Lake res.	5	-0.091
	22	-0.938
	30	-1.999

DNA coliphage

The predicted days for 2-log decline from DNA coliphage (combined male-specific and somatic) experiments in natural water sources are shown in Appendix 5-E, while charts of the data and model curves are in Appendices 3-E and 3-G. In several cases, inactivation of DNA coliphage was quite a bit slower in natural water than F+ RNA coliphage and the two bacterial populations. In five cases, all in the pasteurized water sources, inactivation was so slow that the days predicted for 2-log decline was capped and the maximum for these analyses of variance of 200 days was used for comparison.

Results of ANOVA tests on both surface and ground water sources are shown in Appendix 6-I. Results of ANOVA considering both water types combined indicated that strong significance may be attributed to the effects of temperature and pasteurization independently ($p < 0.01$), but no interaction between the two factors was apparent. The effects of these factors were that higher temperature decreased survival, and pasteurization increased survival. Inclusion of water type as a variable in a three-way ANOVA revealed that water type was statistically significant at the 90% level independently, and the interaction of water type and treatment was statistically significant to the 95% level. As with the F+ RNA coliphage, inactivation of DNA coliphage was more rapid on average in ground water than in surface water when considering both pasteurized and raw water across the three temperature regime. However, there were larger differences at each temperature between raw and pasteurized conditions in surface water than in ground water, particularly in surface water at higher temperatures, thus the interaction of water type and treatment. But while temperature was significant as an independent factor, the interactive effect of temperature on the relative difference due to pasteurization between the two water types was not significant (no three-way interaction as with enterococci).

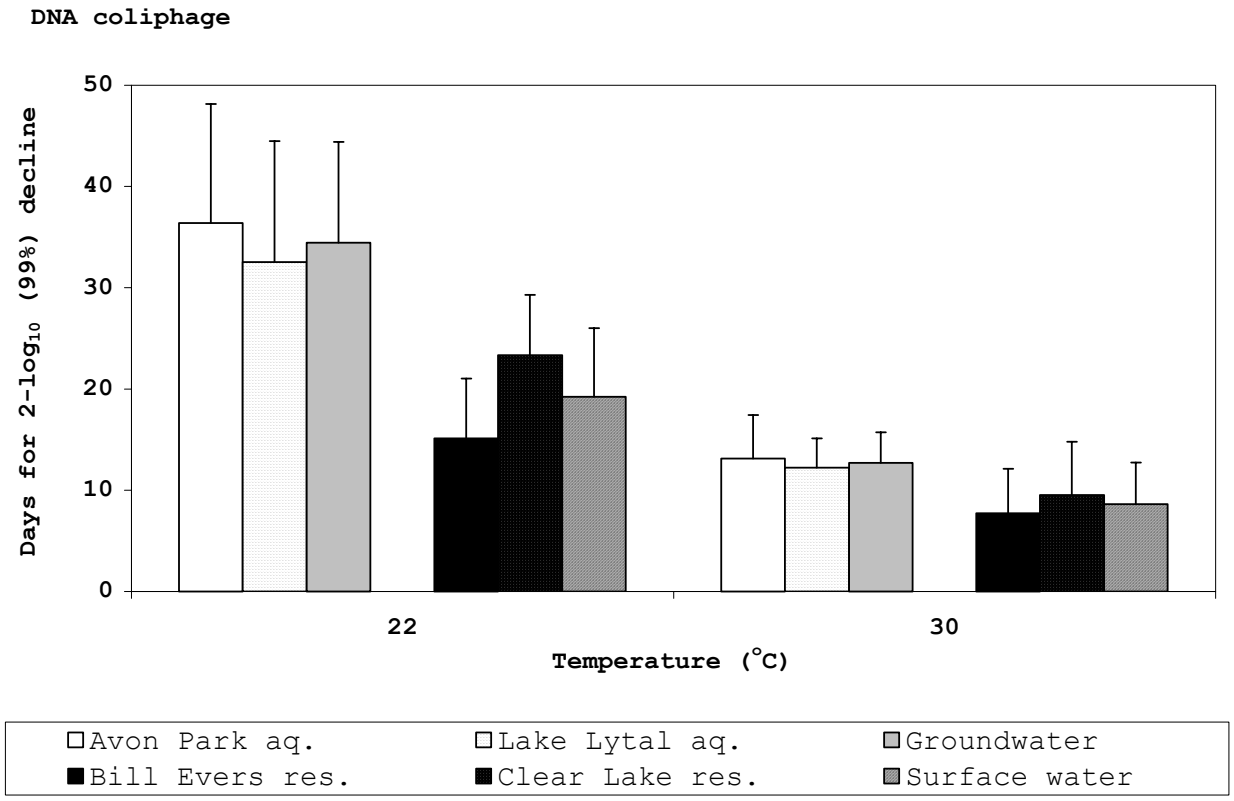
When considering only raw water samples, temperature was statistically-significant ($P < 0.05$) for affecting inactivation, but water type and the interaction were not. There was little difference between water types in raw water, but mean inactivation periods in surface water were slightly shorter when all temperatures were combined (see Appendix 6-I). In actuality, the differences due to water type which were statistically significant in a 3-way ANOVA considering both pasteurized and raw water were due to differences in the pasteurized water

samples between water types. Also, the trend was reversed; inactivation in raw water was more rapid in surface water than in ground water.

Results of statistics for comparisons of the two water types taken independently are also in Appendix 6-I. In surface water and ground water, in both comparisons (raw and pasteurized or raw only), temperature was statistically significant to the 95% level, with more rapid inactivation at higher temperature. The interaction of water type and treatment is explained further in these tests; for surface water sources, pasteurization had a significant effect on inactivation ($p < 0.05$), but was not statistically significant in ground water ($p = 0.12$). Nonetheless survival still increased on average in pasteurized ground water. This difference was more notable at higher temperatures (taken from comparison of values in Appendix 5-E).

Figure 5 depicts the means days for 2-log inactivation from conditions in these trials that were raw water samples (unpasteurized) at temperatures representative of actual Floridan aquifer system conditions (22º and 30º C). This figure shows graphically the large difference between survival of F+ RNA coliphage (Figure 4) and DNA coliphage in aquifer and reservoir water sources at ambient temperatures found in Florida. The mean values at each temperature are less for surface water than for ground water, as was seen with the other types of organisms. However, as ANOVA results on combined raw water sources demonstrated, the effect of water type was not statistically significant if comparing the means at all three temperatures (5° C is not included in Figure 5). The actual ranges of predicted 2-log inactivation days in these more-realistic conditions are shown in Table 12. There appeared to be more variability between the two trials in the ground water samples at 22º C than in other condition sets. It also appears that the difference between the two water types was larger at 22° C.

Figure 5. Chart of mean days for 2-log inactivation for DNA coliphage in raw aquifer and surface water at ambient temperatures for Florida



Note: The ground water and surface water bars are the combined data from Avon Park and Lake Lytal aquifers AND Bill Evers and Clear Lake reservoirs, respectively.

Table 12. Table of days predicted for 2-log inactivation of DNA coliphage in raw aquifer and surface water at ambient temperatures for Florida

Sample Site	Temp. °C	Range of days for 99% decline
Avon Park aq.	22	28-45
	30	10-16
L. Lytal Park aq.	22	24-41
	30	10-14
Bill Evers res.	22	11-19
	30	5-11
Clear Lake res.	22	19-28
	30	6-13

First-order inactivation rates were also computed from DNA coliphage inactivation experiments. Charts of combined data points for raw water at all temperatures are given in Appendices 3-F and 3-H; these combined values of Log N/N_0 for each experiment were used for linear regression, to obtain first order inactivation rates as for the other organisms. These inactivation rate constants are given in Table 13.

Table 13. First-order inactivation rates of DNA coliphage in raw aquifer and surface water sources

Water source	Temperature (C)	Linear inact. rate (log_{10}/d)
Avon Park aq.	5	-0.036
	22	-0.064
	30	-0.132
Lake Lytal aq.	5	-0.035
	22	-0.072
	30	-0.148
Bill Evers res.	5	-0.037
	22	-0.117
	30	-0.167
Clear Lake res.	5	-0.017
	22	-0.092
	30	-0.16

PRD-1 bacteriophage

The bacteriophage PRD-1, which is a virus that infects the bacterium *Salmonella typhimurium*, is generally considered more resistant to environmental stresses than most enteric viruses and bacteria. However, it is useful as a tracer of virus movement through the subsurface for these reasons and may be a good predictor of survival for the more-resistant hepatitis A virus than F+ RNA coliphage (3). Thus, it was evaluated in these trials to gauge its persistence under the conditions as may be found in aquifer injection scenarios in Florida. As was found for temperature-TDS trials, inactivation of this organism was slow, compared to the other indicator organisms. The statistical comparisons of factors affecting survival for PRD-1 employed first-

order inactivation rates. Although a first-order model may not be the best for describing kinetics of decline in all cases, it was adequate for instances found here, because there was not a case of severe deviation from first-order kinetics.

Plots of the PRD-1 data from natural water sources are displayed in Appendices 3-I and 3-K. These charts allow inspection of kinetics of decline and show that in most cases, a linear model is the most useful. Tables of inactivation rate constants are presented in Appendix 5-F. Aside from the different statistic used for comparison of factors affecting PRD-1 survival, the analyses that were performed on the data for PRD-1 were the same as those for the other organisms. Thus, ANOVA result tables were created for these trials as well and are shown in Appendix 6-J. In a 2-way ANOVA considering temperature and treatment effects for all water sources, the increase of inactivation rates with temperature was statistically-significant to the 90% level. However, while inactivation rates decreased in pasteurized water over raw water, the effect was just beyond the range of statistical significance with $p = 0.141$. In a 3-way ANOVA that added differences due to water type, temperature and water type significantly affected inactivation rates ($p < 0.05$), while treatment effects were still not quite significant with $p = 0.107$. Mean inactivation rates steadily increased with temperature, when both water types and both treatments were combined. Although not statistically significant, inactivation was more rapid in raw water than in pasteurized, as with all other organisms evaluated.

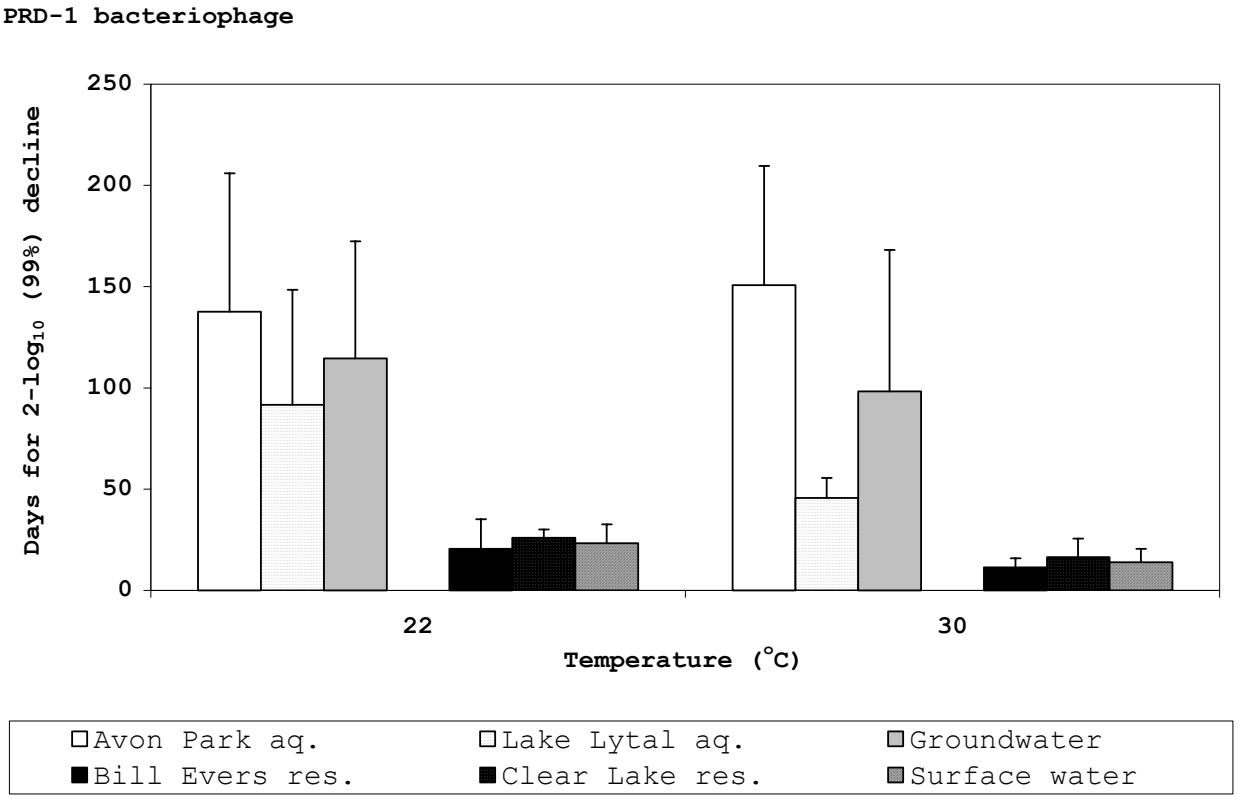
In a 2-way ANOVA that compared temperature and water type effects in only the unpasteurized, raw water samples, temperature, water type, and the interaction of these factors were all significant to the 95% level. Inactivation was much more rapid in surface water than in ground water. As detailed below, increased inactivation due to temperature was mostly due to more rapid inactivation at higher temperatures in surface water but not in ground water sources (raw).

ANOVA were also performed for PRD-1 inactivation rates from each of the water types taken independently. In surface water, the increase of inactivation rates with temperature was significant to the 90% level when averaging both raw and pasteurized water, and to the 95% level in raw water considered alone. Pasteurization of surface water resulted in a decreased average inactivation rate, by about ½, but the effect was not significant ($p = 0.152$). In ground water, neither treatment nor temperature resulted in statistically-significant effects on PRD-1 inactivation rates. There was a consistent increase of rate with temperature such that in raw

ground water, the inactivation rate at 5° was about ½ that at 30° C. However, variability in these cases resulted in fairly high p values, all in excess of 0.3 for these ANOVA.

Although many of the conditions resulted in slow inactivation with predicted days for 2-log decline, like the other organisms PRD-1 inactivation was generally most rapid at the higher temperatures of 22° and 30° C in raw water, conditions which most closely emulate the environment of concern. Thus, for comparison, Figure 6 presents predicted 2-log days in a graphical format. These predictions were converted from the inactivation rates used in comparative analyses by dividing 2 (for 2-log inactivation) by the rate in $\log_{10}$ decline per day. The number of days was much greater on average than that predicted for the other groups of organisms, thus the y-axis scale on Figure 6 is different than on similar figures for the other organisms. This is important to note when comparing this to the other graphs. Table 14 shows the values used for Figure 6, so that the actual range of predicted inactivation periods is easily seen. It is important to keep in mind that these values are predictions which assume a first-order decline at the same rate as that observed during the 28 days of the experiment, and thus may not actually be the time that 2-log decline takes to occur.

Figure 6. Chart of mean days for 2-log inactivation for PRD-1 in raw aquifer and surface water at ambient temperatures for Florida, converted from observed first-order decay rates.



Note: The ground water and surface water bars are the combined data from Avon Park and Lake Lytal aquifers AND Bill Evers and Clear Lake reservoirs, respectively.

Table 14. Table of days (ranges) predicted for 2-log inactivation of PRD-1 in raw aquifer and surface water at ambient temperatures for Florida

Sample Site	Temp. °C	Range of days for 99% decline
Avon Park aq.	22	89-186
	30	109-192
L. Lytal Park aq.	22	52-132
	30	39-53
Bill Evers res.	22	10-31
	30	8-15
Clear Lake res.	22	23-25
	30	14-23

From Table 14 it seems that larger apparent variability was observed for PRD-1 than for the other organisms in raw water samples. Once again however, these values are based on predictions derived from rates over a shorter period than those depicted in the figure and table. In keeping with data from the other organisms, PRD-1 data from the two trials were combined for raw water conditions and fit with linear regression curves to determine average inactivation rates. Linear regression rates of combined data for the above conditions plus at 5° C are given in tabular format in Table 15.

Table 15. First-order inactivation rates of PRD-1 in raw aquifer and surface water sources

Water source	Temperature (C)	Linear inact. rate (log_{10}/d)
Avon Park aq.	5	-0.009
	22	-0.017
	30	-0.015
Lake Lytal aq.	5	-0.015
	22	-0.027
	30	-0.045
Bill Evers res.	5	-0.047
	22	-0.103
	30	-0.153
Clear Lake res.	5	-0.034
	22	-0.084
	30	-0.115

Cryptosporidium parvum

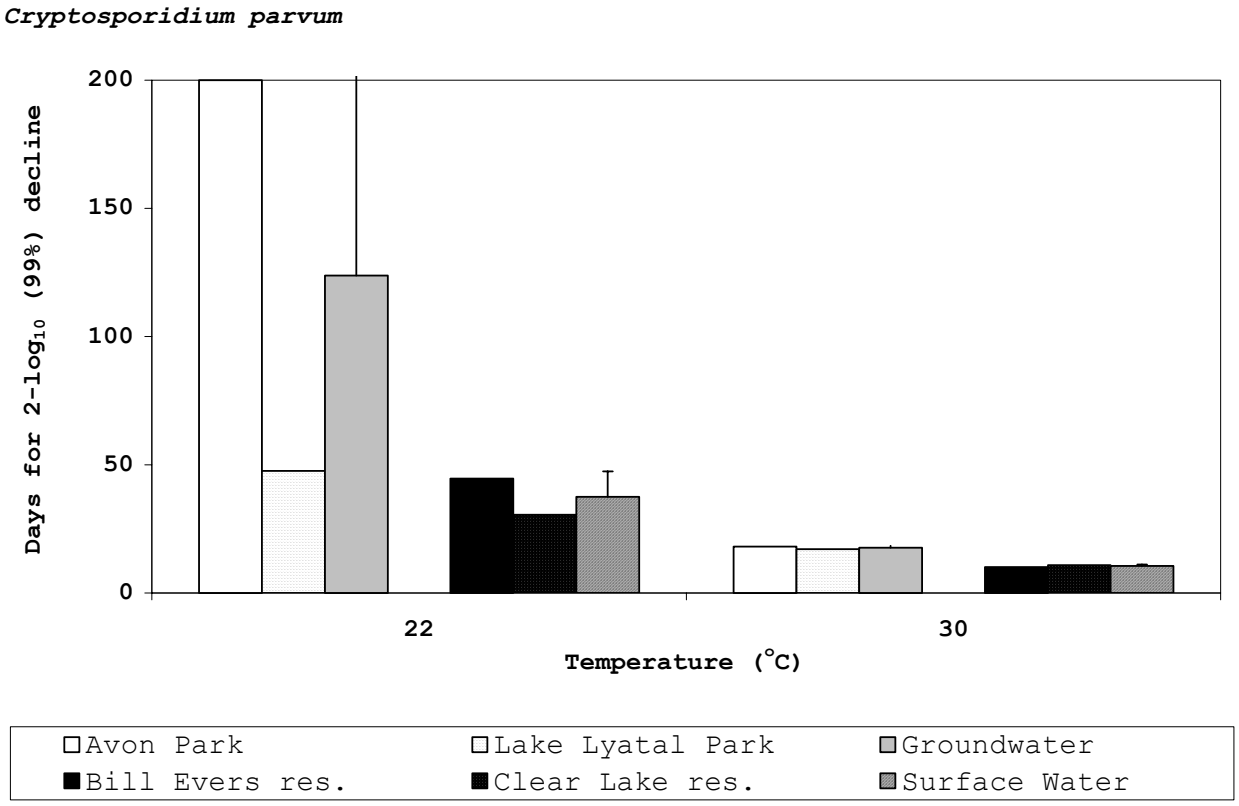
Survival studies were performed with *C. parvum* oocysts and *G. lamblia* cysts in samples of the representative surface water and ground water sources as were done with the indicator organisms. One trial was performed in each water source at the three temperatures of 5°, 22° and 30° C. *C. parvum* survival was determined with infectivity analyses in cell culture as described above, the results of infectivity analyses over time from each trial were modeled with a first-order variant of Equation 1 only, such that the exponent *m* of Equation 1 was 1. Charts of observed data over time and associated model curves are presented in Appendix 3-M. No pasteurized or other treated water samples were used for the intestinal parasites *Cryptosporidium* and *Giardia*.

As was the case for PRD-1, numerous data sets had predicted days for 2-log decline in excess of 200 days, so data for *C. parvum* were expressed in terms of first-order inactivation rates and the rate from each trial at each condition set was used as the statistic for comparative statistical analyses. These inactivation rates are given in tabular format in Appendix 5-G. Rates are shown for each site, and grouped by water type. Rates were used for statistical analysis to compare the effect on survival of temperature and water type. ANOVA results from this comparison are in Appendix 6-L. Significant effects on variability of *C. parvum* inactivation rates in these trials were attributable to both temperature and water type, and the interaction of the two factors ($p < 0.05$).

Essentially, no decline was observed in *C. parvum* infectivity at 5° C over the course of the 25-day experiment. The mean rate of change across all water types was actually a positive rate, but was very low and is thus most likely due to no inactivation and variability in the assay. The pattern of increased infectivity at 22° C as was observed for *C. parvum* in TDS-temperature trials was not seen in these experiments. Inactivation was statistically significantly more rapid in surface water sources compared to ground water sources. Also, a statistically significant interaction between water type and temperature was observed; the relative effect of one variable varied with the other.

In Figure 7, estimated days for 2-log decline based on observed inactivation rates are shown for the two higher temperatures, 22° and 30°, which are typical for the Floridan aquifer system. Also, the average for each water type at these temperatures is given. The model equation for oocysts inactivation in Avon Park well water at 22° C predicted greater than 200 days for 2-log decline, as indicated by this bar. As done with other results, this value was set as a maximum as it is well outside the experimental duration. The average for ground water at 22° C reflects the mean using 200 days for Avon Park trial. Error bars reflect standard deviation of the mean of the two sites for each water type. The values used in this chart are shown in Table 16.

Figure 7. Predicted days for 2-log decline of *C. parvum* oocyst infectivity in raw aquifer and surface water at model temperatures for Florida ground water



Note: The ground water and surface water bars are the combined data from Avon Park and Lake Lytal aquifers AND Bill Evers and Clear Lake reservoirs, respectively.

Table 16. Table of days predicted for 2-log inactivation of *Cryptosporidium parvum* in raw aquifer and surface water at ambient temperatures for Florida

Sample Site	Temp. °C	Predicted days for 99% decline
Avon Park aq.	22	> 200
	30	18
L. Lytal Park aq.	22	48
	30	17
Bill Evers res.	22	45
	30	10
Clear Lake res.	22	30
	30	11

From this chart and this table, one can see that there was a large difference in survival between the water from Avon Park and the Lake Lytal Park wells at 22º C. The reason for this difference is not known, with no inactivation observed over the 25 days in Avon Park water at 22° C. This difference is responsible for the statistically significant interaction of temperature and water type, and if only Lake Lytal Park water were considered, survival at 22° C would be very similar to that observed in surface water. Very little difference existed in survival between the two types of water at 30° C, with inactivation in water from all sites being fairly similar. First-order inactivation rates are shown here in Table 17. This is useful for comparison to the observed results from indicator organism survival described above. This table quantitatively describes the difference between survival in Avon Park water and Lake Lytal Park, with an inactivation rate about ¼ of that in the other water samples.

Table 17. First-order inactivation rates of *C. parvum* in natural water samples at three temperatures

Water source	Temperature (C)	Linear inact. rate (log_{10}/d)
Avon Park aq.	5	0.0088
	22	-0.0010
	30	-0.11
Lake Lytal aq.	5	0.00090
	22	-0.042
	30	-0.12
Bill Evers res.	5	-0.0017
	22	-0.045
	30	-0.20
Clear Lake res.	5	-0.0037
	22	-0.066
	30	-0.18

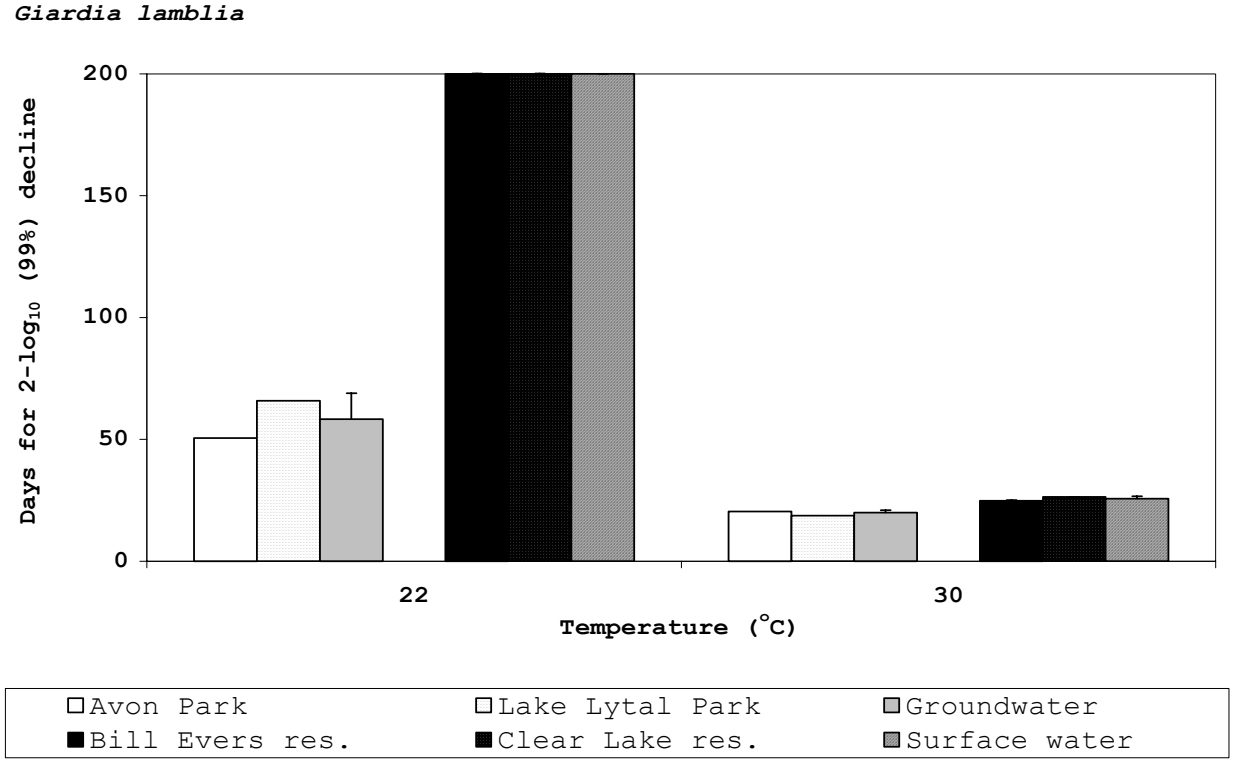
Giardia lamblia

Giardia cyst inactivation in representative surface and ground water samples was measured as in TDS-temperature experiments. One trial was performed for seeded cysts in each water sample at three temperatures. Results of observed viability concentrations over time and

model curves are shown on charts in Appendix 3-N. As with *C. parvum* and PRD-1, slow inactivation necessitated the use of first-order inactivation rate constants rather than days for 2-log decline with *G. lamblia*. These inactivation rates, and averages for each water type, are shown in Appendix 5-H. Statistical analysis of inactivation rates (Appendix 6-M) revealed that, like *Cryptosporidium*, temperature and water type were statistically significant factors in affecting variability of *G. lamblia* inactivation rates, as was the interaction of the two ($p < 0.01$). Inactivation was more rapid on average in ground water than in surface water, in contrast to *C. parvum*. This effect was also related to temperature, with greater differences between the two water types at temperatures of 22° C, but close similarity at 5° and 30°. As with *C. parvum*, first-order models were used for all natural water data sets, and virtually no inactivation was visible at 5° and at 22° C in some cases (Appendix 3-N). However, the inactivation rate did increase on average at 22° C from 5° C.

Inactivation rates were converted to predictions of days to achieve 2-log inactivation at 22° and 30° C, and these values are summarized for each site in Figure 8. The values for each water type are averaged as well. The values depicted in this chart are also given in Table 18. Once again, these values were predicted based on observed inactivation rates, and thus the actual observation of 2-log decline was not made for those periods greater than 25 days.

Figure 8. Predicted days for 2-log decline of *G. lamblia* cyst viability in raw aquifer and surface water at model temperatures for Florida ground water



Note: The ground water and surface water bars are the combined data from Avon Park and Lake Lytal aquifers AND Bill Evers and Clear Lake reservoirs, respectively.

Table 18. Table of days predicted for 2-log inactivation of *Giardia lamblia* in raw aquifer and surface water at ambient temperatures for Florida

Sample Site	Temp. °C	Predicted days for 99% decline
Avon Park aq.	22	51
	30	20
L. Lytal Park aq.	22	66
	30	19
Bill Evers res.	22	> 200
	30	25
Clear Lake res.	22	> 200
	30	26

Observed kinetics at 22° C resulted in predictions for 2-log decline of over 200 days in surface water. The difference between ground water and surface water at this temperature was responsible for the interactive effect of temperature and water type on variability in inactivation rate described by the ANOVA for these data. Inactivation rates were very similar for each site and water type at 30° C. The actual first-order inactivation rate constants are shown in Table 19. Even though they were low, rates were quite similar for surface water at 22° C between the two sites. Inactivation rates for the two sites of each water type were similar at the other temperatures as well.

Table 19. First-order inactivation rates of *G. lamblia* in natural water samples at three temperatures

Water source	Temperature (C)	Linear inact. rate (log_{10}/d)
Avon Park aq.	5	-0.0042
	22	-0.0395
	30	-0.098
Lake Lytal aq.	5	-0.0050
	22	-0.0304
	30	-0.11
Bill Evers res.	5	-0.0034
	22	-0.0050
	30	-0.081
Clear Lake res.	5	-0.0034
	22	-0.0042
	30	-0.076

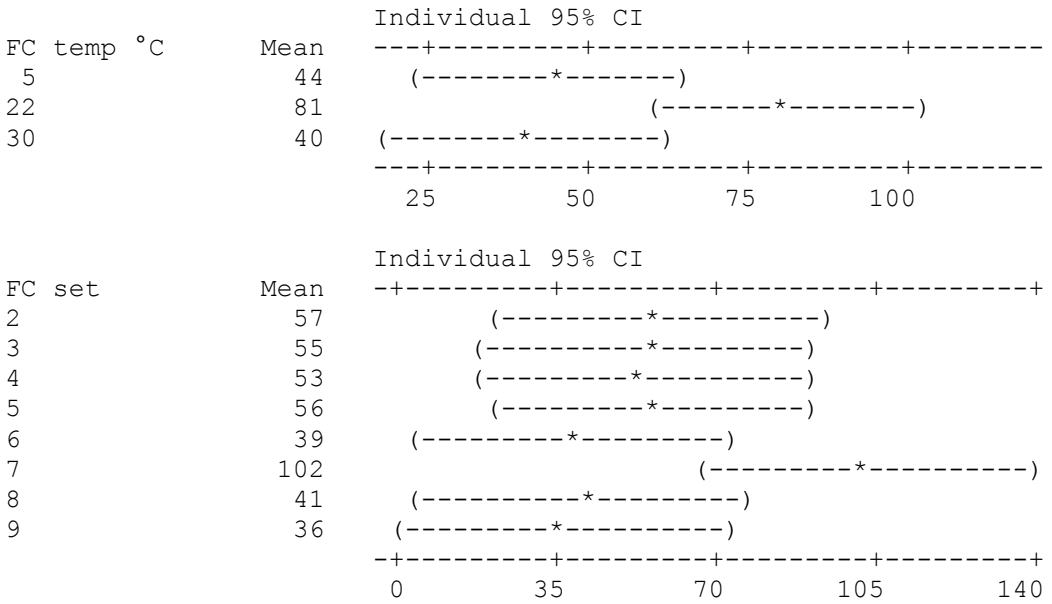
Results of buffered saline controls

For each experimental run with the indicator organisms, inactivation in control microcosms of PBS was also tested alongside each experimental set to assess variability amongst batches of seed organisms or due to other unknown reasons, as described in the STUDY METHODS section. PBS-control microcosms were used for each temperature with each experiment, with the exception of the first trial for bacteria, in the first of TDS-temperature trials at 500 and 3000 mg/L for which the PBS control was only at 5° C. Charts with data from viability counts at each time point for the PBS sets are included in Appendix 4 with results for each organism in sections A-E. To gauge the overall variability in PBS control microcosms,

statistical analyses of variance were performed as was done for the results of actual experimental conditions, using the days until a predicted decline of 2-log or the first-order inactivation rate constant for PRD-1. The statistical analysis for 2-log days for the bacteria appear in Appendix 4-A for fecal coliform and Appendix 4-B for enterococci; similar results for the RNA and DNA coliphage are in Appendices 4-C and 4-D respectively, and Appendix 4-E displays the ANOVA results for PRD-1 inactivation rates in PBS.

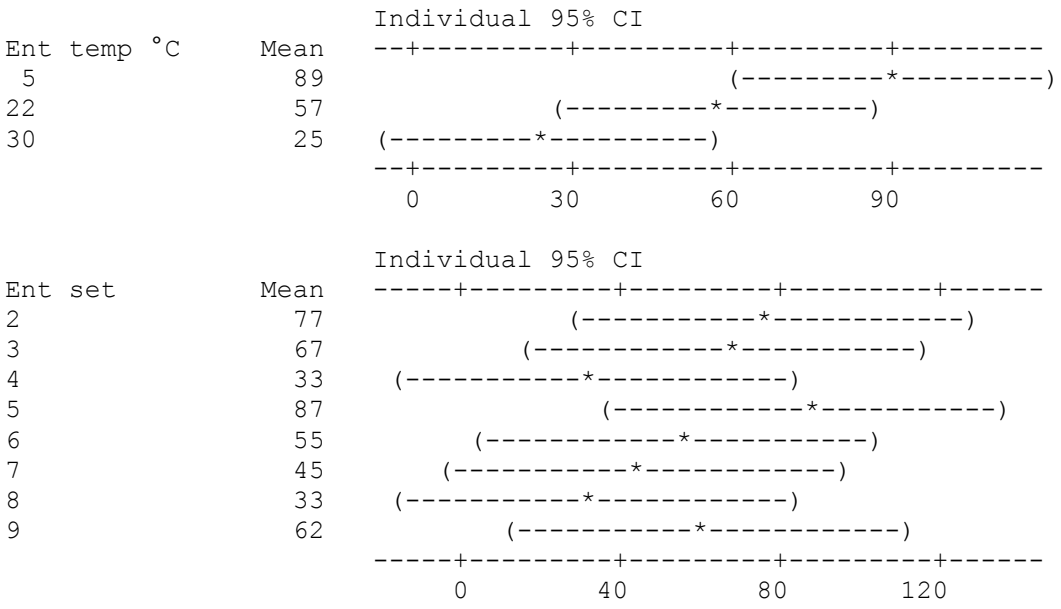
Results for fecal coliform indicate that, for experimental sets 2 through 9 (excluding set 1 which was only at 1 temperature), statistically significant differences existed between predicted days for 2-log inactivation at the three temperature levels, but statistically significant variability was not attributed to the individual sets. The diagrams of means shown in Figure 9 indicates that for fecal coliform, one set (7) does stand out as having, on average for all three temperatures, longer periods for 2-log decline. The rest of the means are all fairly close together. The trend of days in response to temperature is interesting in that the longer periods for predicted 2-log decline are at 22° rather than at 5° or 30°. Growth of some members of the fecal coliform population was observed in many cases, it appeared that most likely it was the *Klebsiella* species, based on the differences in colony morphology. These appeared to become much more numerous relative to the other colony types and the overall effect was evidenced as a slower decline in total fecal coliform population numbers. Growth of these sub-types occurred frequently in PBS at 22° and 30°, but decline was slower at 22° C.

Figure 9. Fecal coliform days for 2-log inactivation in PBS controls at three temperatures



For enterococci, differences between sets were not statistically significant; in looking at a diagram of the mean inactivation for each set in Figure 10, no particular set or sets seem to stand out as being much more rapid or slower than the rest. The temperature trend was more typical of what occurred in the experimental microcosms, with increasing inactivation at higher temperatures (indicated by fewer days for 2-log decline). Colony morphologies for the enterococci were too similar to allow any observation on the dynamics of the sub-types within the population, but there was no indication that enterococci re-grew in any of the control or experimental microcosms.

Figure 10. Enterococci days for 2-log inactivation in PBS controls at three temperatures



Results of ANOVA for the F+ RNA coliphage showed that statistically significant differences in the inactivation of F+ RNA coliphage existed between sets, so much so that the trend due to temperature was overshadowed and determined to be not statistically significant. As can be seen in Figure 11, the overall slow decline in set 2 seems to stand out from the rest, this set was used for TDS-temperature trials at 500 and 3000 mg/L. Variability between the sets also existed for DNA coliphage, but only set 8 seems to be notably different from the rest on average as shown in Figure 12. Set 8 was used for the second of two trials in artificial sea salts at 3,000 mg/L for the DNA coliphage. Temperature was also statistically significant in these controls, following the typical trend of decreasing number of days for 2-log inactivation with increasing temperature. For PRD-1, variability could be attributed to temperature ($p < 0.01$), but not to differences among sets. The inactivation rate means were variable, but with no real stand-outs as the diagram in Figure 13 indicates.

Figure 11. F+ RNA coliphage days for 2-log inactivation in PBS controls at three temperatures

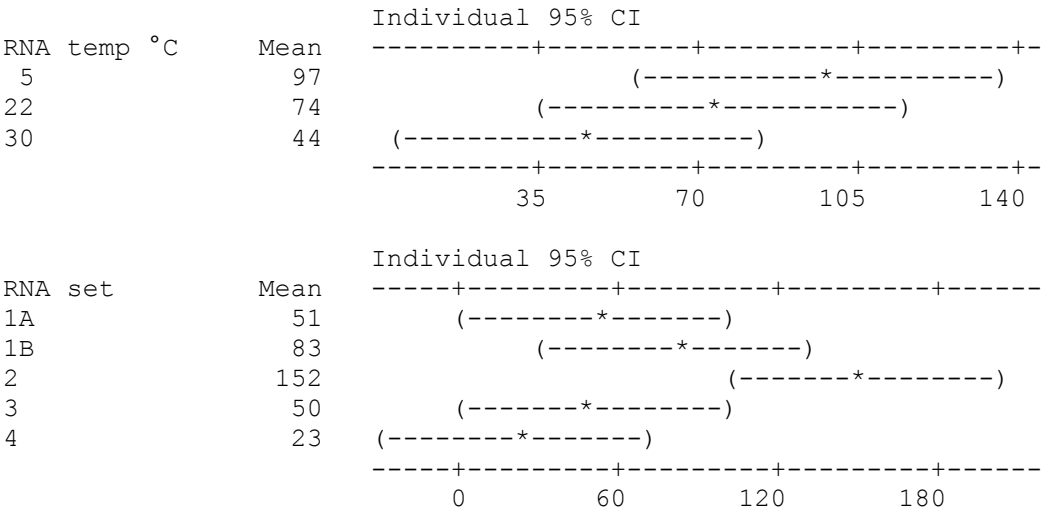


Figure 12. DNA coliphage days for 2-log inactivation in PBS controls at three temperatures

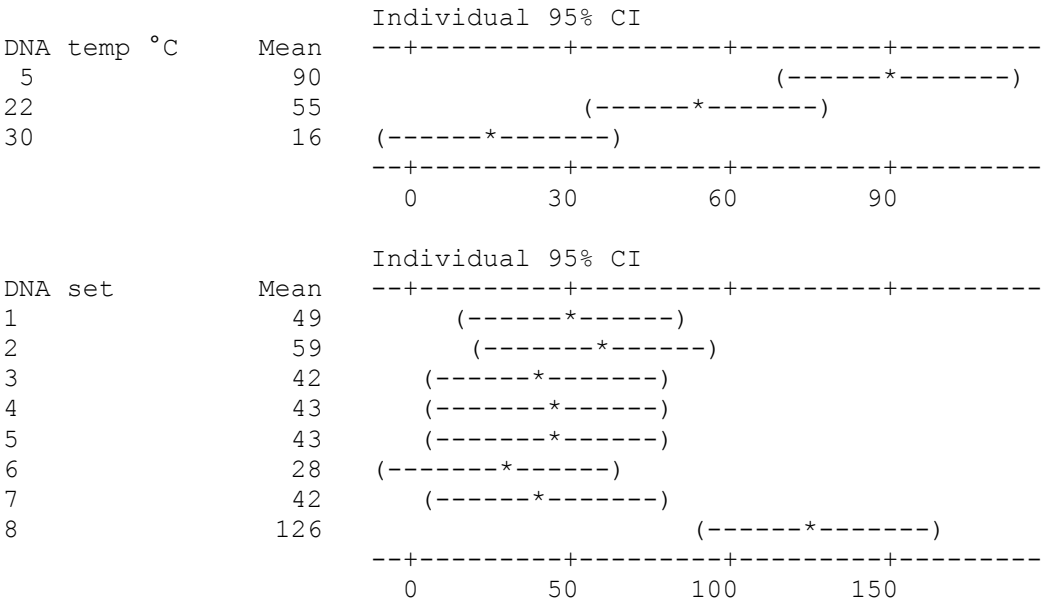
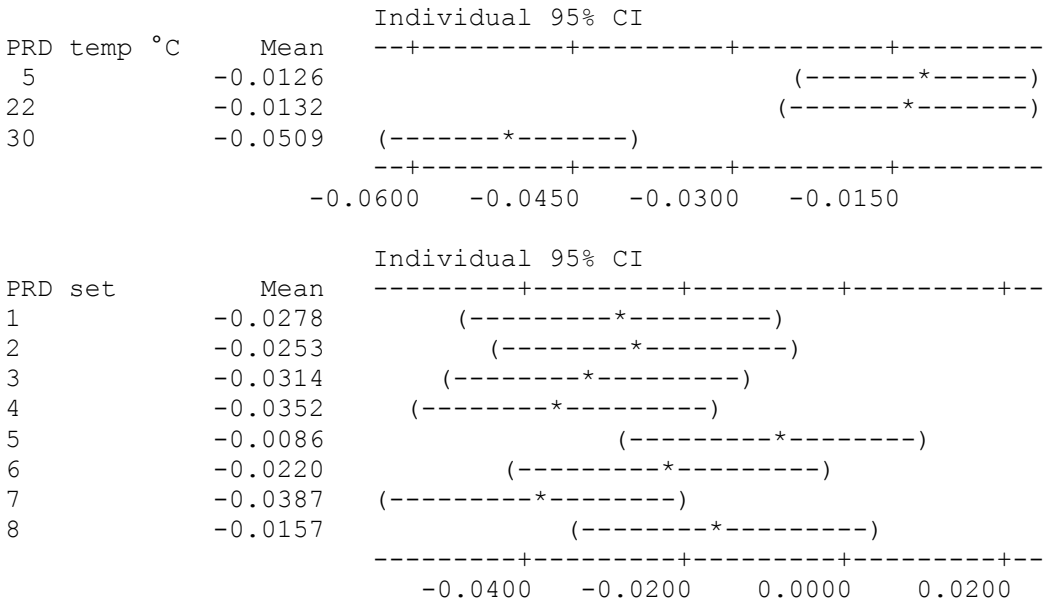


Figure 13. PRD-1 coliphage days inactivation rates in PBS controls at three temperatures



CONCLUSIONS

Two main branches of laboratory investigations were performed for this project: survival studies in controlled conditions to isolate the effects of temperature and total dissolved solids (TDS), and survival studies in both raw and pasteurized samples of representative aquifer and reservoir water to characterize survival in these types of water, with additional interest in effects of temperature and reduced background microbial levels. Modeling of observed viable concentrations of the seven organisms provided estimates of days to achieve 2-log (99%) reductions in concentrations or first-order inactivation rate constants. These periods or rates were used to compare variability between conditions with analyses of variance (a statistical test), which revealed factors that were determined to be statistically significant in affecting survival. For temperature-TDS comparisons, factors which were statistically significant to the 90% or 95% level are listed for each organism group in Table 20.

Table 20. Statistical significance of TDS and temperature for indicator organisms and *Cryptosporidium*

Organism	TDS range (mg/L)	Significant factors (95% unless noted)
fecal coliform	200 - 3000	**temp**
	200 - 1000	**temp**
enterococci	200 - 3000	**temp, TDS (90%)**
	200 - 1000	**temp**
F+ RNA coliphage	200 - 3000	**temp, TDS, interact**
	200 - 1000	**temp**
DNA coliphage	200 - 3000	**temp (90%)**
	200 - 1000	**temp (90%)**
PRD-1	200 - 3000	**none**
	200 - 1000	**none**
Cryptosporidium	200 - 1000	**temp**

Since only one replicate was performed with *Giardia lamblia*, an analysis of variance was not performed. However, inactivation rates were much larger at 30° C than at 5° and 22° C, and there was a decline in inactivation rates going from 200 to 1000 mg/L TDS at 30° C. With the exception of PRD-1, which is typically quite resistant to environmental conditions, survival of all other organism groups was significantly affected by temperature. The temperature ranged in these trials from 5° to 30° C, and survival was inversely related to temperature in cases where it was significant. However, significance of temperature on survival of DNA coliphage was significant to only the 90% level, meaning the effect was not as obvious as those with temperature significant to the 95% level. TDS was a significant variable for enterococci and F+ RNA, but only when considering the high TDS concentration of 3000 mg/L. In the case of F+ RNA coliphage, the interaction of TDS and temperature was also significant. This indicates an interdependence of the relative effect of each. Among the lower TDS concentrations evaluated, ranging from 200 - 1000 mg/L, TDS was not a significant variable for survival of any organism group.

Regarding trends observed in decline of the indicator organism populations, between the two populations of bacteria, fecal coliform had greater days for 2-log inactivation than enterococci, more notably at higher temperatures and in the lower TDS concentrations. This indicates perhaps a less pronounced temperature-based inactivation of fecal coliform than enterococci, especially in the less-saline water conditions. F+ RNA decline in these trials was generally more rapid on average than DNA coliphage, but they were fairly close in most cases. The difference did not appear dependent on temperature, but larger differences between the two phage populations were observed at 3000 mg/L TDS. Tables of mean days for 2-log decline for these organisms are shown in Appendix 5-A for inspection. Due to the stability of PRD-1 and thus low rates of inactivation even at higher temperatures, neither TDS nor temperature variation in the ranges examined for this study resulted in significant effects on survival for this bacteriophage. However, mean inactivation rates across all TDS concentrations did increase with respect to temperature for PRD-1. These rates ranged from -0.016 log/d at 5° to -0.070 log/d at 30° C.

For the evaluation of factors affecting survival in environmental water studies, ANOVA were used to compare predicted inactivation times or rates in both combined and separate tests. The results in terms of significant factors are summarized in the following tables for each

indicator organism group. The tables give water types and treatments included in each comparison, the factors compared for significance, and which of those were significant in affecting variability in predicted inactivation times. For the significant factors, each listing indicates that particular factor was significant independently, if interactions were also determined, those are spelled out as well. Table 21 summarizes the comparison results for fecal coliform.

Table 21. Significant variables affecting fecal coliform survival in natural water sources

Fecal Coliform

Water type	Treatment	Comparison parameters	Significant factors (95% unless noted)
surface & ground	raw and pasteurized	2-way: temp, treatmnt	**temp, treatmnt**
surface & ground	raw and pasteurized	3-way: temp, treatmnt, type	**temp, treatmnt, type**
surface & ground	raw only	2-way: temp, type	**temp**
surface water	raw and pasteurized	2-way: temp, treatmnt	**temp, treatmnt**
surface water	raw only	1-way: temp	**temp**
groundwater	raw and pasteurized	2-way: temp, treatmnt	**treatmnt**
groundwater	raw only	1-way: temp	**not sign. (but clear trend)**

Highlights to note are that temperature significantly affected decline in general, as when considering both water types combined. Treatment was also significant for fecal coliform, indicating that a reduction of background microbial populations via pasteurization increased survival (increased the number of days for 99% inactivation) of introduced fecal coliform bacteria. This was the case for both water types when considered independently as well as when combined. Water type was also a significant factor, but only when considering both raw and pasteurized conditions. In raw water only, there was not a statistically significant difference between inactivation in the two water types, but the mean for surface water was greater than in ground water. Also, in raw ground water trials, temperature was not a statistically significant

variable although there seemed to be a trend with increasing inactivation resulting from increasing temperatures.

Summarized results of analyses for factors affecting enterococci survival are shown in Table 22. These tests revealed that temperature strongly affected enterococci inactivation in all comparative analyses. Also, when considering all the conditions at once, statistically significant interactions were noted, namely that water type and treatment effects were interrelated, and an interaction of all three variables of temperature, water type, and treatment existed. The treatment effect was more pronounced in surface water than in ground water, since pasteurization reduced die-off in surface water but not in ground water. In raw water of both types, the two types were significantly different, with faster inactivation in surface water, and this effect was related to temperature as well. In other words, the temperature effect was relatively more pronounced in surface water than ground water, or the differences between the two water types were greater at higher temperature and vice-versa.

Table 22. Significant variables affecting enterococci survival in natural water sources

Enterococci

Water type	Treatment	Comparison parameters	Significant factors (95% unless noted)
surface & ground	raw and pasteurized	2-way: temp, treatmnt	**temp**
surface & ground	raw and pasteurized	3-way: temp, treatmnt, type	**temp, treatmnt (90%), type-tmnt interact, 3-way interact**
surface & ground	raw only	2-way: temp, type	**temp, type, interatction (90%)**
surface water	raw and pasteurized	2-way: temp, treatmnt	**temp, treatmnt, interaction**
surface water	raw only	1-way: temp	**temp**
groundwater	raw and pasteurized	2-way: temp, treatmnt	**temp**
groundwater	raw only	1-way: temp	**temp**

Table 23. Significant variables affecting F+ RNA coliphage survival in natural water sources

F+ RNA coliphage

Water type	Treatment	Comparison parameters	Significant factors (95% unless noted)
surface & ground	raw and pasteurized	2-way: temp, treatmnt	**temp**
surface & ground	raw and pasteurized	3-way: temp, treatmnt, type	**temp, type (90%), 3-way interact (90%)**
surface & ground	raw only	2-way: temp, type	**temp, type (90%), interact (90%)**
surface water	raw and pasteurized	2-way: temp, treatmnt	**temp**
surface water	raw only	1-way: temp	**temp**
groundwater	raw and pasteurized	2-way: temp, treatmnt	**temp, treatmnt, interact**
groundwater	raw only	1-way: temp	**temp (90%)**

The summary of F+ RNA coliphage survival in reservoir and aquifer water sources shown above in Table 23 indicates that temperature significantly increased inactivation in all comparisons. Water type was significant to a lower level of stringency in both combined raw and pasteurized water sources, and in raw water only. Also, the three-way interaction of temperature, water type, and treatment was statistically significant to the 90% level. But differences due to water type and treatment were primarily due to the differences at 5° C, a consistent trend was not present. Pasteurization increased survival at 5° in ground water and decreased survival at 5° in surface water. Thus, while pasteurization produced a statistically-significant reduction in inactivation in ground water and did not in surface water, this trend was due to variability at low temperature and is of limited importance when considering our environment of concern. This may be inferred from examining mean values for 2-log decline in Appendix 5. Inactivation was more rapid for the RNA coliphage in raw ground water than in surface water. But while survival was less on average in ground water compared to surface water in both raw and combined raw and pasteurized analyses, this effect was due to differences at 5° more so than at the higher temperatures of concern to the Florida subsurface. This may also be inferred from examining mean 2-log inactivation days from Appendix 5.

Significant factors in DNA coliphage decline are summarized in Table 24.

Table 24. Significant variables affecting DNA coliphage survival in natural water sources

DNA coliphage

Water type	Treatment	Comparison parameters	Significant factors (95% unless noted)
surface & ground	raw and pasteurized	2-way: temp, treatmnt	**temp, treatmnt**
surface & ground	raw and pasteurized	3-way: temp, treatmnt, type	**temp, treatmnt, type (90%), type-trmnt interact**
surface & ground	raw only	2-way: temp, type	**temp**
surface water	raw and pasteurized	2-way: temp, treatmnt	**temp, treatmnt**
surface water	raw only	1-way: temp	**temp**
groundwater	raw and pasteurized	2-way: temp, treatmnt	**temp**
groundwater	raw only	1-way: temp	**temp**

As with other organisms, higher temperature consistently resulted in statistically significant decreases of 2-log inactivation times for DNA coliphage. Also, pasteurization resulted in statistically significant increases in inactivation times. When evaluating water type and treatment effects as well (3-way test), all three variables were statistically significant (water type only to the 90% level) along with the interaction of water type and treatment. But, when considering only raw water samples, temperature was statistically-significant ($P < 0.05$), and water type and the interaction were not. Due to this observation and from looking at the mean 2-log inactivation times in Appendix 5, it is apparent that the differences due to water type that were statistically significant in a 3-way ANOVA with both pasteurized and raw water were due to differences in the pasteurized water samples between water types. Independent tests revealed that treatment had more of an effect on inactivation times in surface water than in ground water.

Table 25 shows significant factors for variability in first-order inactivation rates (in $\log_{10}$ decline per day) for PRD-1. Inactivation rates were significantly greater with increasing temperature on average, but really this effect was statistically significant due to behavior in surface water and not in ground water. Inactivation was on average more rapid in raw vs. pasteurized water, but this effect was not statistically significant. Water type was a significant factor also, with much more rapid inactivation in surface water than in ground water.

A large difference was observed in inactivation rates between the first and second replicate in the pasteurized Bill Evers reservoir water source for PRD-1, along with the other organisms' inactivation in these water samples. Naturally, other variables present in the water sources, and unknown differences between batches of seed organisms, could cause variability not attributable to the examined causes.

Table 25. Significant variables affecting PRD-1 survival in natural water sources

Water type	Treatment	Comparison parameters	Significant factors (95% unless noted)
surface & ground	raw and pasteurized	2-way: temp, treatmnt	**temp (90%)**
surface & ground	raw and pasteurized	3-way: temp, treatmnt, type	**temp, type**
surface & ground	raw only	2-way: temp, type	**temp, type, interaction**
surface water	raw and pasteurized	2-way: temp, treatmnt	**temp (90%)**
surface water	raw only	1-way: temp	**temp**
groundwater	raw and pasteurized	2-way: temp, treatmnt	**none**
groundwater	raw only	1-way: temp	**none**

For the natural water sources, temperature had the most consistently noticeable effect for all indicator organisms. However, the other factors, when compared across the different organisms, did not present trends that were as obvious. The effect of pasteurization was significant in a number of cases, but did not seem to be more important for the bacteria over the viruses, or vice versa. However, in general there was a least some decrease of inactivation in the pasteurized water samples compared to raw water, even in cases where the difference was not statistically significant. When considering only raw, unpasteurized water, water type was significant for the enterococci, F+ RNA coliphage, and PRD-1, but not for fecal coliform or DNA coliphage.

In raw water, fecal coliform, enterococci, DNA coliphage, PRD-1 and *Cryptosporidium* all experienced greater inactivation in surface water than in groundwater. In contrast, RNA coliphage and Giardia experienced greater inactivation in ground water than surface water. In

these raw water experiments, water type (surface versus ground water) was a statistically significant variable. Overall, the most consistently important determinant of survival from these experiments was shown to be temperature. Temperature ranges for these trials included 5° C, which is much colder than seen in subtropical ground water environments such as the Floridan aquifer system. Also, frequently when the average days for 2-log decline were categorized by temperature, the mean inactivation days were closely grouped at 22° and 30°, and the mean for 5° was set apart. This can be seen from ANOVA results in Appendix 6 for each organism. Specifically, if considering mean days for 2-log inactivation in raw surface water and raw ground water as independent categories, this noticeable grouping by temperature is obvious for all the organisms in both water types except for fecal coliform in ground water and PRD-1. Thus, inactivation rates in the subsurface will likely be more rapid in higher temperatures observed in subtropical environments like Florida when compared to other areas of the world that are much colder.

While observations on the factors which impact survival of these indicator microbes are useful for understanding possible processes in the environment, the actual periods necessary to allow a certain concentration to die out and rates of decline are very important for gauging the risk of ground water contamination or injection of harmful microorganisms. Naturally, the most interest lies with survival and inactivation in water conditions as would be found in the environments of concern. Figures in the RESULTS section depict days predicted for 2-log decline in raw water sources for each organism. These figures provide a summary picture of survival in raw water at 22° and 30° C, temperatures that would be observed in subtropical aquifers like the Floridan aquifer. Important trends are visible in these figures. For both bacteria groups, DNA coliphage, and PRD-1, inactivation was more rapid overall in surface water than in ground water at these temperatures. While the same cannot be said for F+ RNA coliphage, averaged periods for inactivation are similar and low between the two water types, such that the difference is nearly indistinguishable.

At these temperatures, fecal coliform and enterococci inactivation seem to be similar in Figures 2 and 3. From Tables 6 and 8, estimated days for 2-log decline are similar for these two bacteria as well. For fecal coliform, 2-log inactivation was predicted over periods on the order of 2 - 6 weeks in ground water and 1 - 2 weeks in surface water; enterococci predictions ranged slightly less at around 1 - 5 weeks in ground water and about 1 - 2 week in surface water sources.

A comparison of the two types of coliphage reveals that DNA coliphage were much hardier in the conditions evaluated, as Figures 4 and 5 show. F+ RNA had the shortest periods for 2-log decline, thus the most rapid inactivation in these experiments. At subtropical temperatures, 2-log inactivation in these water sources both surface and ground water) would be predicted in 1 week or less for F+ RNA coliphage. DNA coliphage results, on the other hand, indicated 2-log inactivation over periods on the order of 2 - 6 weeks in both water types.

As expected, PRD-1 in these conditions more indicative of actual conditions was the most stable of the organisms evaluated. As can be seen in Figure 6, larger differences between the two ground water sites were observed than for the indicator organisms, but results in surface water were similar. Also, inactivation in surface water was much more rapid on average than in ground water. Table 14 in the RESULTS section shows predicted periods for inactivation for PRD-1, but even in these more harsh conditions inactivation during the experiments was limited and predicted periods for 2-log decline are well outside the experimental duration in some cases. For instance, periods for 2-log decline in Avon Park well water were predicted at up to 6 months at 22° or 30° based on observed inactivation rates over 28 days. Predicted durations were shorter in other water sources, and were within experimental durations for the surface water at 2 -3 weeks.

If the parasite inactivation rates are extrapolated to 99% inactivation, between 10 to over 200 days and 24 to over 200 days would be needed for 99% inactivation of *Cryptosporidium* and *Giardia*, respectively, when combining results from both water types. However, these long predicted periods for decline should be defined more accurately since they are an extrapolation of inactivation kinetics from the 25 day experiments. Also, the *Giardia* data are somewhat suspect due to some other reasons. One, as stated previously, the test used for *Giardia* cyst survival in these experiments does not truly reflect its viability and may thus not be a very accurate determination of its actual longevity. Also, due to the need for staining cysts in suspension and the need for very high concentrations of cysts to enable microscopic viewing, small volumes of test water (< 1 ml) were mixed with *Giardia* cyst suspensions. Thus, these small volumes may not have been representative of the conditions in bulk water volumes.

Intestinal parasite inactivation rates (*Cryptosporidium* and *Giardia*) were generally lower than those of the indicator organisms. For ground water survival, very low rates were observed for both parasites at 5° C. Table 26 compares inactivation rates of the 7 groups of organisms in

ground water at 22° and 30° C. At water temperatures more representative of the Floridan aquifer system, *Cryptosporidium* inactivation rates were much slower than the bacterial indicators in the Avon Park samples at 22° C, but were more similar (although slightly less) to those of the two bacterial indicators in Lake Lytal Park well water at 22° and in both ground water sources at 30° C. *Giardia* inactivation rates were about ½ those of bacterial indicators in ground water at 22° and 30° C, except they were fairly similar in Lake Lytal Park water at 30° C. With the exception of Avon Park water at 22° C, inactivation rates of the two parasites were fairly similar in ground water at these temperatures.

Table 26. First-order inactivation rates of all organism groups in ground water at 22° and 30° C

Avon Park 22° C		Lake Lytal Park 22° C		Avon Park 30° C		Lake Lytal Park 30° C	
C. parvum	-0.0010	PRD-1	-0.027	PRD-1	-0.015	PRD-1	-0.045
PRD-1	-0.017	*G. lamblia*	-0.030	*G. lamblia*	-0.098	*G. lamblia*	-0.11
G. lamblia	-0.040	*C. parvum*	-0.042	*C. parvum*	-0.11	*C. parvum*	-0.12
DNA coliphage	-0.064	enterococci	-0.062	DNA coliphage	-0.13	enterococci	-0.13
fecal coli	-0.10	fecal coli	-0.065	fecal coli	-0.17	fecal coli	-0.15
enterococci	-0.16	DNA coliphage	-0.072	enterococci	-0.25	DNA coliphage	-0.15
F+ RNA phage	-0.51	F+ RNA phage	-0.45	F+ RNA phage	-1.6	F+ RNA phage	-2.4

A similar comparison may be drawn for observed inactivation rates of these organisms in surface water at temperatures representative of Florida's subsurface. Table 27 shows first-order inactivation rates from (raw) surface water at temperatures of 22° and 30° C. At 22° C, *Giardia* inactivation was very low and similar between the two sites. *C. parvum* inactivation was also similar between the two sites at 22° C, and was about ½ that of PRD-1 and about $1/10^{th}$ of the inactivation rates of the bacterial indicators. Thus, these two bacterial groups were not good proxies for parasite inactivation in the surface water samples at the lower temperature. At 30° C, *Giardia* inactivation rates were still the lowest of the seven organisms, and were about half those of *C. parvum*. *C. parvum* rates were about ½ to 1/3 those of the bacteria, but similar to DNA coliphage and PRD-1. However, viruses are generally not used as indicators for parasites due to the vast difference in size and generally poor correlation of occurrence and survival.

Table 27. First-order inactivation rates of all organism groups in surface water at 22° and 30° C

Bill Evers 22° C		Clear Lake 22° C		Bill Evers 30° C		Clear Lake 30° C	
G. lamblia	-0.0050	*G. lamblia*	-0.0042	*G. lamblia*	-0.081	*G. lamblia*	-0.076
C. parvum	-0.045	C. parvum	-0.066	*PRD-1*	-0.15	*PRD-1*	-0.12
PRD-1	-0.10	*PRD-1*	-0.084	DNA coliphage	-0.17	DNA coliphage	-0.16
DNA coliphage	-0.12	DNA coliphage	-0.092	C. parvum	-0.20	C. parvum	-0.18
F+ RNA phage	-0.25	fecal coli	-0.17	F+ RNA phage	-0.63	fecal coli	-0.30
enterococci	-0.38	enterococci	-0.27	enterococci	-0.77	enterococci	-0.50
fecal coli	-0.42	F+ RNA phage	-0.94	fecal coli	-1.0	F+ RNA phage	-2.0

Since inactivation rates of similar organisms from a number of studies in environmental water were compiled for the literature review, results of these studies may be compared to those ranges of values. However, these comparisons should be considered only a general, gross comparison for a qualitative picture of how data from this report correlate to previously reported survival information on similar organisms, rather than a direct comparison. A direct comparison is by no means appropriate since information presented in the context of the survival review was derived from many disparate studies using different organisms and different conditions. More specifically, these reviewed studies all evaluated organisms that were not necessarily the same as those used here, which were isolates from environmental sources. The review pooled together data from studies on similar but not necessarily the same species or strains of bacteria and viruses for a more-broad scope. Also, each of the reviewed studies potentially employed different methods for culturing organisms, different experimental methods and conditions, different water types evaluated (and from different sources), potentially different viability analysis methods or media, and different data analysis and reporting of their results.

Nonetheless, comparisons for the sake of gauging the relative range of the data from this study with others are included here. Coliform bacteria in reviewed studies had inactivation rates ranging from about 0.01 to 0.1 log / d at temperatures of 0 - 10°, with a mean of 0.05; fecal coliform in raw water trials at 5° in the present study had inactivation rates of about 0.02 to 0.07 log / d, near the mean of reviewed studies, except for inactivation in Lake Lytal Park water at 5° which was more rapid at 0.14 log / d (Table 7). At higher temperature, reviewed rates ranged from 0.9 to 0.35 log / d (mean ~0.2) at 21-25° C, and rates from our study were at 0.1 in ground water and 0.3 log / d in surface water, both at 22°.

Enterococci inactivation rates observed in this study were also within the ranges of reviewed rates, and near the means of respective temperature ranges; the mean of reviewed rates

at 0° - 10° was 0.08 log/d, and most enterococci inactivation rates at 5° from this study were around 0.05 log / d, except in Lake Lytal Park water which was much lower at 0.01 log / d. At higher temperature, the mean from reviewed papers at 21 - 25° was 0.24 log / d, from our work at 22° C enterococci inactivation rates were about those of fecal coliform, 0.1 log / d in ground water and 0.3 log / d in surface water. For reference to some pathogenic bacteria, inactivation rates for *Salmonella* and *Shigella* spp. bacteria were near these for temperatures over 20° C.

Coliphage inactivation rates from reviewed studies as means for studies at each temperature range were about 0.03 log/d at 0 - 10°, 0.4 log/d at 21 - 25°, and 0.4 log/d at 26 - 30°. Almost all phage inactivation rates were from studies in ground water. The inactivation rates of DNA coliphage at 5° closely match the average from reviewed studies at about 0.02 to 0.03 log/d in both water types, while RNA coliphage rates at this temperature were faster at 0.075 to 0.1, but still within the range of observed studies of 0 - 0.1 log/d. At 22° C, RNA coliphage fell more in line with reviewed rates, with mean inactivation rates in both water types at 0.5 log/d, and DNA coliphage rates were much slower at about 0.07 to 0.1 log/d. At 30° temperatures, RNA coliphage inactivation was on the high side of the range from reviewed studies, at around 1 - 2 log/d with rates from reviewed studies ranging from 0.2 to 2.5 log/d. However, DNA coliphage inactivation was below the reviewed rates average and the lower bound of the range at about 0.15 log/d in both water types.

PRD-1 inactivation rates observed from the work described in this chapter were near those found in reviewed studies at low temperature (0 - 10° C), such that rates in this study were about 0.01 log/d in ground water and 0.04 log/d in surface water, while the mean of reviewed PRD-1 inactivation rates at low temperatures was 0.02 log/d. At temperatures around 21 - 25° C, rates from this study were below rates from reviewed studies, which ranged from 0.05 to 0.8 log/d (mean 0.32). Our results indicated PRD-1 inactivation rates at 22° were around 0.02 to 0.03 in ground water and about 0.1 log/d in surface water.

Interestingly, although our PRD-1 inactivation rates in ground water at 22° were lower than rates from reviewed studies, they were a good approximation to hepatitis A virus inactivation rates from two studies which evaluated its survival in ground water and ground water/soil microcosms (9, 19). At temperatures of 20 - 25° C, these HAV rates ranged from 0.015 to 0.14, with an average of 0.05. This comparison suggests that both PRD-1 and DNA coliphage survival from this study (at 22° C) could be indicative of hepatitis A survival. Others

have found that PRD-1 survival may be a good model for that of hepatitis A in ground water (3). Compiled poliovirus inactivation rates averaged somewhere in between RNA coliphage and DNA coliphage rates, at about 0.27 log/d at 21 - 25° C and about 1 log/d at 26 - 30° C.

It is important to make some observations about experimental variability between replicates of the same conditions for this study. Occasionally large differences in observed inactivation behavior between trials at the same condition sets were observed. Unfortunately, examination of the instances where differences were observed did not reveal consistent patterns or causes. In general, large differences between trials were more frequent in sterile TDS-temperature trials particularly or in pasteurized natural water sources. F+ RNA coliphage experiments were performed such that the two replicates were performed simultaneously; variability between replicates for these trials was much lower than for the other organisms. It is possible that differences in batches of seeded bacteria or viruses or bottle effects from the microcosm containers were largely responsible for observed variability in activation.

Variability was consistent in some cases. For instance, for both types of bacteria, observed inactivation behavior in the two trials at 3000 mg/L was vastly different at the higher temperatures. Also, organisms died off much more rapidly in the pasteurized Bill Evers reservoir water on the second trial, for all except the RNA phage which were tested in separate trials. Thus, for natural water sources, it appears differences in the microbial communities or compounds present may cause differences in the survival ability of a wide range of introduced microbes. Therefore, it is important to use caution in interpreting the results of this study for the context of survival of contaminant pathogens or indicators in the actual environment. If bottle effects or culturing and processing procedures can lead to the variability observed, similar or perhaps larger degrees of variability could be expected from survival of pathogens in the ground water environment where a multiplicity of additional factors that could affect survival may be present. One encouraging point though, is that qualitatively, variability was the least in raw reservoir or aquifer water sources, particularly at higher temperatures. Thus, it may be that the minor variations which caused variability of inactivation have a much greater impact in the relatively controlled, isolated conditions such as in TDS-temperature trials where natural factors controlling inactivation are absent.

The results of this study have several implications for ASR projects. First, if untreated surface water or waters containing fecal indicator or enteric pathogenic microorganisms are to be

used for storage, both the nature of the water, the native biological flora, and particularly the temperature during storage will influence how much attenuation of the microorganisms is to be expected. As the microorganisms migrate into the native ground water, the inactivation rate may change again. Storage over time and differential in temperatures between surface and ground waters will also produce a gradient of conditions for a range of inactivation rates.

Different microorganisms will survive at different rates. The protozoa appear to be the most robust and may thus survive the longest; the indicator bacteria and phage do not appear to be adequate predictors of the reduction of levels of these types of pathogens. For viruses, there are many different types that may have different survival rates. PRD-1 can be used as a good field tracer virus and is quite stable, but is not found naturally in contaminated waters. However, the DNA coliphage are much more robust and could be used to evaluate the attenuation of raw surface waters during storage. In any case, due to the variability observed in this study, and to questions surrounding differences in survival between the laboratory bench top and actual field conditions, site-specific monitoring of any injection sites with non-disinfected water would seem to be necessary, at least until attenuation of potential pathogens and/or indicators is better defined in particular circumstances.

The naturally occurring levels of fecal indicators in surface waters (see Table 3) suggest that anywhere from 99% to 99.9% inactivation would lead to non-detection. There had been much discussion of the storage time after injection lasting approximately 5 months (180 days) prior to recovery. Under this scenario, the $\log_{10}$ reductions that might be predicted for these microorganisms at 22^{o}C based on the surface water survival data are between 45 and 169 $\log_{10}$ for F+ RNA coliphage, 30 to 75 $\log_{10}$ for fecal coliform bacteria, 48 to 68 $\log_{10}$ for enterococci, 16 to 21 $\log_{10}$ for DNA coliphage; 8 to 12 $\log_{10}$ for *Cryptosporidium*, but less than 1 $\log_{10}$ for *Giardia.* Even greater reductions would be predicted for the higher temperatures. All of these levels of inactivation (except for *Giardia*) are theoretical and could not be measured in the field or laboratory.

REFERENCES

1. Alvarez, M. E., Aguilar, M., Fountain, A., Gonzalez, N., Rascon, O., and Saenz, D. 2000. Inactivation of MS-2 phage and poliovirus in groundwater. Canadian Journal of Microbiology. 46(2): 159-165.

2. APHA, AWWA, and WEF, *Standard Methods for the Examination of Water and Wastewater*. 18th ed, ed. Greenberg, A. E., Clesceri, L. S., and Eaton, A. D. 1992, Baltimore, MD.

3. Blanc, R. and Nasser, A. 1996. Effect of effluent quality and temperature on the persistence of viruses in soil. Water Science and Technology. 33(10-11): 237-242.

4. Campbell, A. T., Robertson, L. J., and Smith, H. V. 1992. Viability of Cryptospordium-Parvum Oocysts - Correlation of Invitro Excystation with Inclusion or Exclusion of Fluorogenic Vital Dyes. Applied and Environmental Microbiology. 58(11): 3488-3493.

5. Hsu, F. C., Shieh, Y. S. C., Vanduin, J., Beekwilder, M. J., and Sobsey, M. D. 1995. Genotyping Male-Specific Rna Coliphages by Hybridization with Oligonucleotide Probes. Applied and Environmental Microbiology. 61(11): 3960-3966.

6. Jenkins, M. B., Anguish, L. J., Bowman, D. D., Walker, M. J., and Ghiorse, W. C. 1997. Assessment of a dye permeability assay for determination of inactivation rates of Cryptosporidium parvum oocysts. Applied and Environmental Microbiology. 63(10): 3844-3850.

7. Kersters, I., Huys, G., VanDuffel, H., Vancanneyt, M., Kersters, K., and Verstraete, W. 1996. Survival potential of Aeromonas hydrophila in freshwaters and nutrient-poor waters in comparison with other bacteria. Journal of Applied Bacteriology. 80(3): 266-276.

8. Medema, G. J., Bahar, M., and Schets, F. M. 1997. Survival of Cryptosporidium parvum, Escherichia coli, faecal enterococci and Clostridium perfringens in river water: Influence of temperature and autochthonous microorganisms. Water Science and Technology. 35(11-12): 249-252.

9. Nasser, A. M. and Oman, S. D. 1999. Quantitative assessment of the inactivation of pathogenic and indicator viruses in natural water sources. Water Research. 33(7): 1748-1752.

10. Neumann, N. F., Gyurek, L. L., Gammie, L., Finch, G. R., and Belosevic, M. 2000. Comparison of animal infectivity and nucleic acid staining for assessment of Cryptosporidium parvum viability in water. Applied and Environmental Microbiology. 66(1): 406-412.

11. Sauch, J. F., Flanigan, D., Galvin, M. L., Berman, D., and Jakubowski, W. 1991. Propidium Iodide as an Indicator of Giardia Cyst Viability. Applied and Environmental Microbiology. 57(11): 3243-3247.

12. Schupp, D. G. and Erlandsen, S. L. 1987. A New Method to Determine Giardia Cyst Viability - Correlation of Fluorescein Diacetate and Propidium Iodide Staining with Animal Infectivity. Applied and Environmental Microbiology. 53(4): 704-707.

13. Slifko, T. R., Friedman, D., Rose, J. B., and Jakubowski, W. 1997. An in vitro method for detecting infectious Cryptosporidium oocysts with cell culture. Applied and Environmental Microbiology. 63(9): 3669-75.

14. Slifko, T. R., Huffman, D. E., Dussert, B., Owens, J. H., Jakubowski, W., Haas, C. N., and Rose, J. B. 2002. Comparison of tissue culture and animal models for assessment of Cryptospridium parvum infection. Experimental Parasitology. 101(2-3): 97-106.

15. Slifko, T. R., Huffman, D. E., and Rose, J. B. 1999. A most-probable-number assay for enumeration of infectious Cryptosporidium parvum oocysts. Applied and Environmental Microbiology. 65(9): 3936-41.

16. Sobsey, M. D., Shields, P. A., Hauchman, F. H., Hazard, R. L., and Caton, L. W. 1986. Survival and Transport of Hepatitis a Virus in Soils, Groundwater and Waste-Water. Water Science and Technology. 18(10): 97-106.

17. Toranzos, G. A. 1991. Current and possible alternate indicators of fecal contamination in tropical waters: a short review. Environmental Toxicology and Water Quality. 6: 121-130.

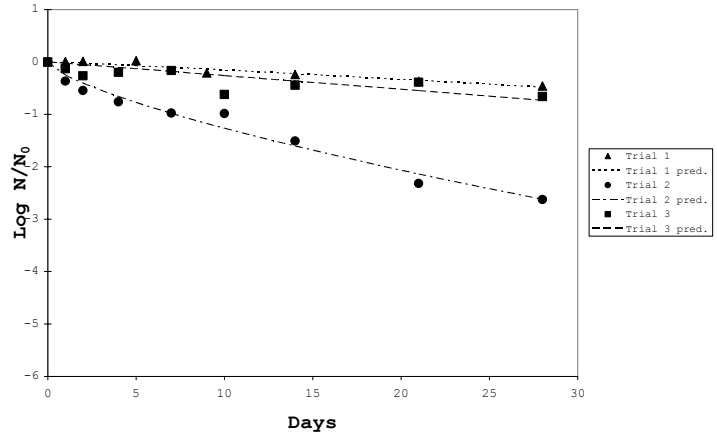
18. Yates, M. V. and Gerba, C. P. 1985. Factors Controlling the Survival of Viruses in Groundwater. Water Science and Technology. 17(4-5): 681-687.

19. Yates, M. V., Gerba, C. P., and Kelley, L. M. 1985. Virus Persistence in Groundwater. Applied and Environmental Microbiology. 49(4): 778-781.

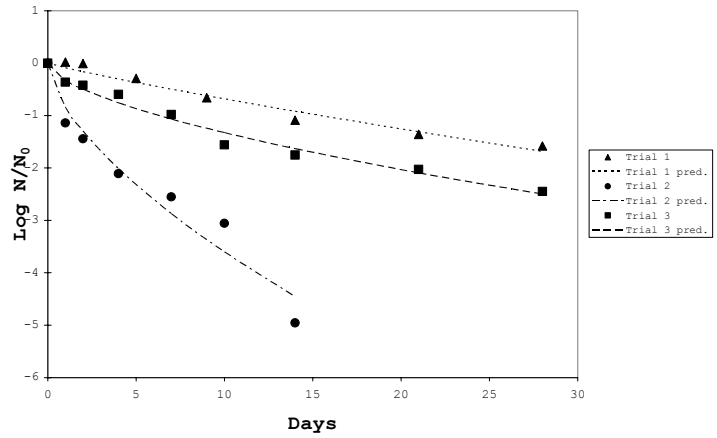
20. Yates, M. V., Stetzenbach, L. D., Gerba, C. P., and Sinclair, N. A. 1990. The Effect of Indigenous Bacteria on Virus Survival in Ground- Water. Journal of Environmental Science and Health Part a- Environmental Science and Engineering & Toxic and Hazardous Substance Control. 25(1): 81-100.

APPENDIX 1. Observed data plots and fitted model curves for TDS-temperature experiments

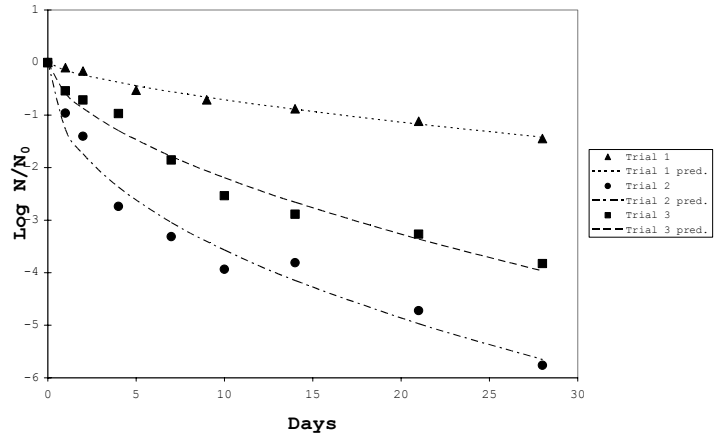
A. Fecal coliform TDS-Temperature experiments



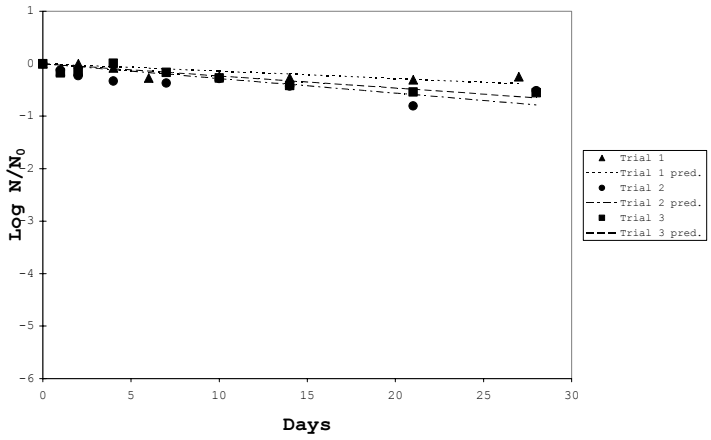
A. Fecal coliform ASW 200 mg/l, 5° C



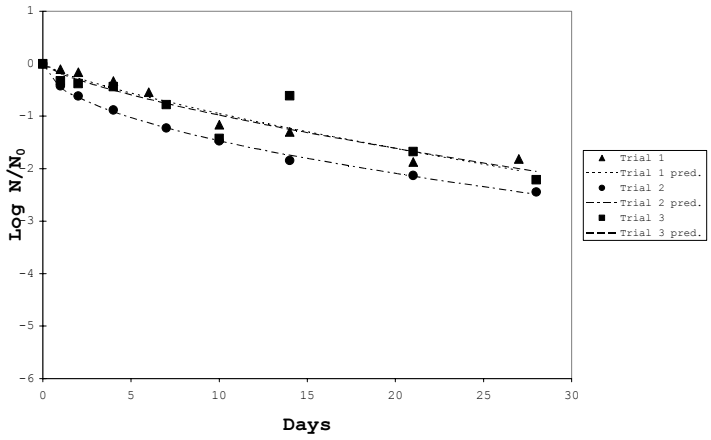
B. Fecal coliform ASW 200 mg/l, 22° C



C. Fecal coliform ASW 200 mg/l, 30° C



Fecal coliform ASW 500 mg/l, 5° C



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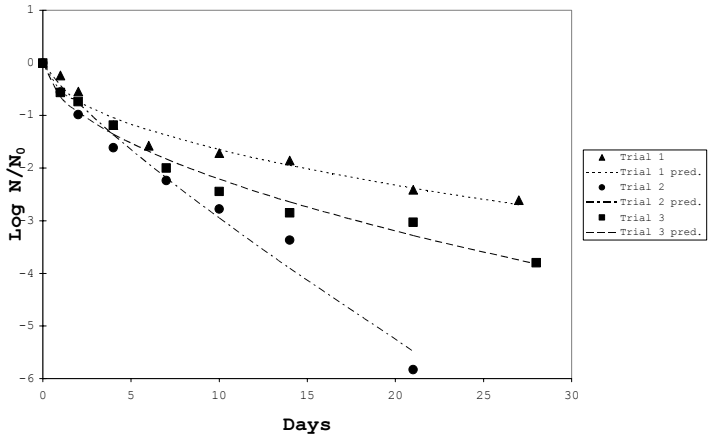

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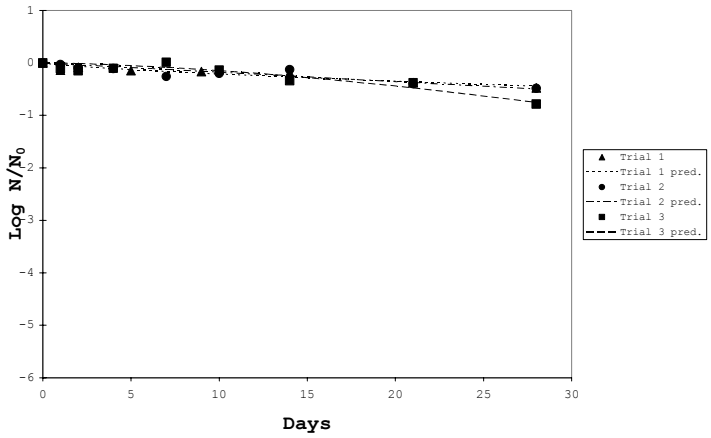
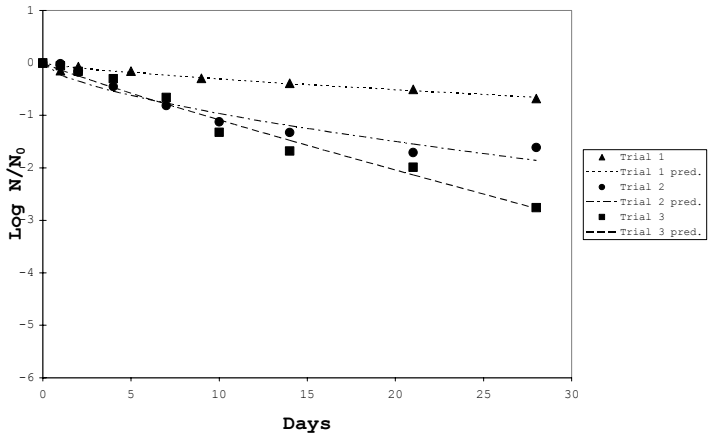
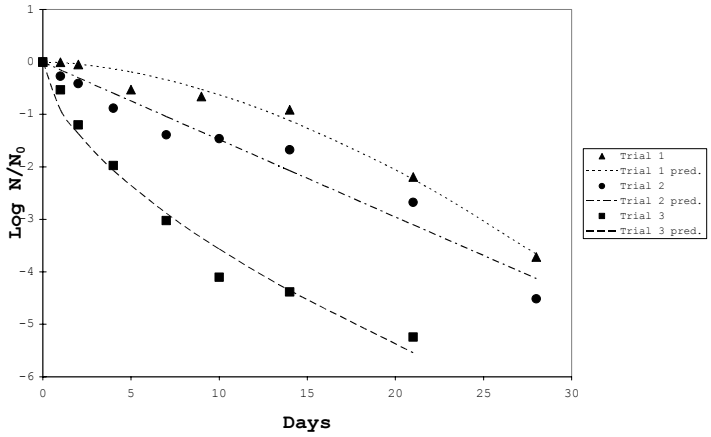


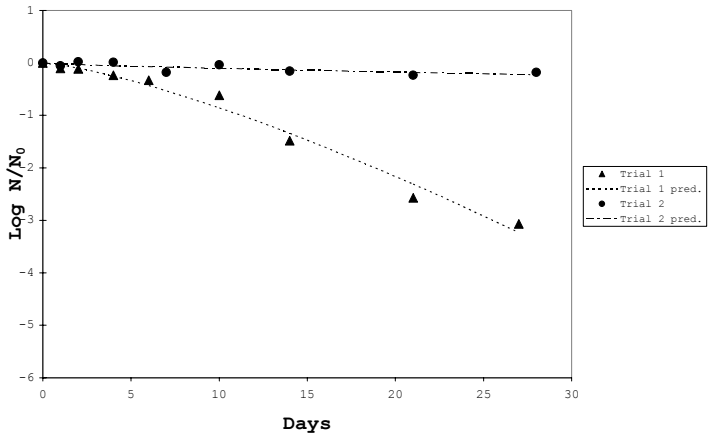
Fecal coliform ASW 1000 mg/l, 5° C



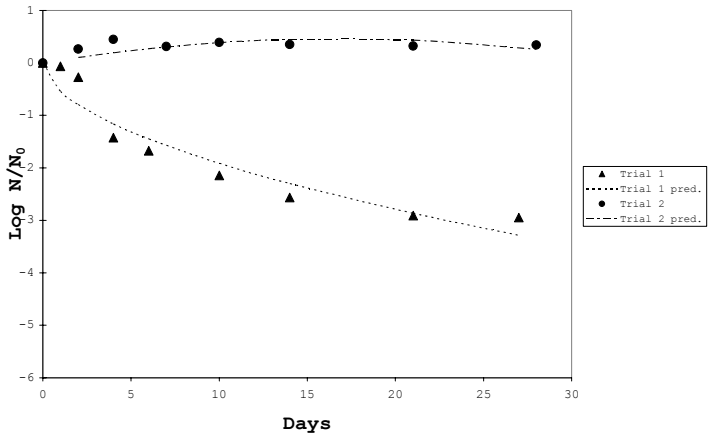
Fecal coliform ASW 1000 mg/l, 22° C



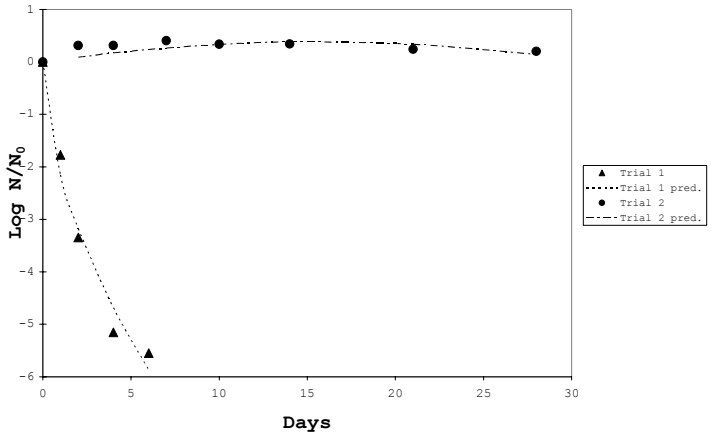
Fecal coliform ASW 1000 mg/l, 30° C



Fecal coliform ASW 3000 mg/l, 5° C

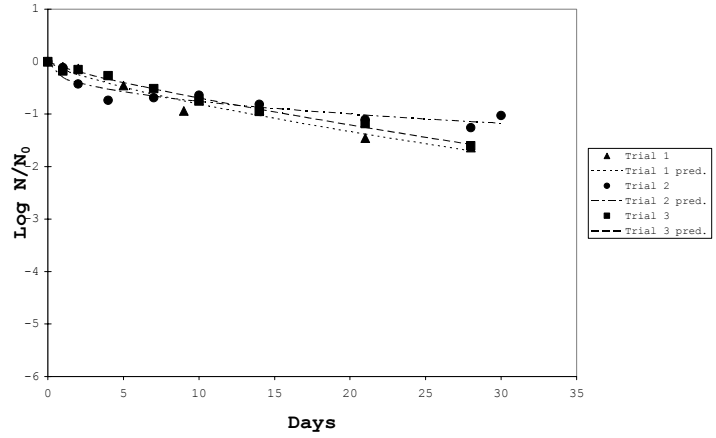


Fecal coliform ASW 3000 mg/l, 22° C

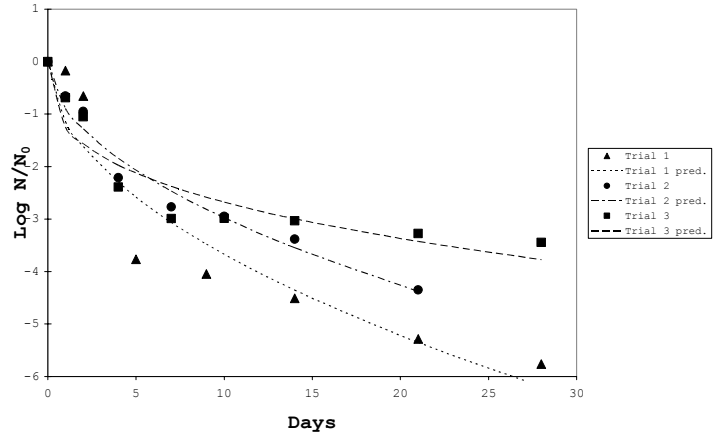


Fecal coliform ASW 3000 mg/l, 30° C

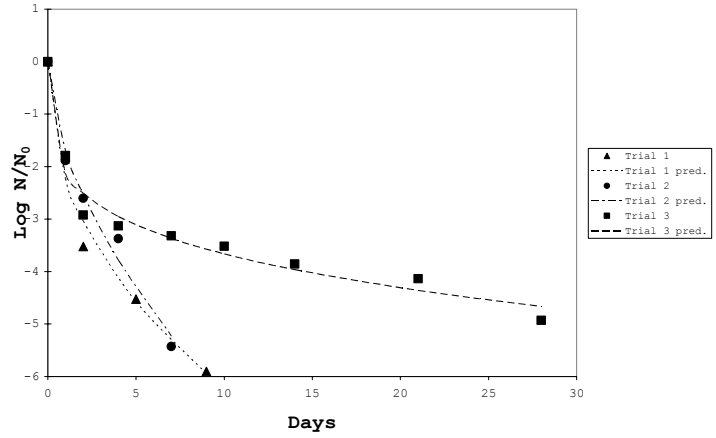
B. Enterococci TDS-temperature experiments



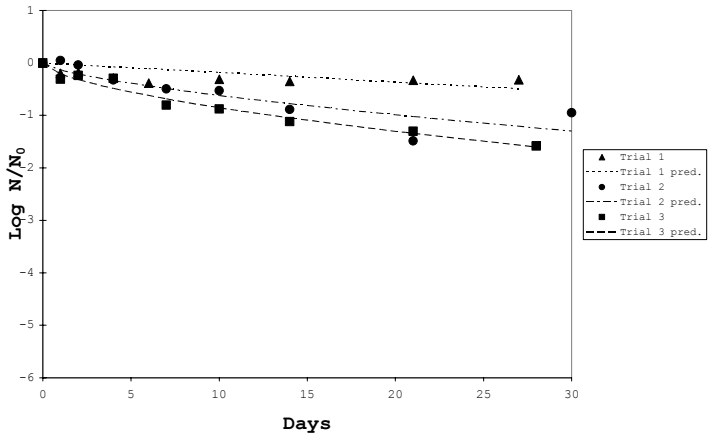
Enterococci ASW 200 mg/l, 5° C



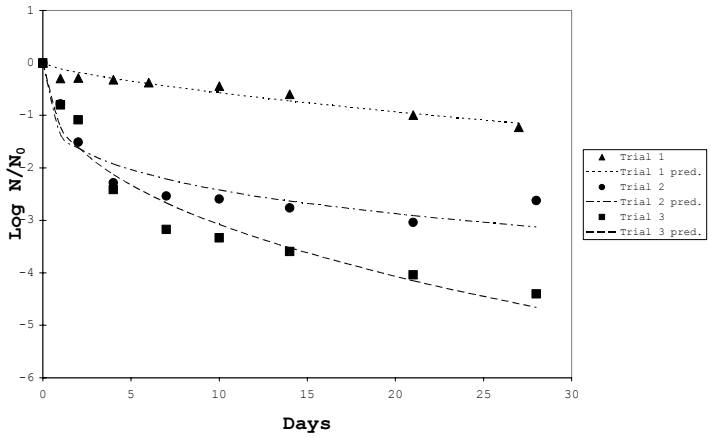
Enterococci ASW 200 mg/l, 22° C



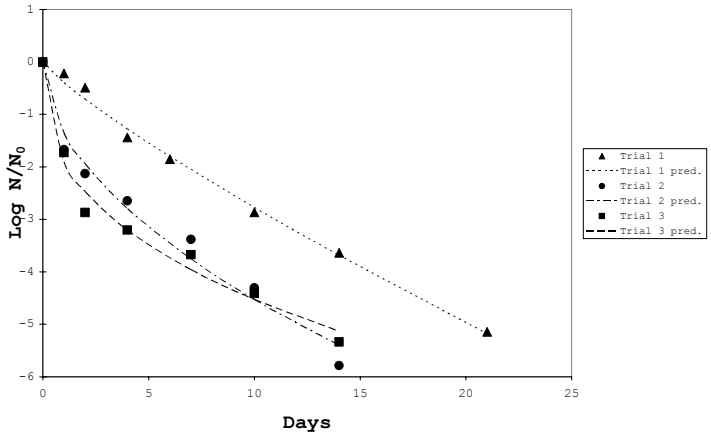
Enterococci ASW 200 mg/l, 30° C



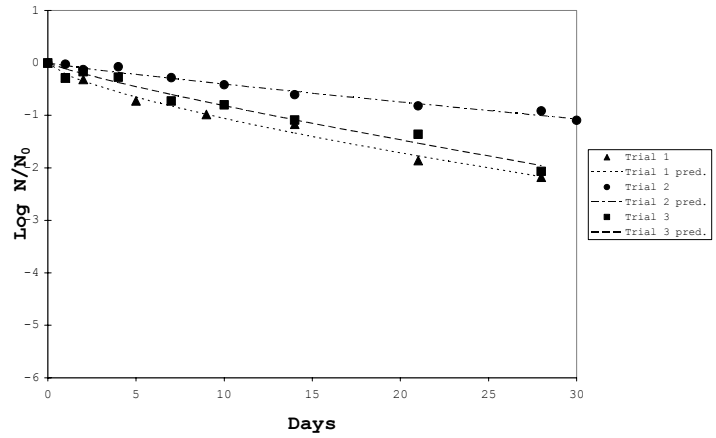
Enterococci ASW 200 mg/l, 5° C



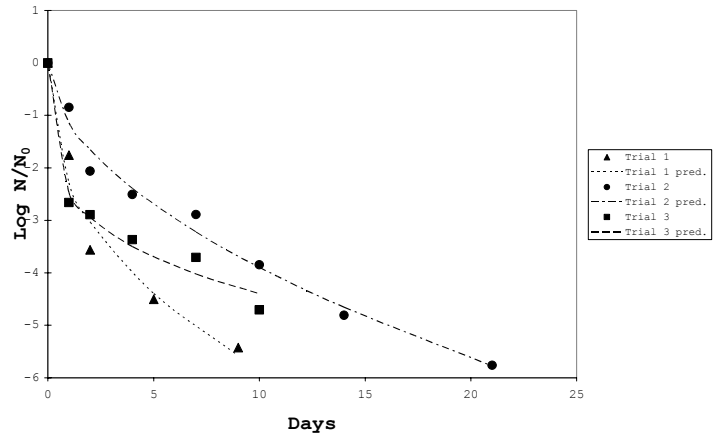
Enterococci ASW 500 mg/l, 22° C



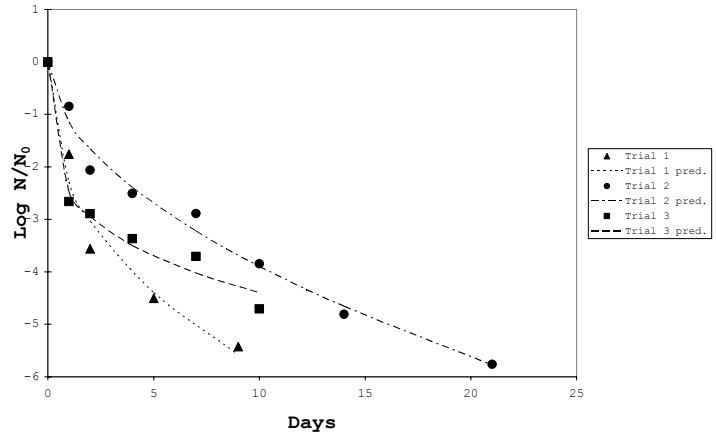
Enterococci ASW 500 mg/l, 30° C



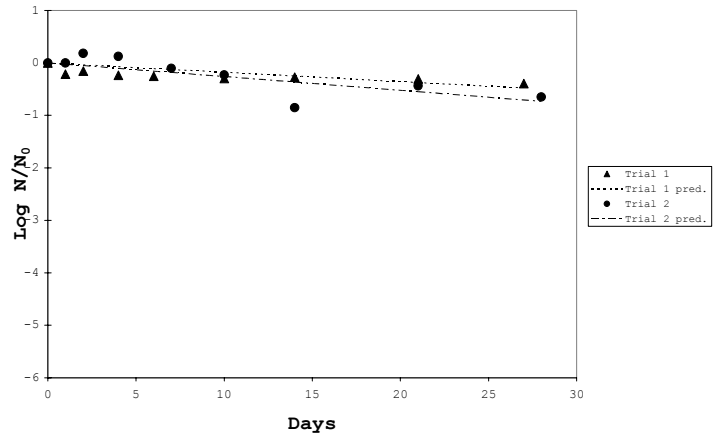
Enterococci ASW 1000 mg/l, 5° C



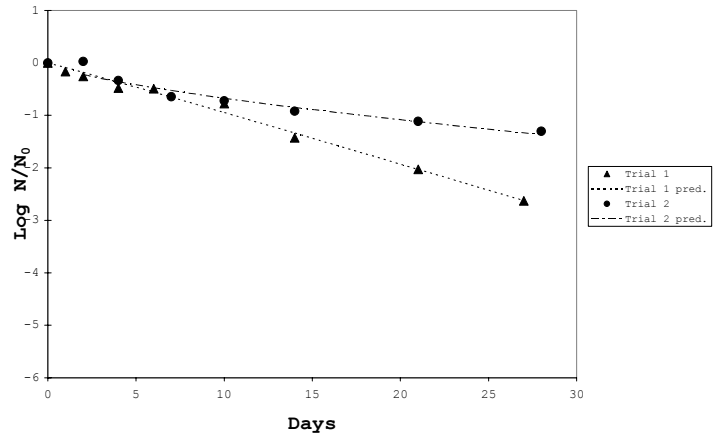
Enterococci ASW 1000 mg/l, 30° C



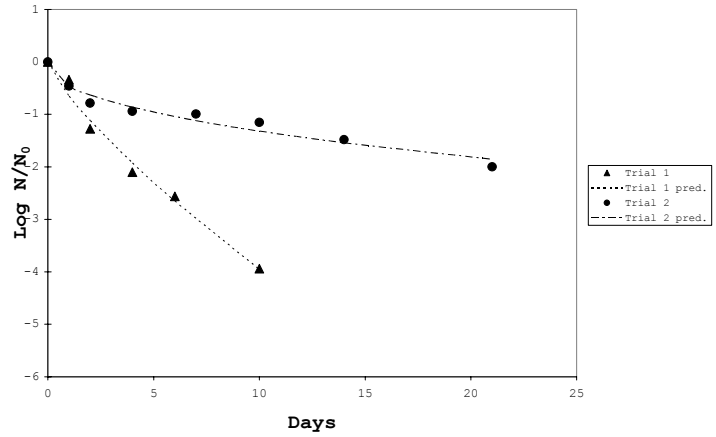
Enterococci ASW 1000 mg/l, 30° C



Enterococci ASW 3000 mg/l, 5° C

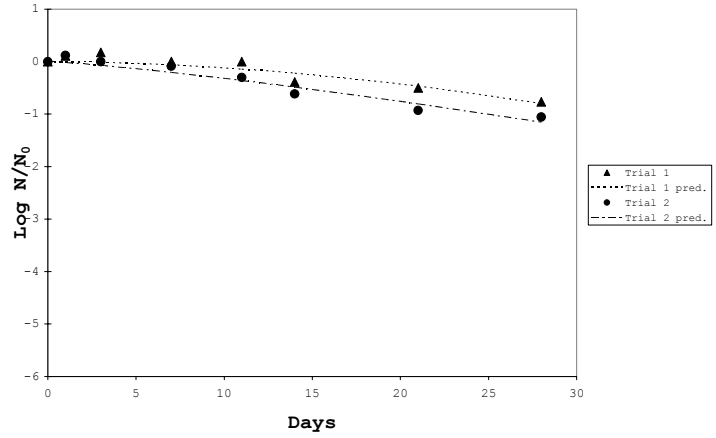


Enterococci ASW 3000 mg/l, 22° C

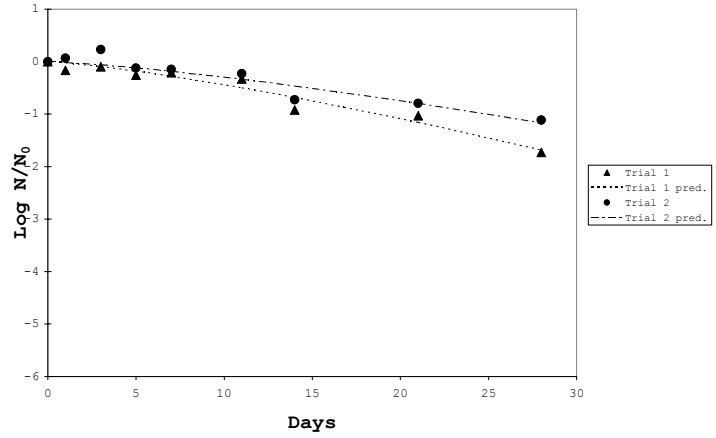


Enterococci ASW 3000 mg/l, 30° C

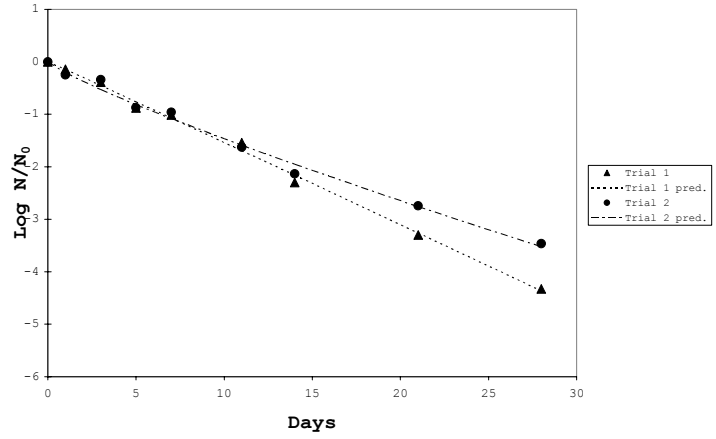
C. F+RNA coliphage TDS-temperature experiments



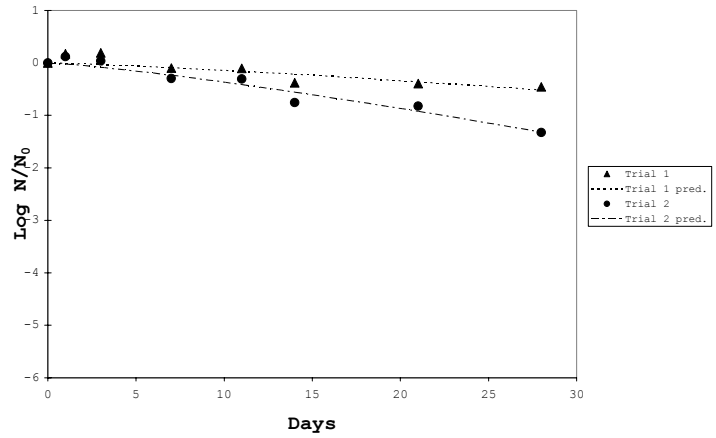
F+ RNA coliphage ASW 200 mg/l, 5° C



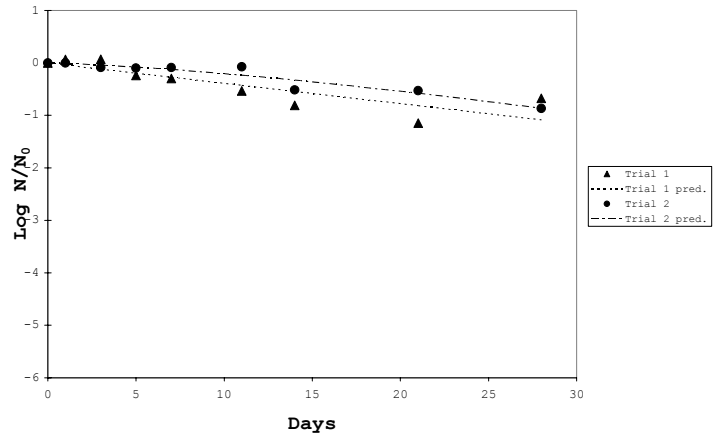
F+ RNA coliphage ASW 200 mg/l, 22° C



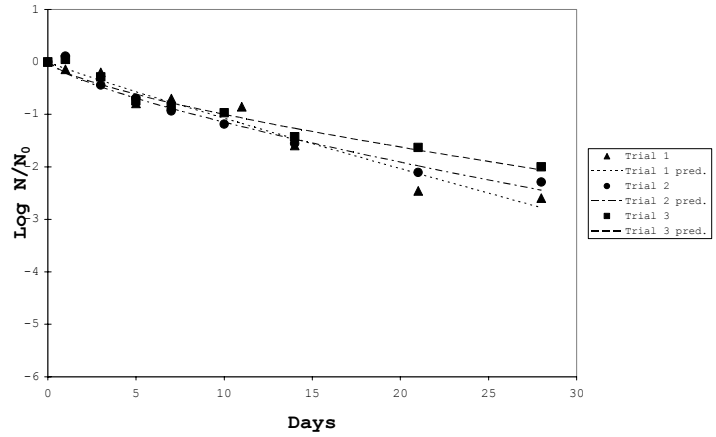
F+ RNA coliphage ASW 200 mg/l, 30° C



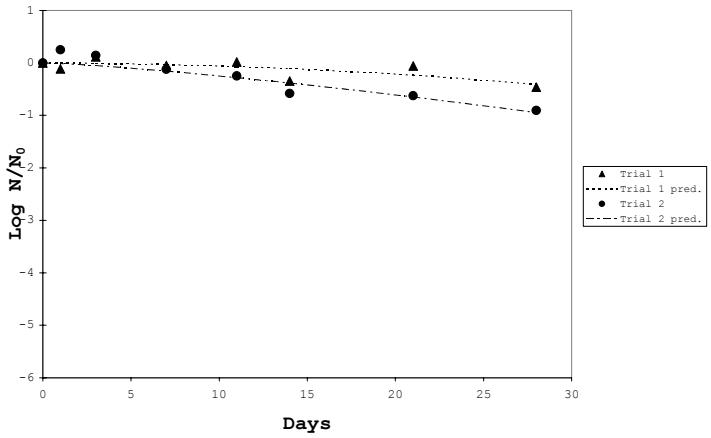
F+ RNA coliphage ASW 500 mg/l, 5° C



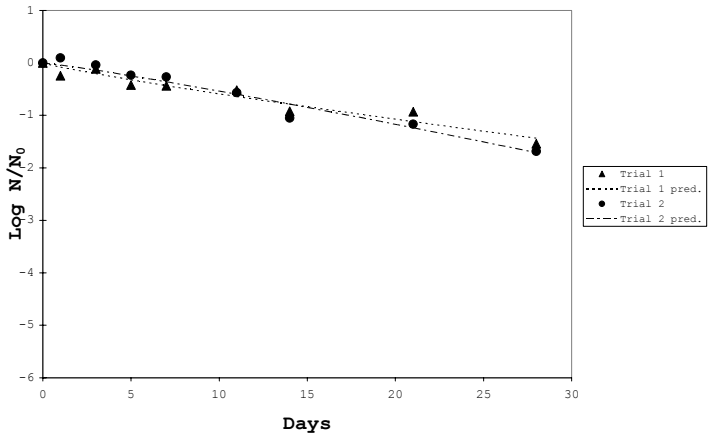
F+ RNA coliphage ASW 500 mg/l, 22° C



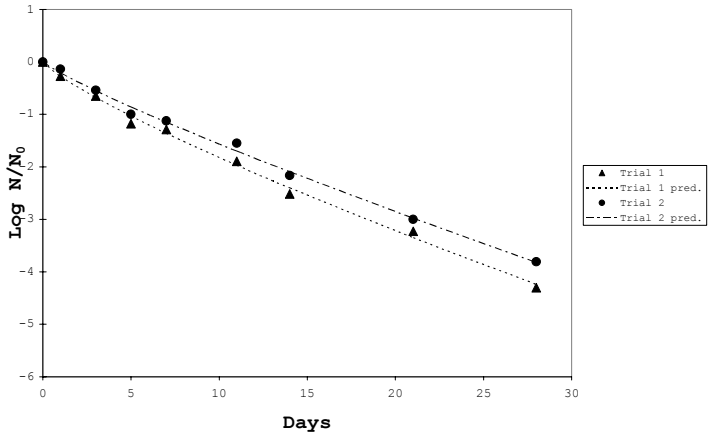
F+ RNA coliphage ASW 500 mg/l, 30° C



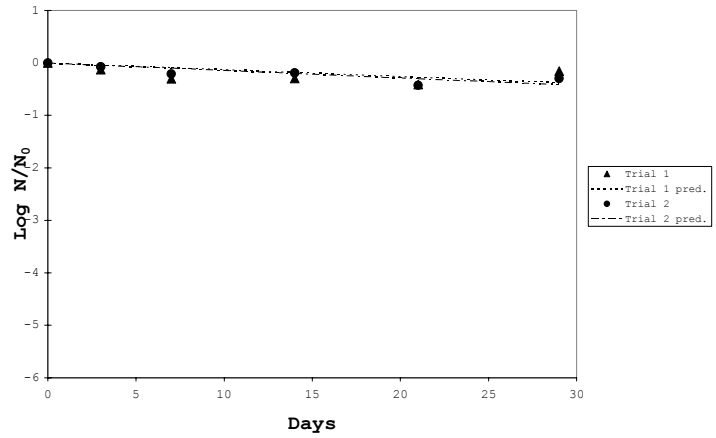
F+ RNA coliphage ASW 1000 mg/l, 5° C



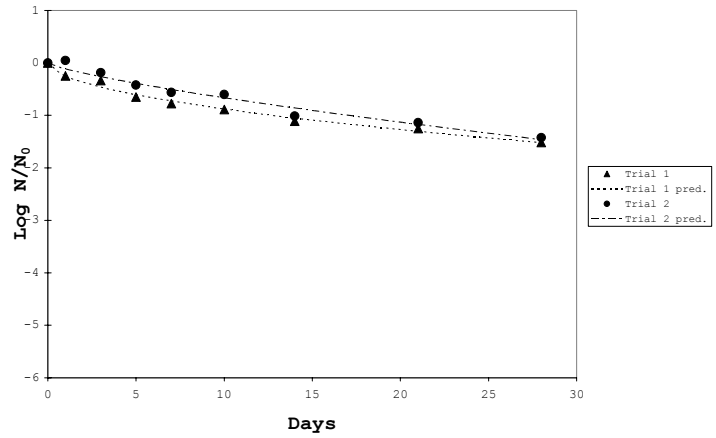
F+ RNA coliphage ASW 1000 mg/l, 22° C



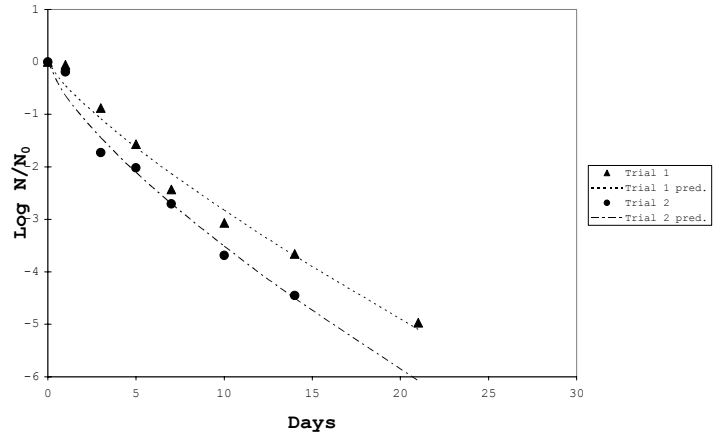
F+ RNA coliphage ASW 1000 mg/l, 30° C



F+ RNA coliphage ASW 3000 mg/l, 5° C

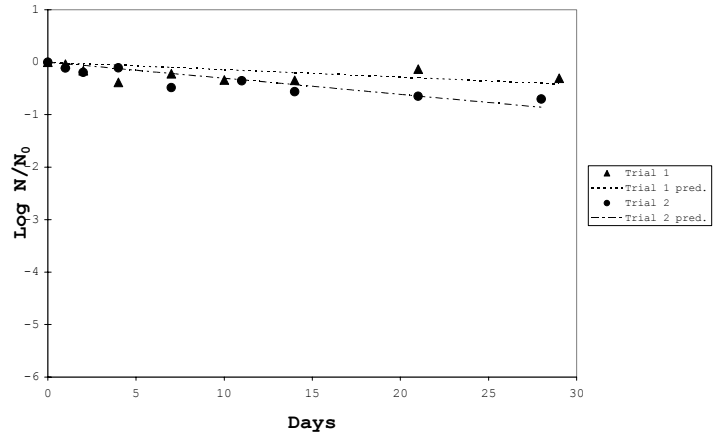


F+ RNA coliphage ASW 3000 mg/l, 22° C

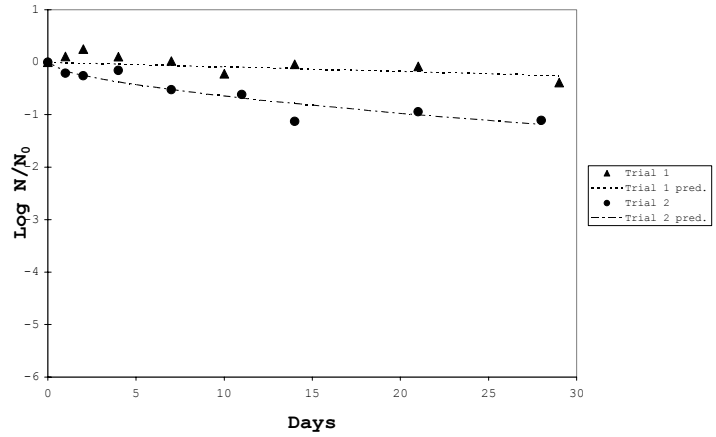


F+ RNA coliphage ASW 3000 mg/l, 30° C

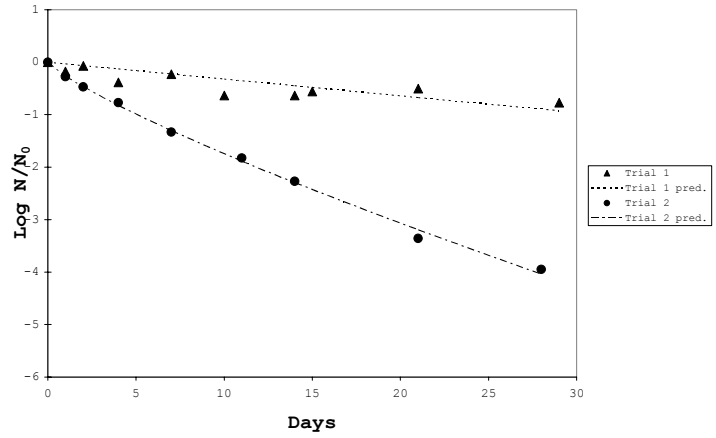
D. DNA coliphage TDS-temperature experiments



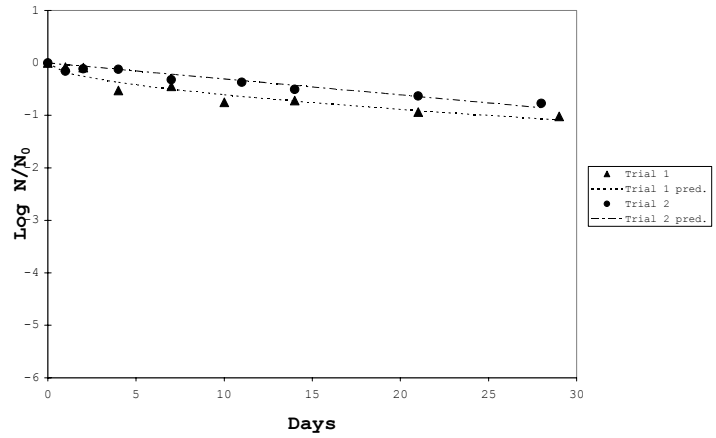
DNA coliphage ASW 200 mg/l, 5° C



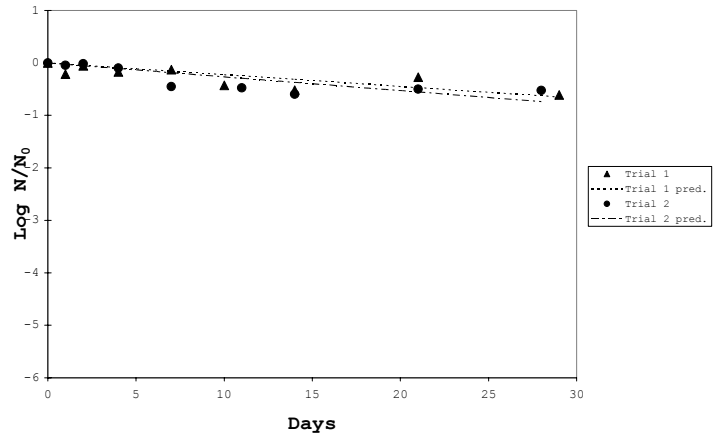
DNA coliphage ASW 200 mg/l, 22° C



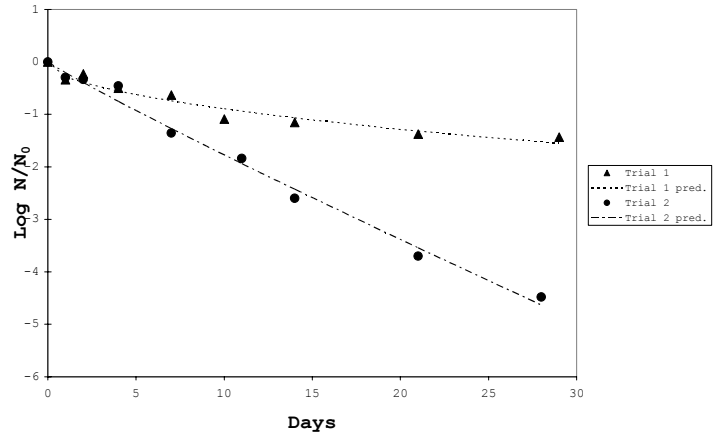
DNA coliphage ASW 200 mg/l, 30° C



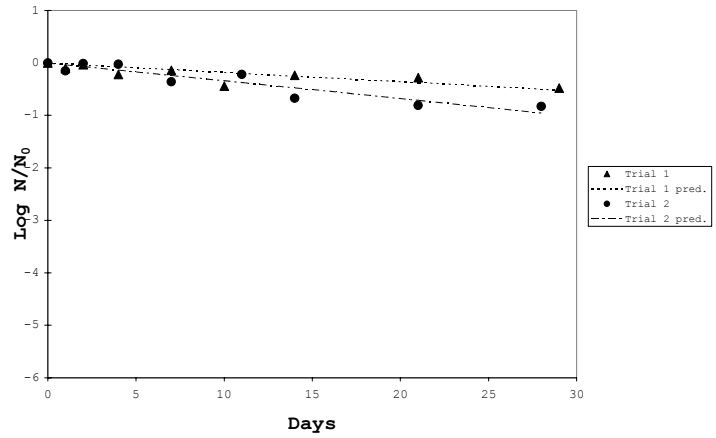
DNA coliphage ASW 500 mg/l, 5° C



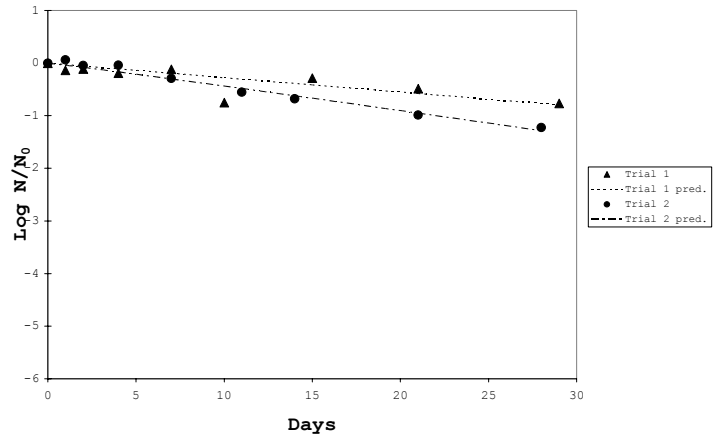
DNA coliphage ASW 500 mg/l, 22° C



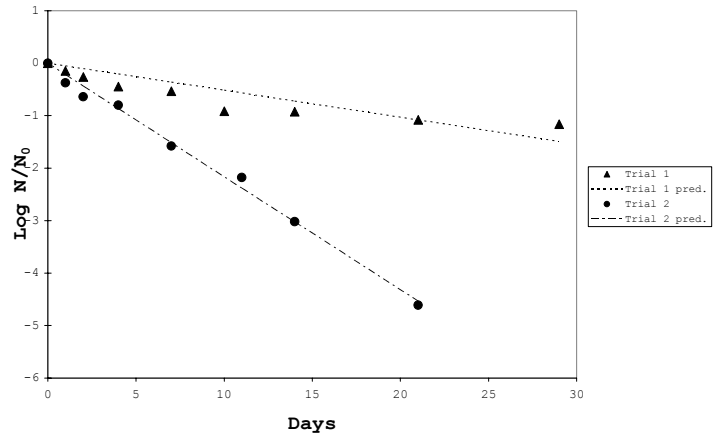
DNA coliphage ASW 500 mg/l, 30° C



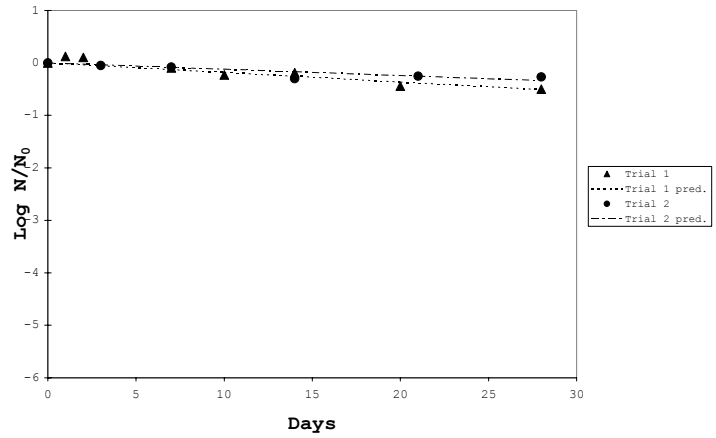
DNA coliphage ASW 1000 mg/l, 5° C



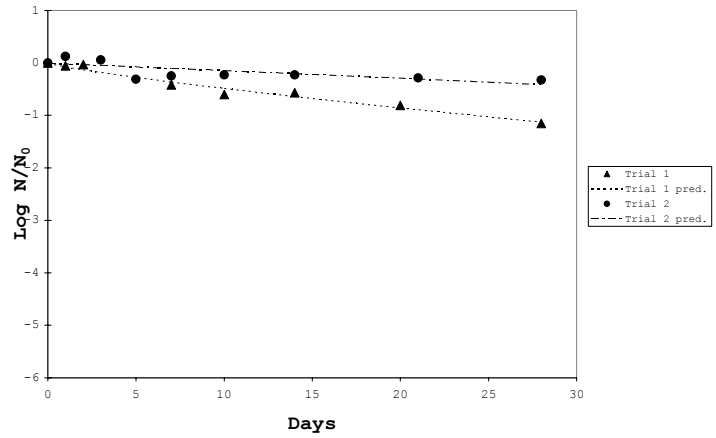
DNA coliphage ASW 1000 mg/l, 22° C



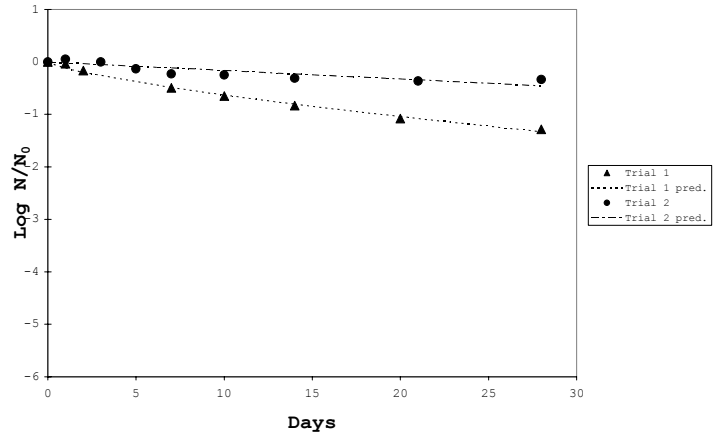
DNA coliphage ASW 1000 mg/l, 30° C



DNA coliphage ASW 3000 mg/l, 5° C

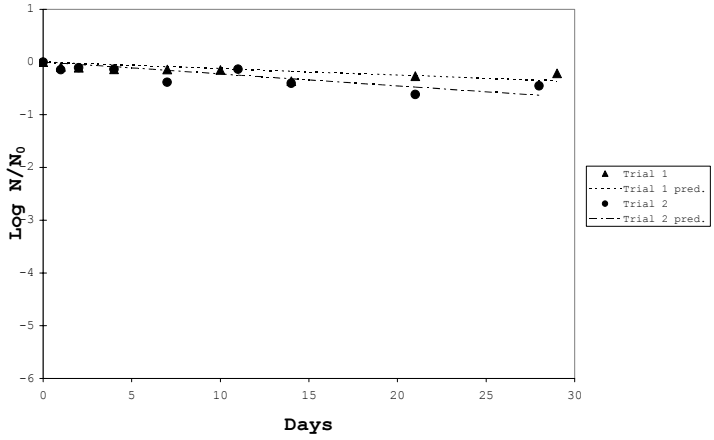


DNA coliphage ASW 3000 mg/l, 22° C

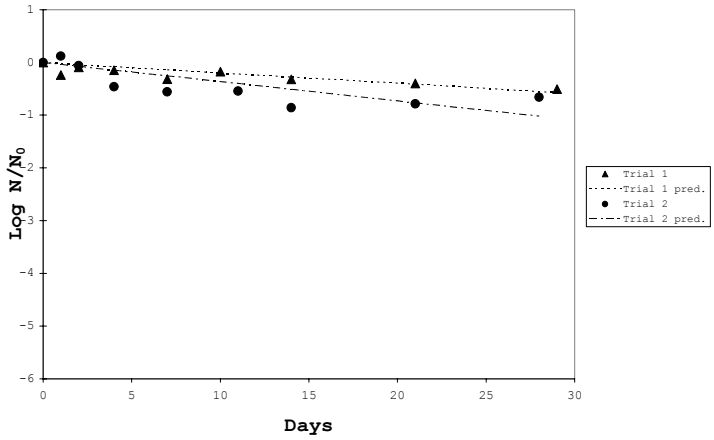


DNA coliphage ASW 3000 mg/l, 30° C

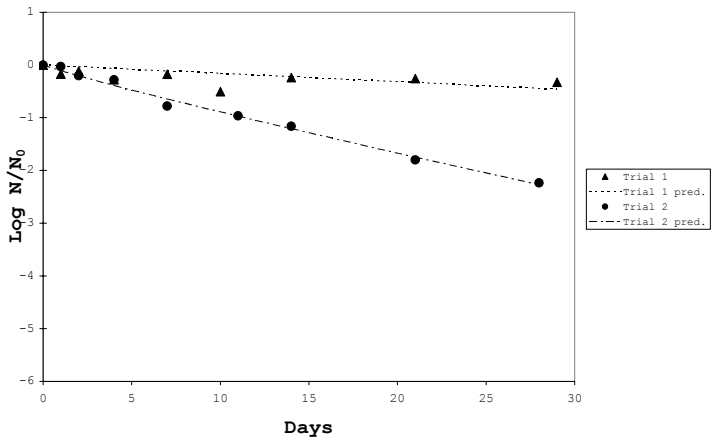
E. PRD-1 TDS-temperature trials



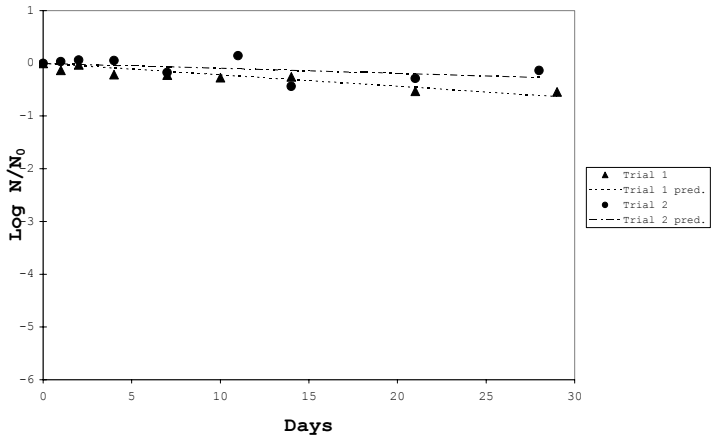
PRD-1 ASW 200 mg/l, 5° C



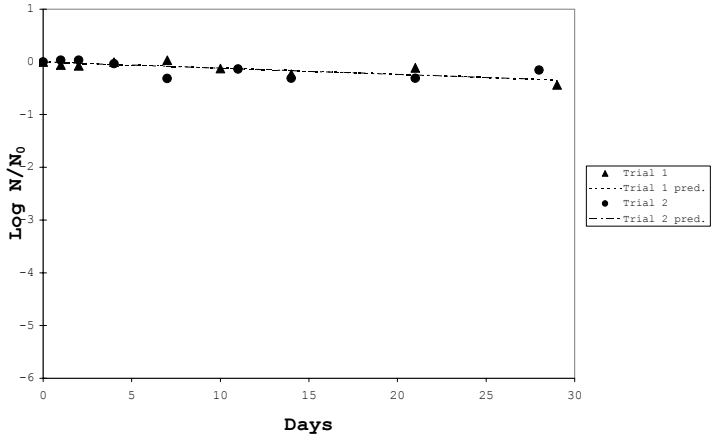
PRD-1 ASW 200 mg/l, 22° C



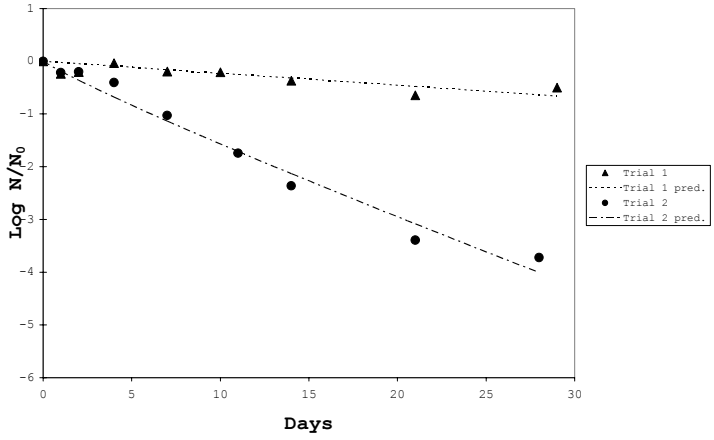
PRD-1 ASW 200 mg/l, 30° C



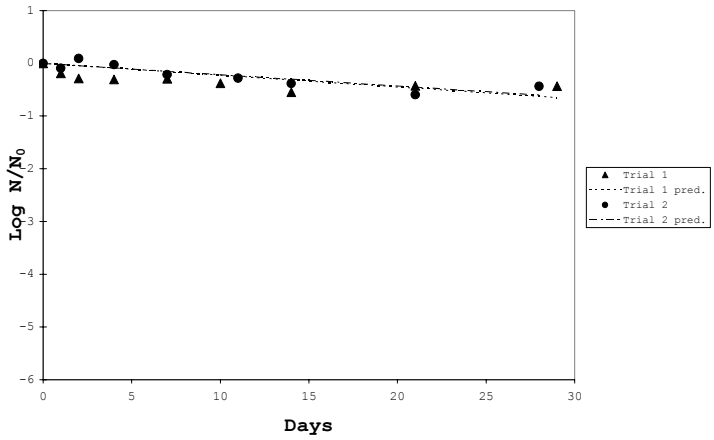
PRD-1 ASW 500 mg/l, 5° C



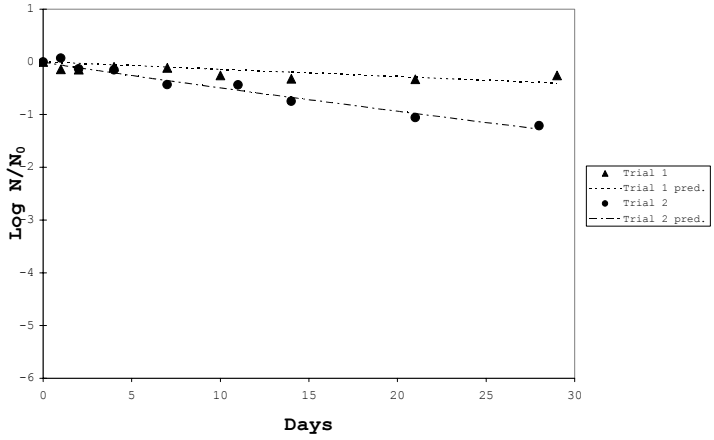
PRD-1 ASW 500 mg/l, 22° C



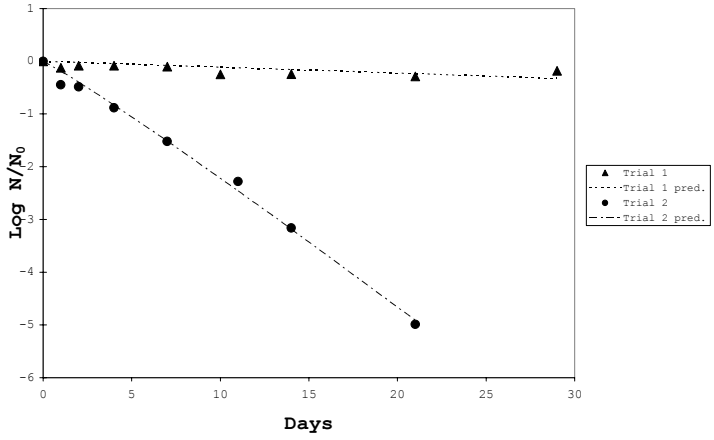
PRD-1 ASW 500 mg/l, 30° C



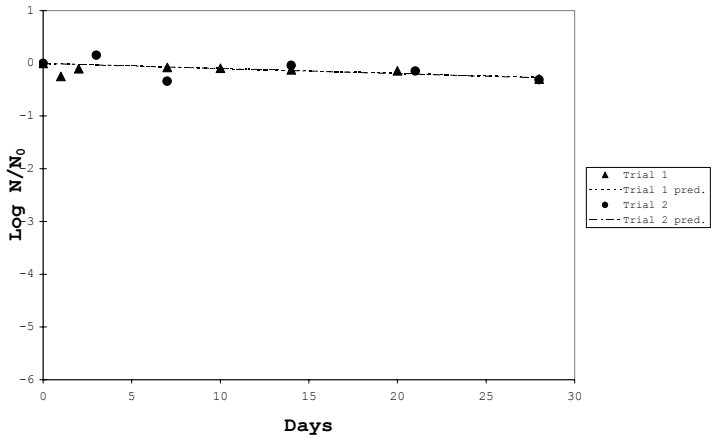
PRD-1 ASW 1000 mg/l, 5° C



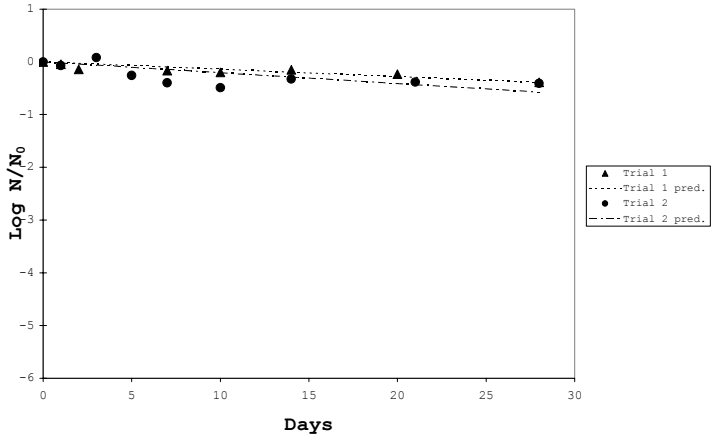
PRD-1 ASW 1000 mg/l, 22° C



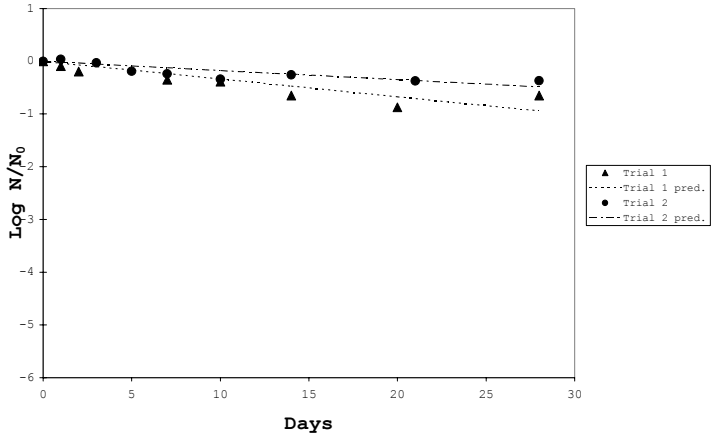
PRD-1 ASW 1000 mg/l, 30° C



PRD-1 ASW 3000 mg/l, 5° C

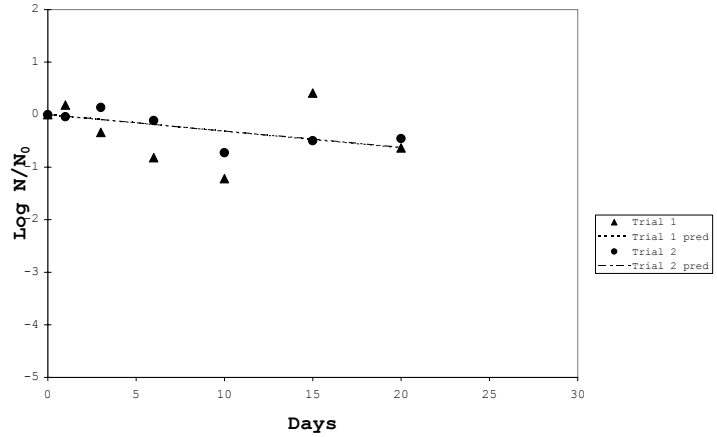


PRD-1 ASW 3000 mg/l, 22° C

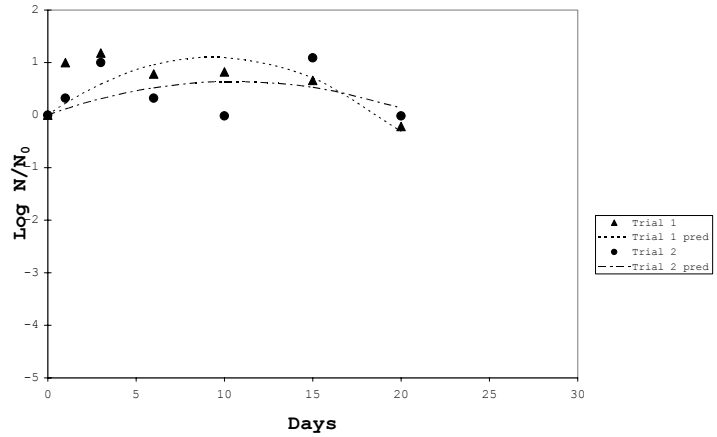


PRD-1 ASW 3000 mg/l, 30° C

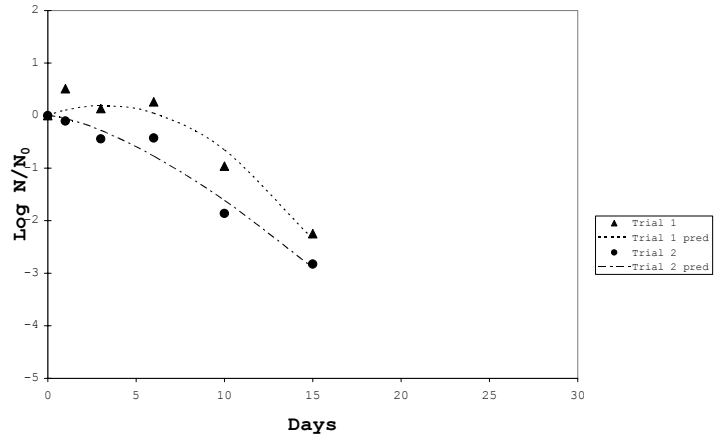
F *Cryptosporidium parvum* TDS-temperature trials



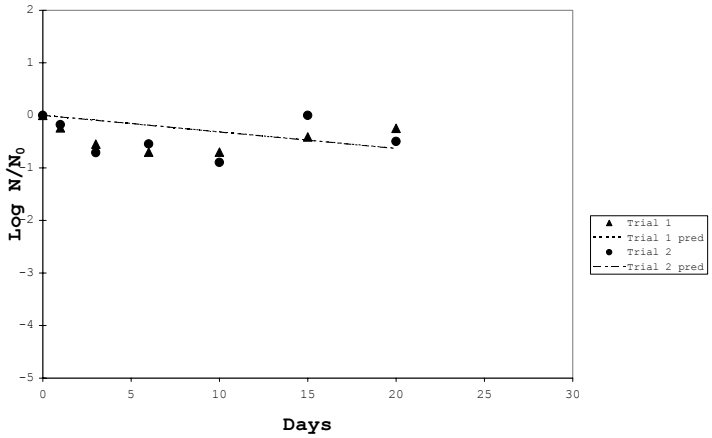
***Cryptosporidium parvum*, ASW 200 mg/L, 5° C**



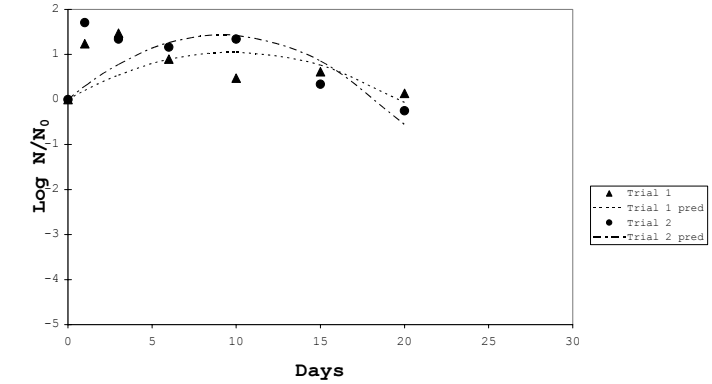
***Cryptosporidium parvum*, ASW 200 mg/L, 22° C**



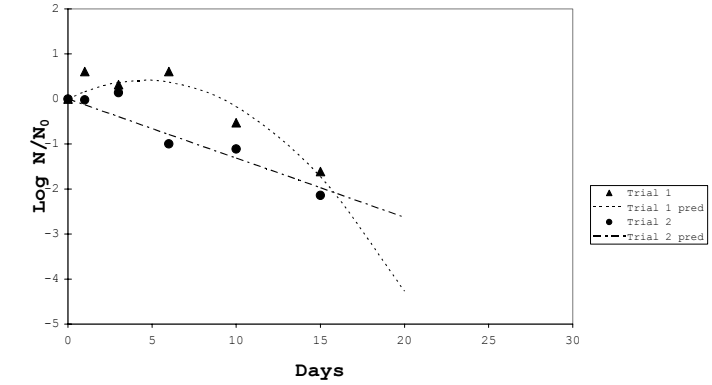
***Cryptosporidium parvum*, ASW 200 mg/L, 30° C**



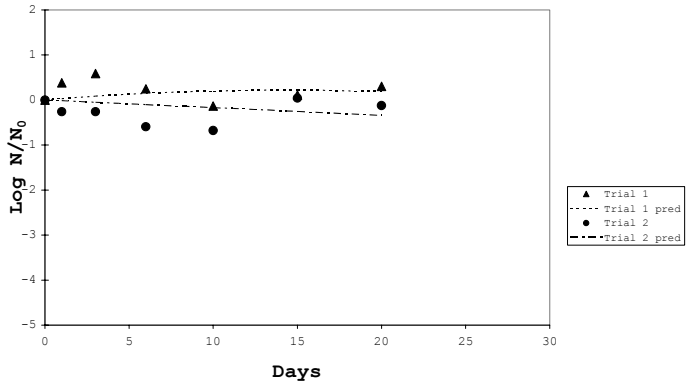
Cryptosporidium parvum, ASW 500 mg/L, 5° C



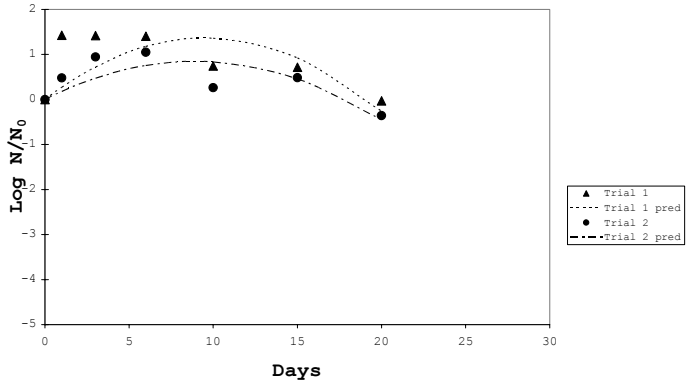
***Cryptosporidium parvum*, ASW 500 mg/L, 22° C**



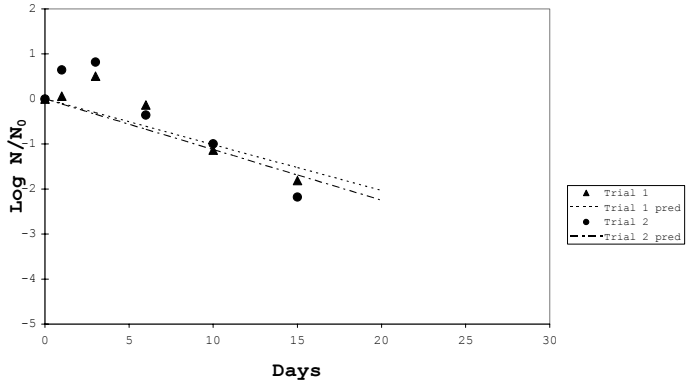
***Cryptosporidium parvum*, ASW 500 mg/L, 30° C**



***Cryptosporidium parvum*, ASW 1000 mg/L, 5° C**

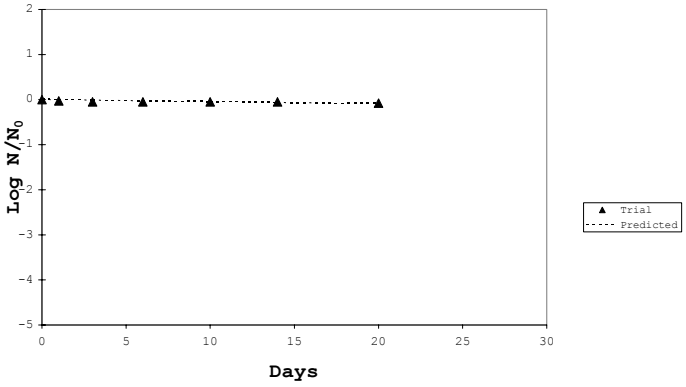


***Cryptosporidium parvum*, ASW 1000 mg/L, 22° C**

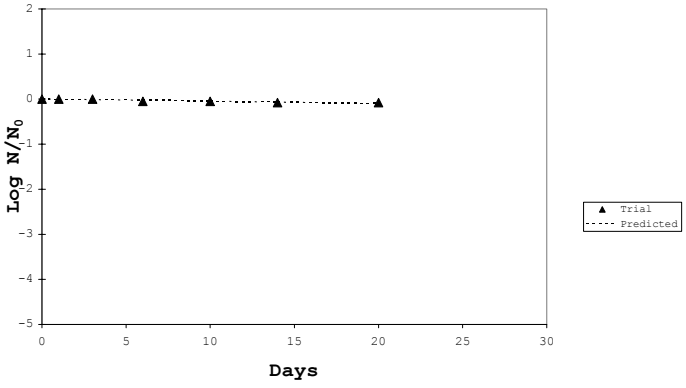


***Cryptosporidium parvum*, ASW 1000 mg/L, 30° C**

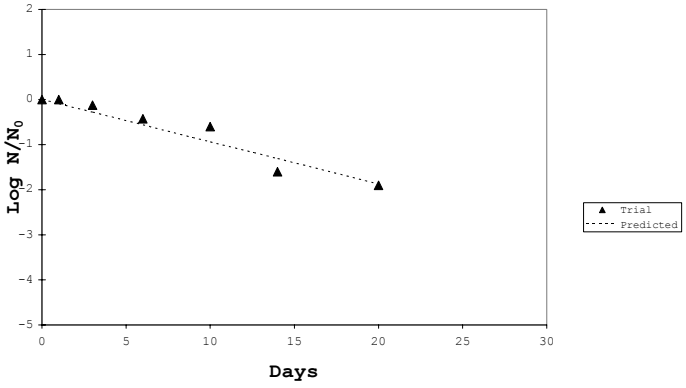
G. *Giardia lamblia* TDS-temperature trials



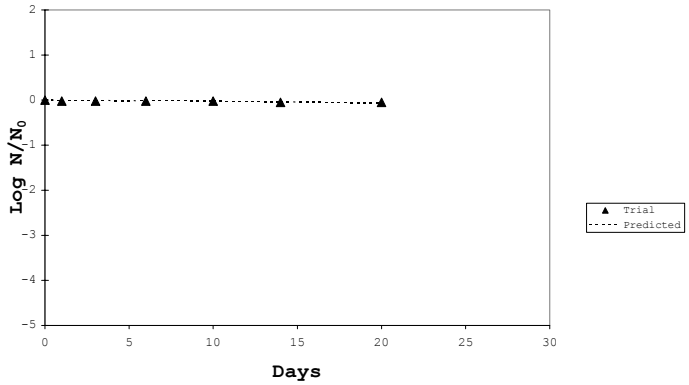
***Giradia lamblia*, ASW 200 mg/L, 5° C**



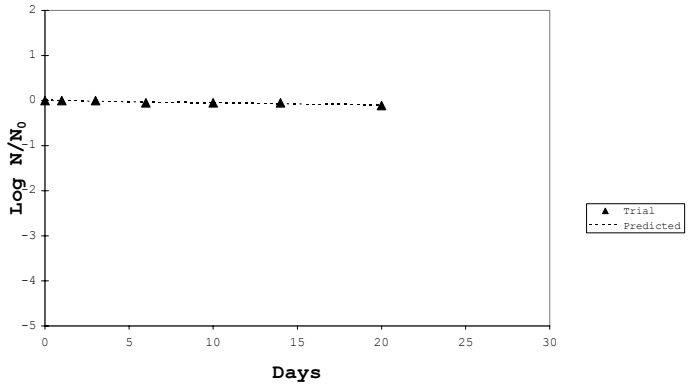
***Giradia lamblia*, ASW 200 mg/L, 22° C**



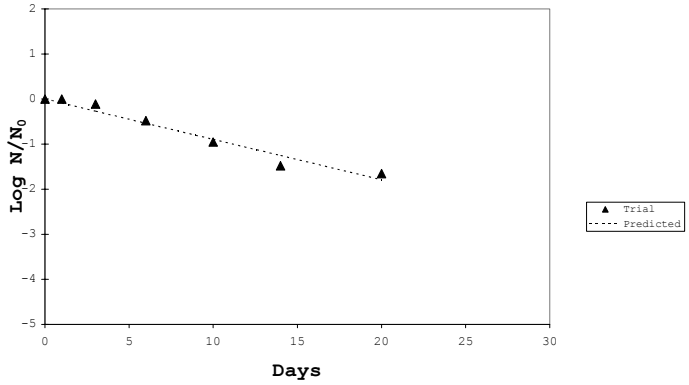
***Giardia lamblia*, ASW 200 mg/L, 30° C**



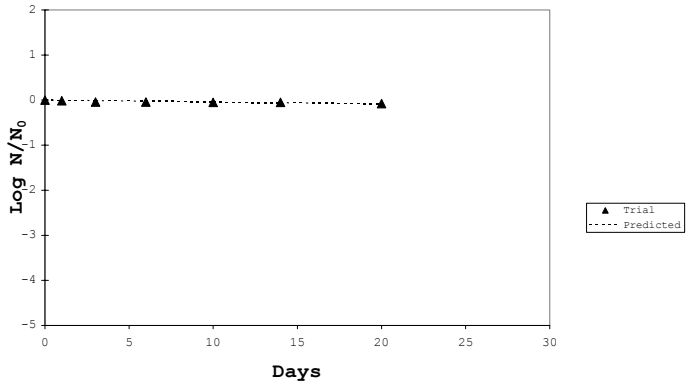
Giardia lamblia, ASW 500 mg/L, 5° C



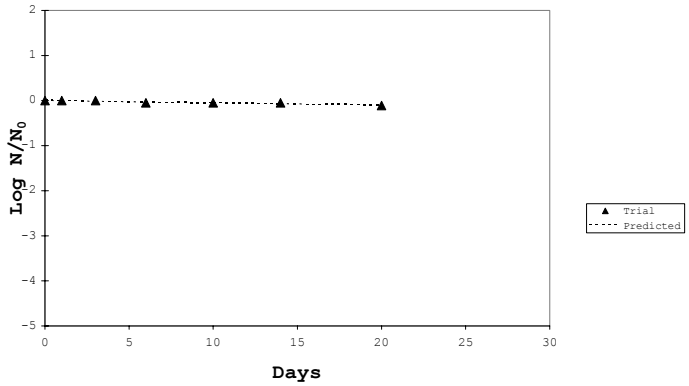
Giardia lamblia, ASW 500 mg/L, 22° C



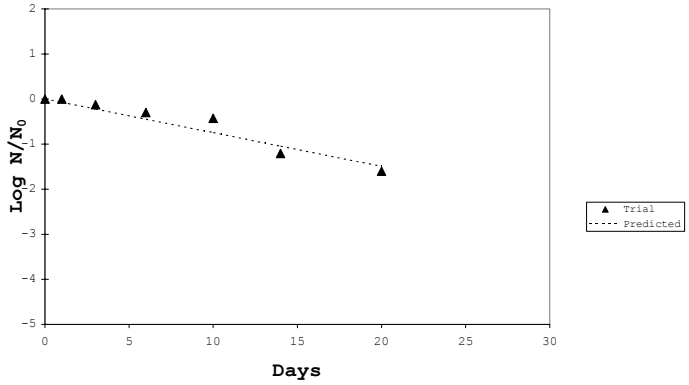
Giardia lamblia, ASW 500 mg/L, 30° C



Giardia lamblia, ASW 1000 mg/L, 5° C



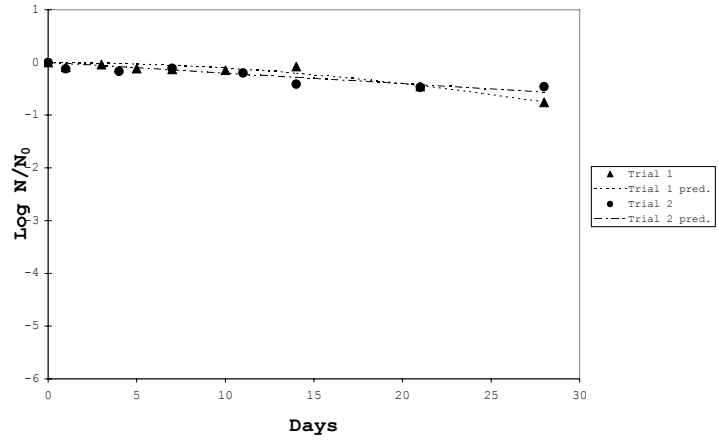
Giardia lamblia, ASW 1000 mg/L, 22° C



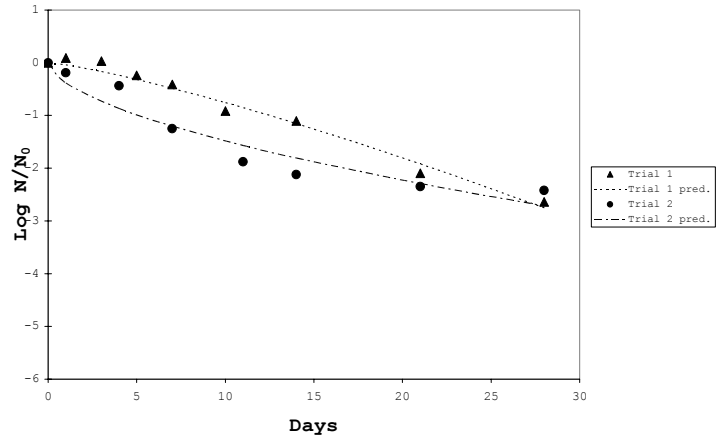
Giardia lamblia, ASW 1000 mg/L, 30° C

APPENDIX 2. Observed data plots and fitted model curves for aquifer and reservoir water sample studies with indicator bacteria populations

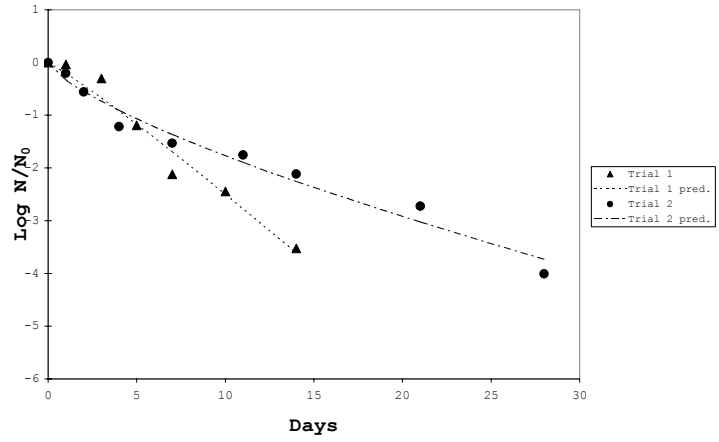
A. Fecal coliform Bradenton site natural water experiments



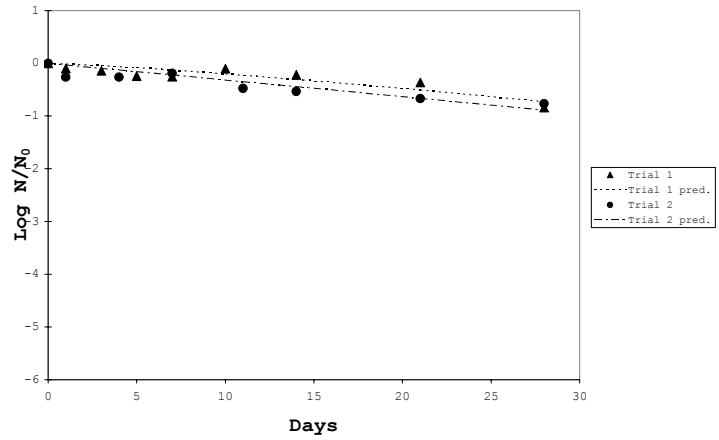
Fecal coliform, Avon Park aquifer, raw, 5° C



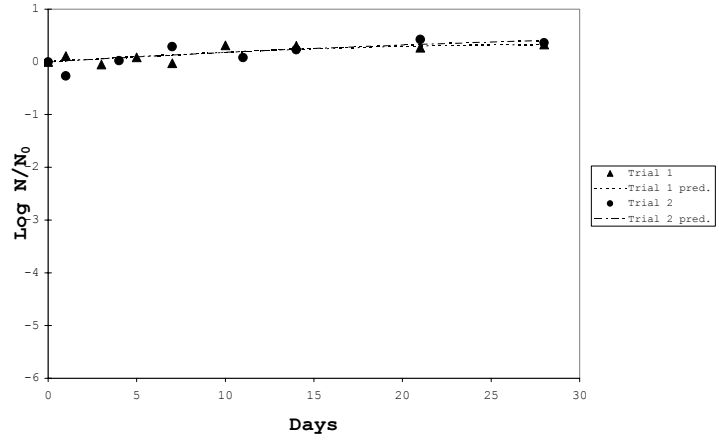
Fecal coliform, Avon Park aquifer, raw, 22° C



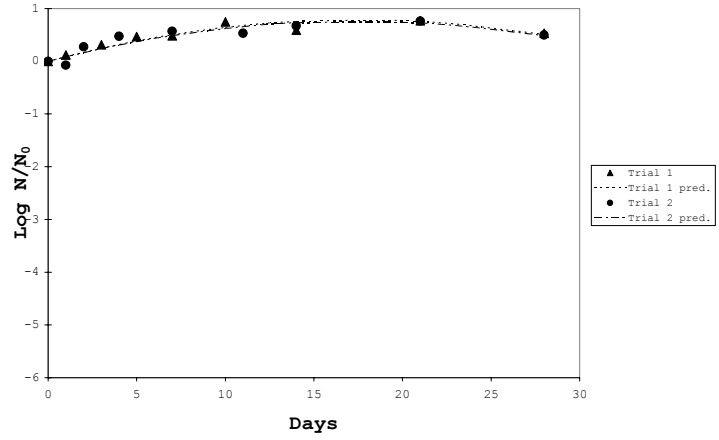
Fecal coliform, Avon Park aquifer, raw, 30° C



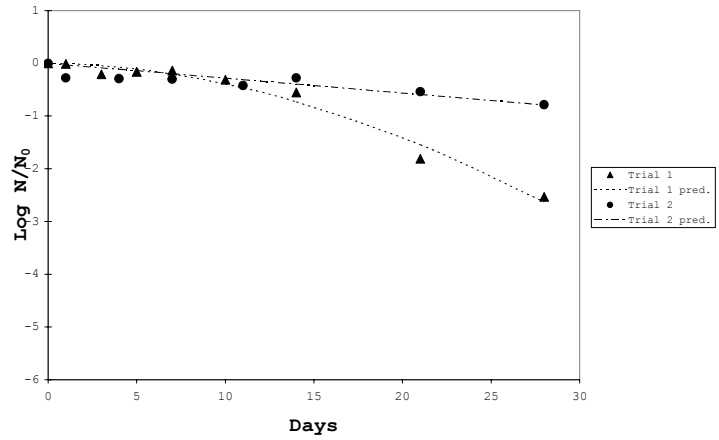
Fecal coliform, Avon Park aquifer, pasteurized, 5° C



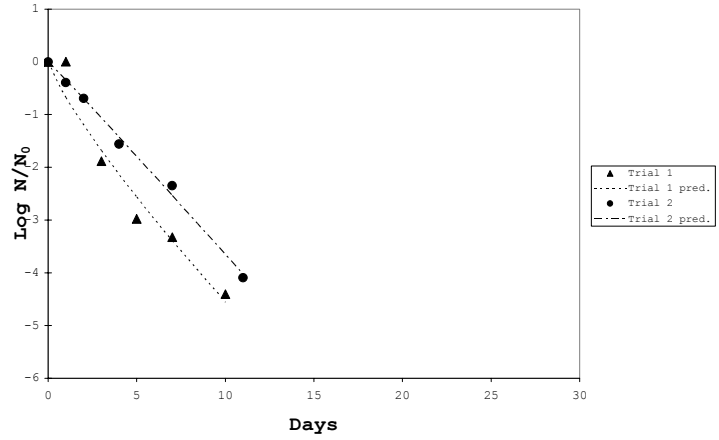
Fecal coliform, Avon Park aquifer, pasteurized, 22° C



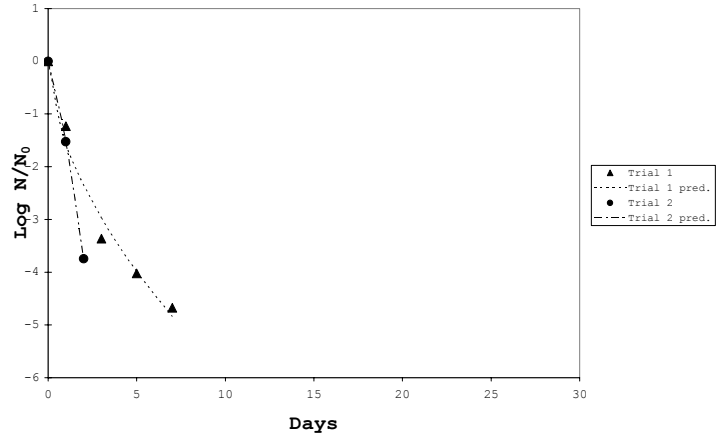
Fecal coliform, Avon Park aquifer, pasteurized, 30° C



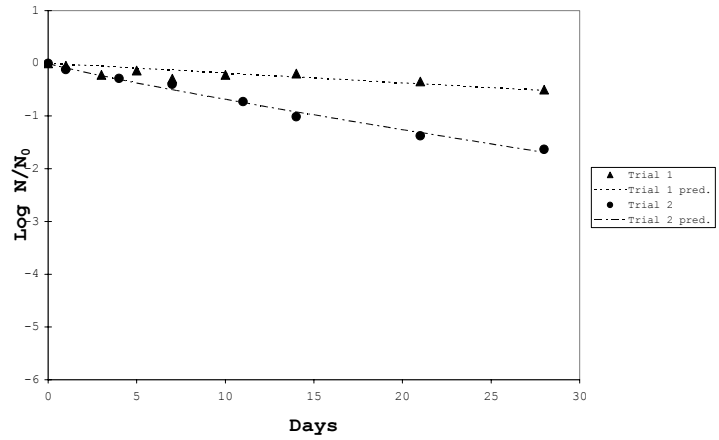
Fecal coliform, Bill Evers reservoir, raw, 5° C



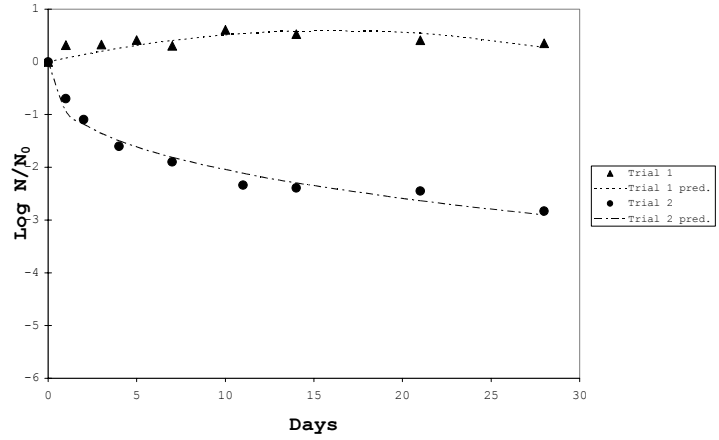
Fecal coliform, Bill Evers reservoir, raw, 22° C



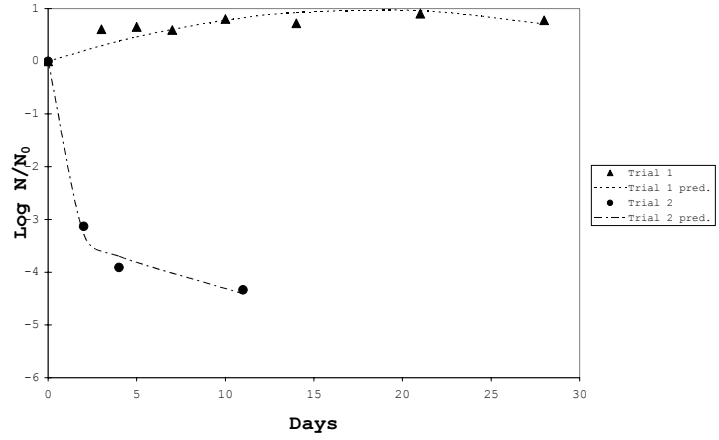
Fecal coliform, Bill Evers reservoir, raw, 30° C



Fecal coliform, Bill Evers reservoir, pasteurized, 5° C

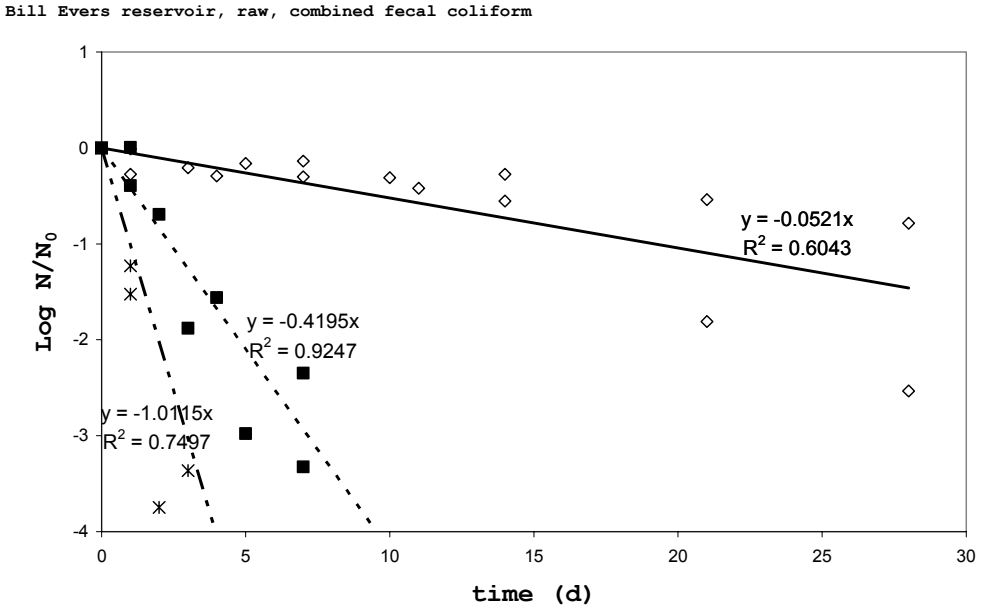


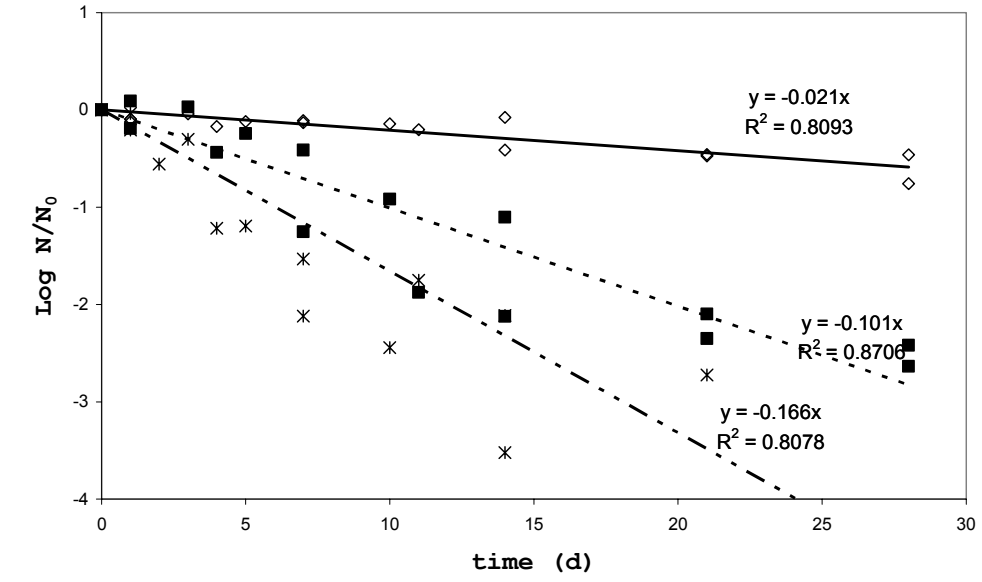
Fecal coliform, Bill Evers reservoir, pasteurized, 22° C

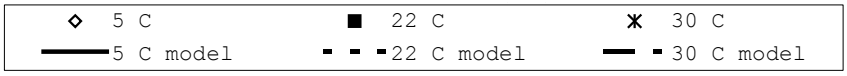


Fecal coliform, Bill Evers reservoir, pasteurized, 30° C

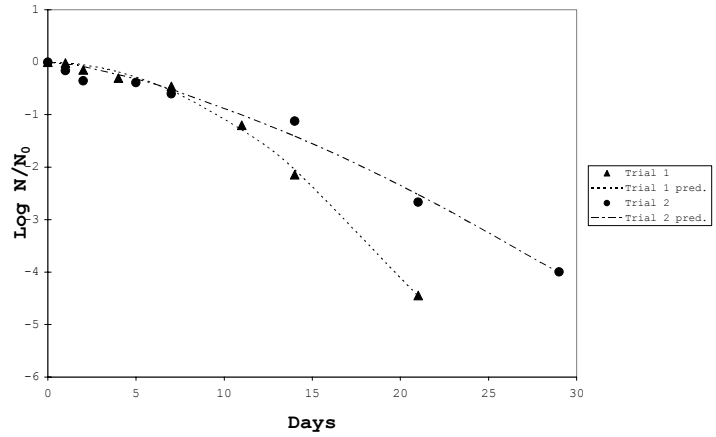
B. Fecal coliform Bradenton site raw water plots and fitted first-order regression models



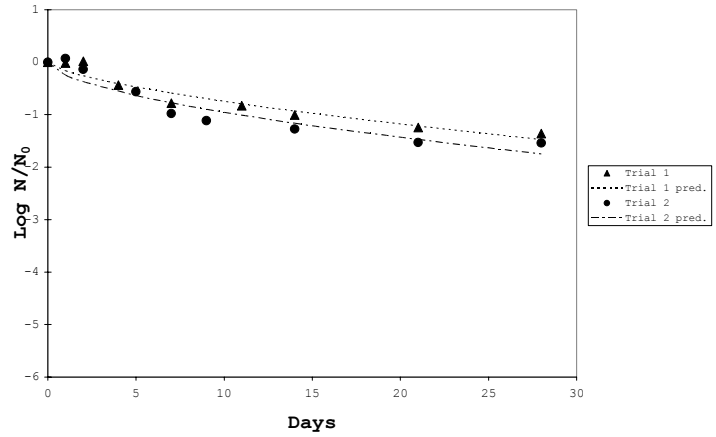




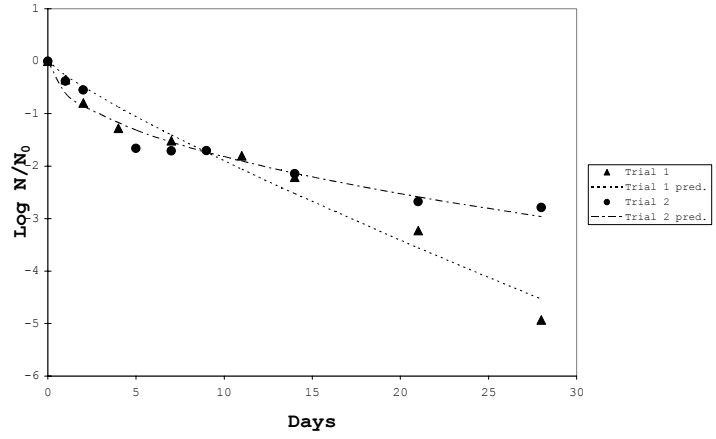
C. Fecal coliform West Palm Beach site natural water experiments



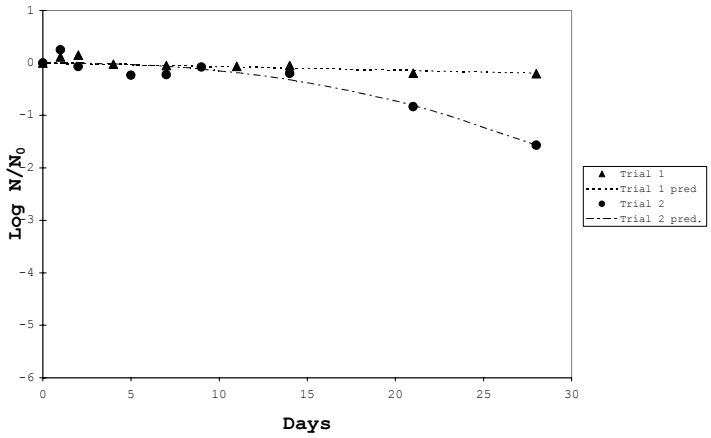
Fecal coliform, Lake Lytal aquifer, raw, 5° C



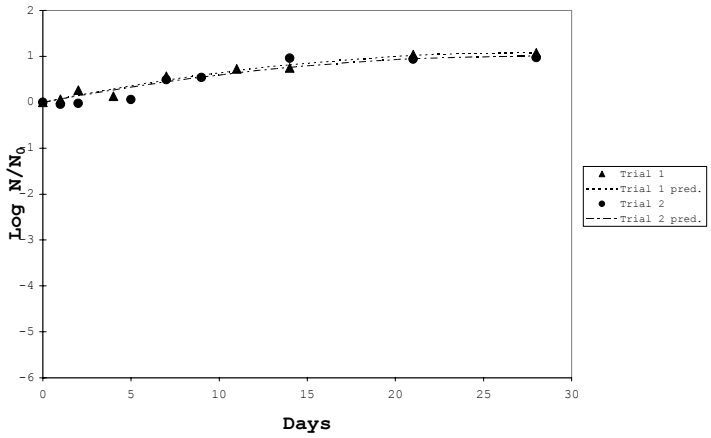
Fecal coliform, Lake Lytal aquifer, raw, 22° C



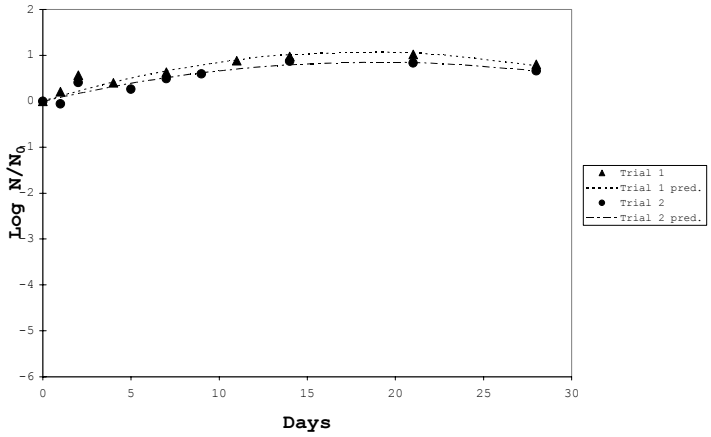
Fecal coliform, Lake Lytal aquifer, raw, 30° C



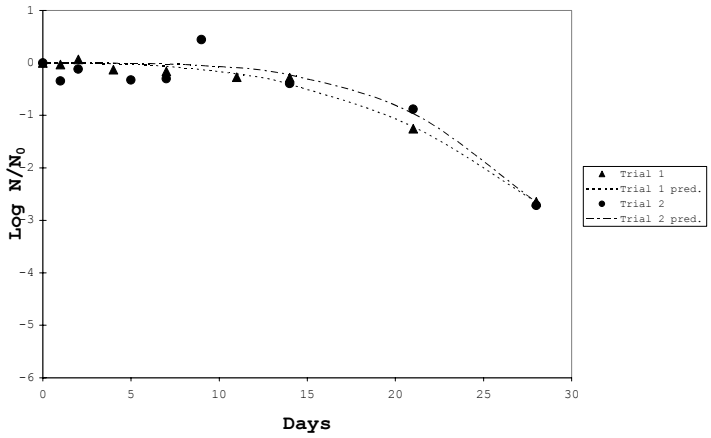
Fecal coliform, Lake Lytal aquifer, pasteurized, 5° C



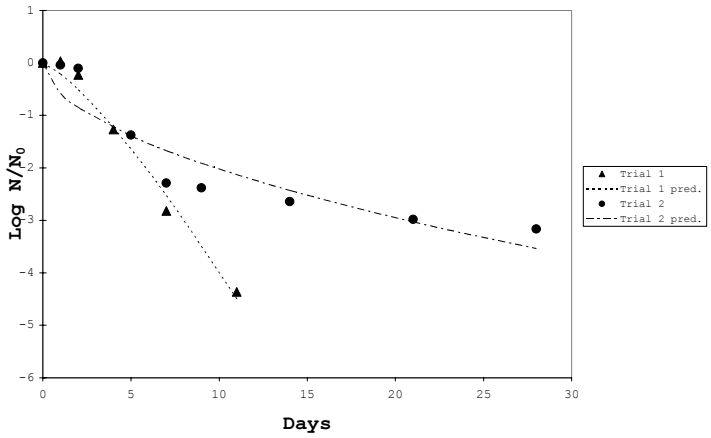
Fecal coliform, Lake Lytal aquifer, pasteurized, 22° C



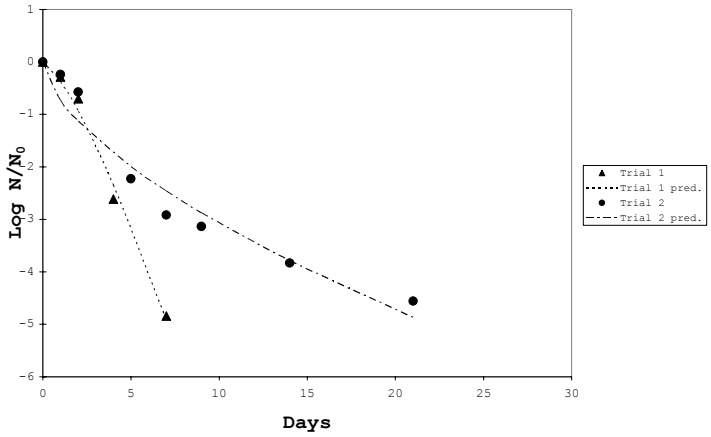
Fecal coliform, Lake Lytal aquifer, pasteurized, 30° C



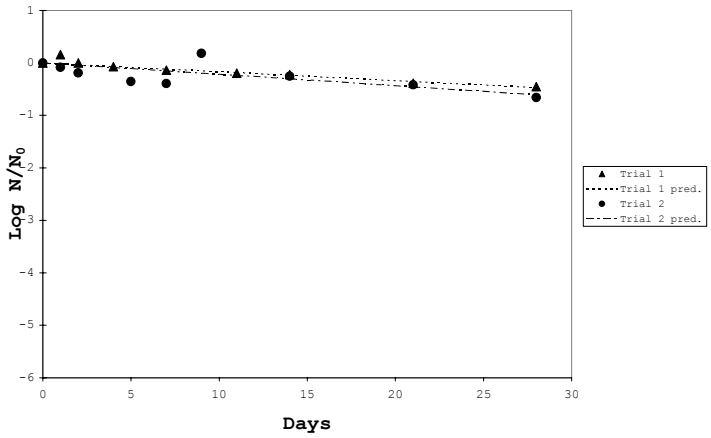
Fecal coliform, Clear Lake reservoir, raw, 5° C



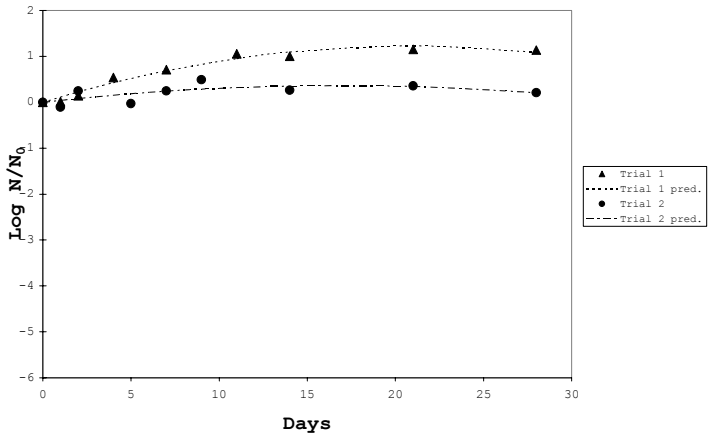
Fecal coliform, Clear Lake reservoir, raw, 22° C



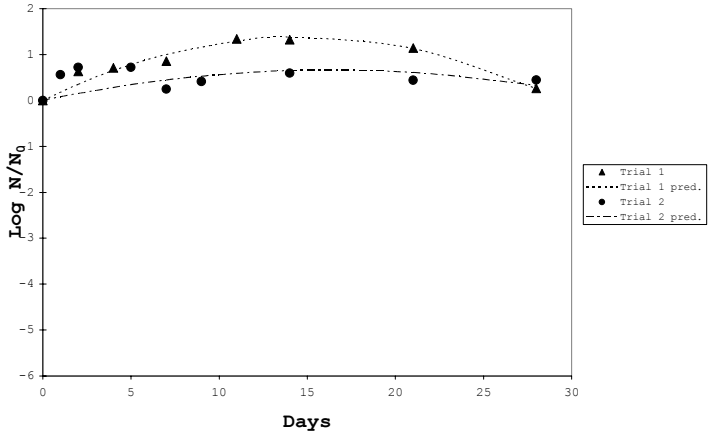
Fecal coliform, Clear Lake reservoir, raw, 30° C



Fecal coliform, Clear Lake reservoir, pasteurized, 5° C



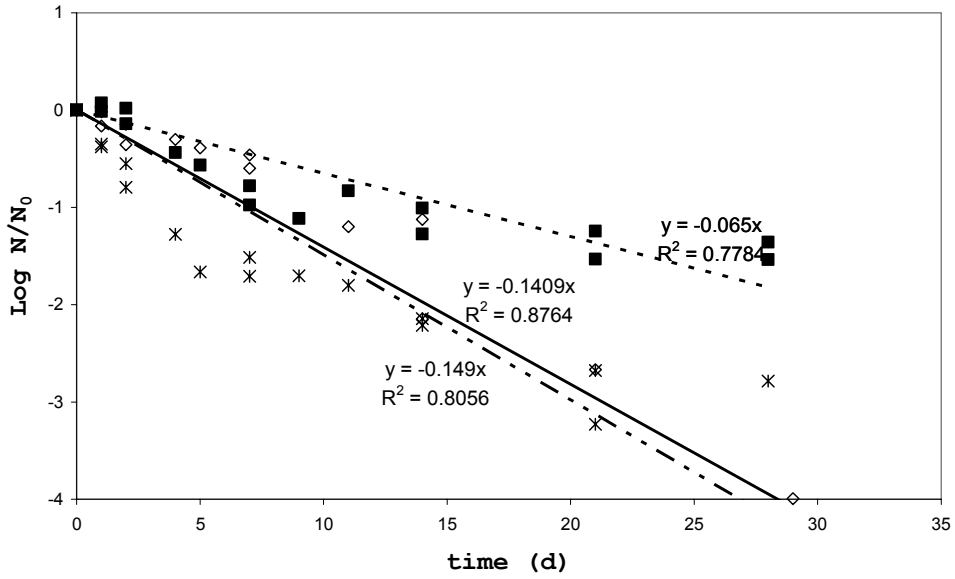
Fecal coliform, Clear Lake reservoir, pasteurized, 22° C



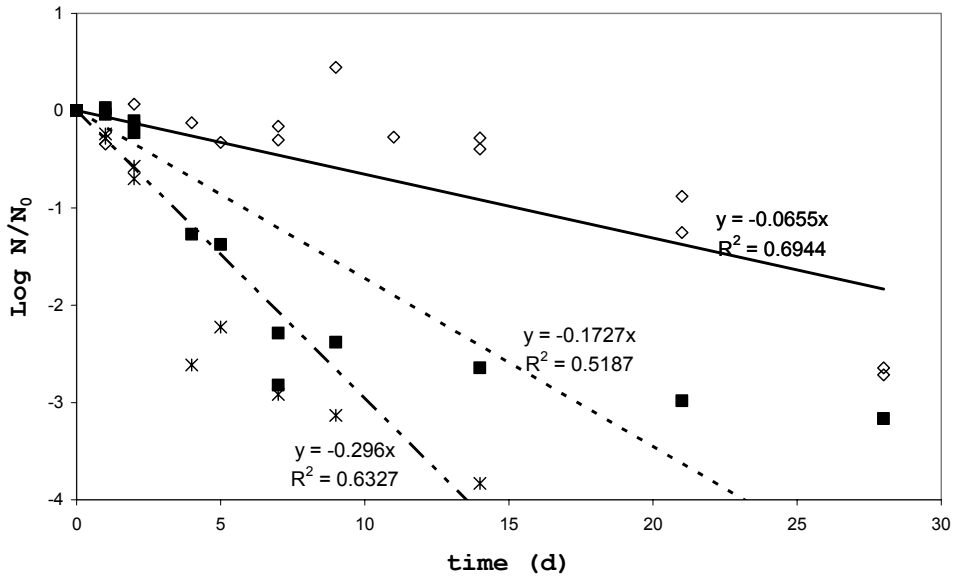
Fecal coliform, Clear Lake reservoir, pasteurized, 30° C

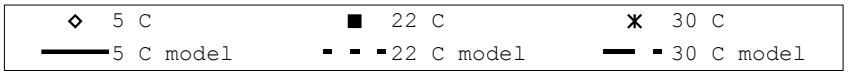
D. Fecal coliform West Palm Beach site raw water plots and fitted first-order regression models

Lake Lytal Park aquifer, raw, combined fecal coliform

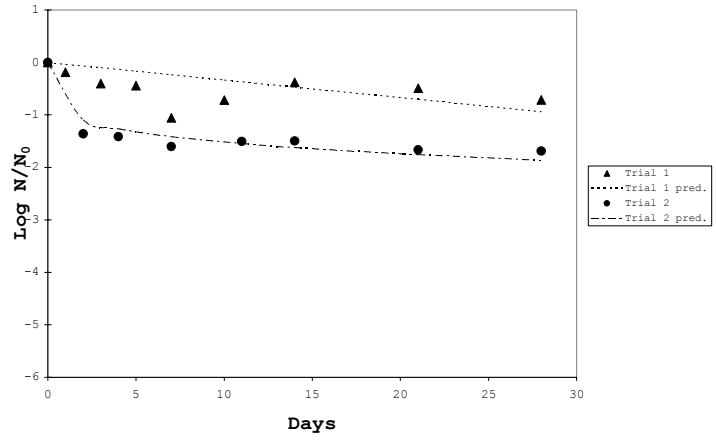


Clear Lake reservoir, raw, combined fecal coliform

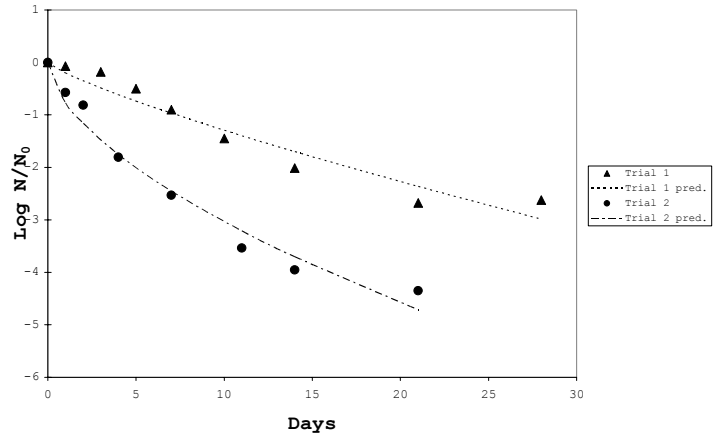




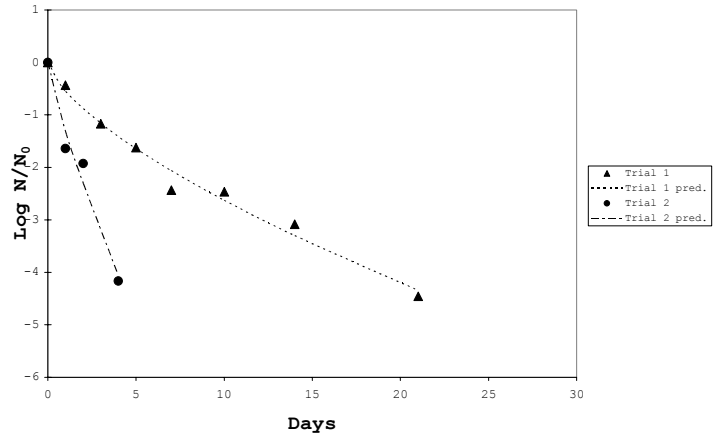
E. Enterococci Bradenton site natural water experiments



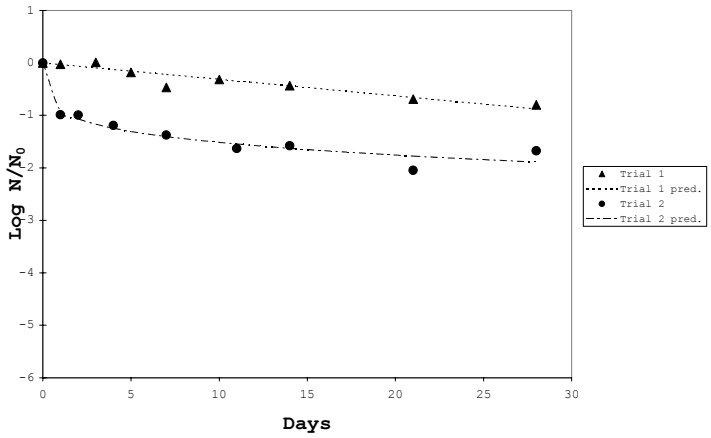
Enterococci, Avon Park aquifer, raw, 5° C



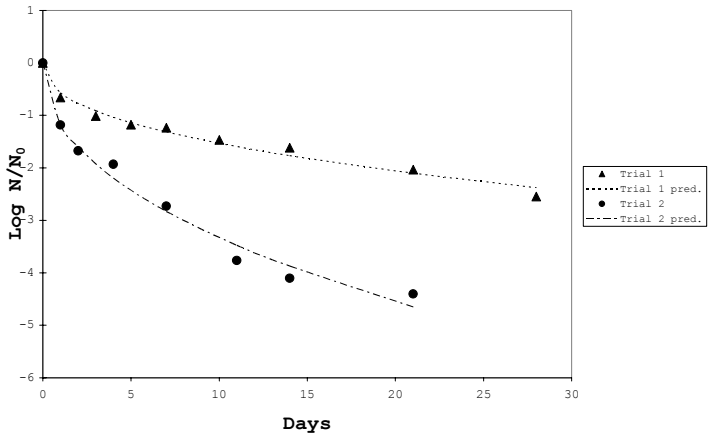
Enterococci, Avon Park aquifer, raw, 22° C



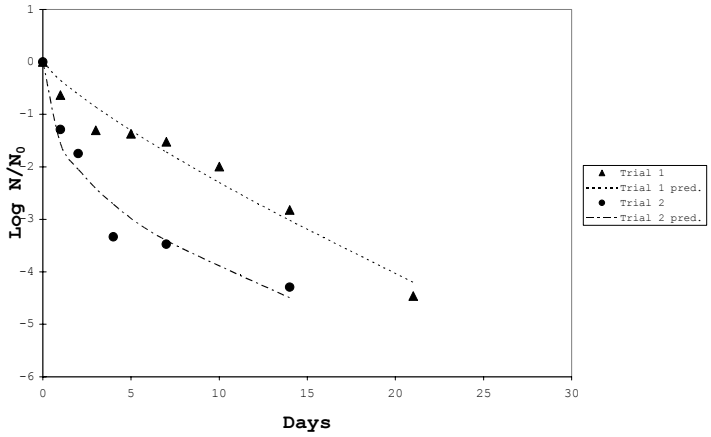
Enterococci, Avon Park aquifer, raw, 30° C



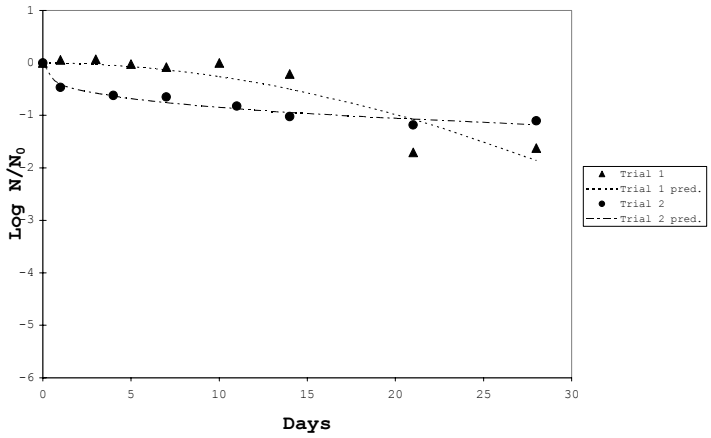
Enterococci, Avon Park aquifer, pasteurized, 5° C



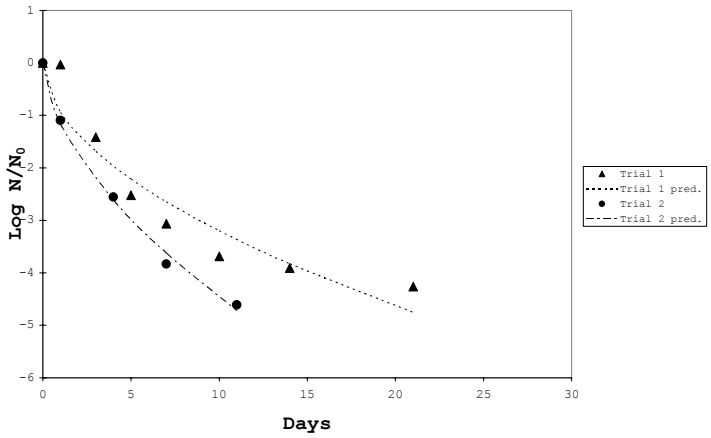
Enterococci, Avon Park aquifer, pasteurized, 22° C



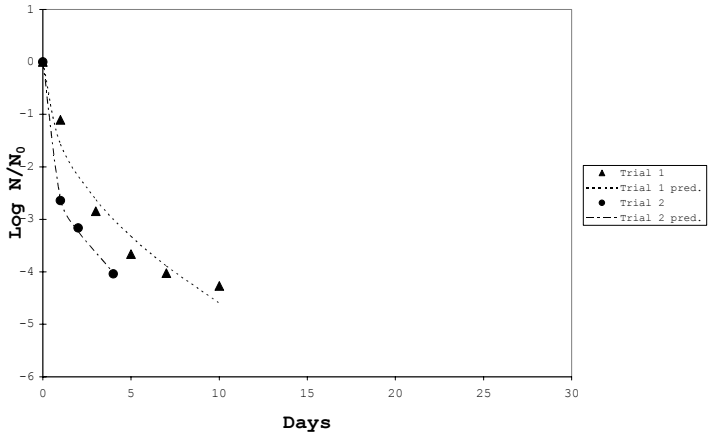
Enterococci, Avon Park aquifer, pasteurized, 30° C



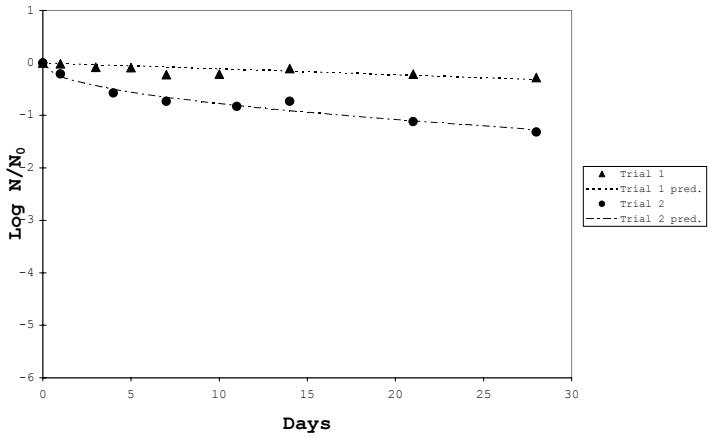
Enterococci, Bill Evers reservoir, raw, 5° C



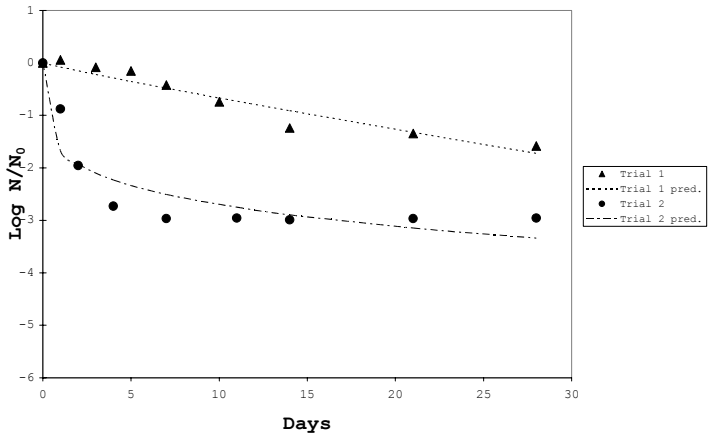
Enterococci, Bill Evers reservoir, raw, 22° C



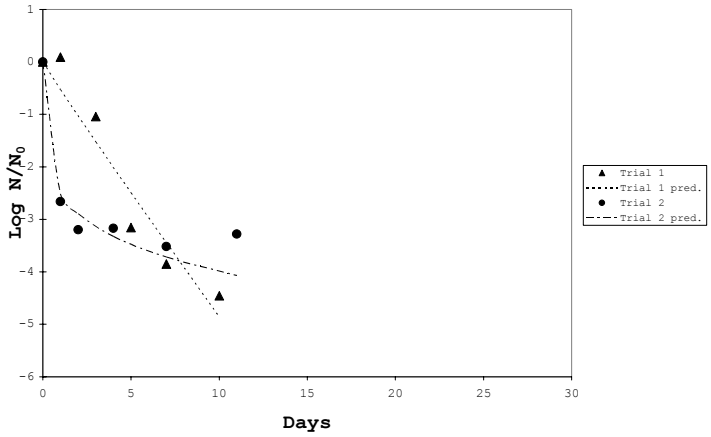
Enterococci, Bill Evers reservoir, raw, 30° C



Enterococci, Bill Evers reservoir, pasteurized, 5° C



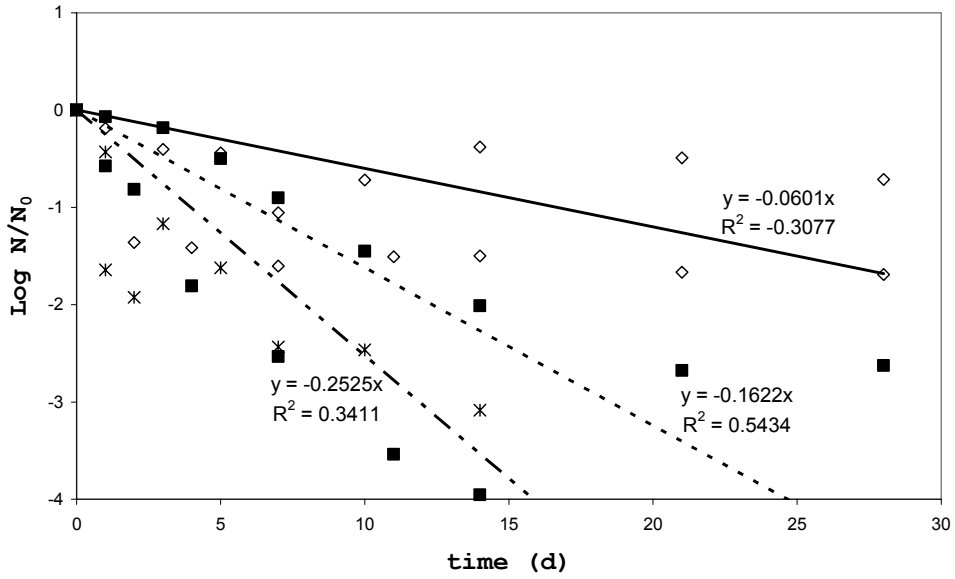
Enterococci, Bill Evers reservoir, pasteurized, 22° C



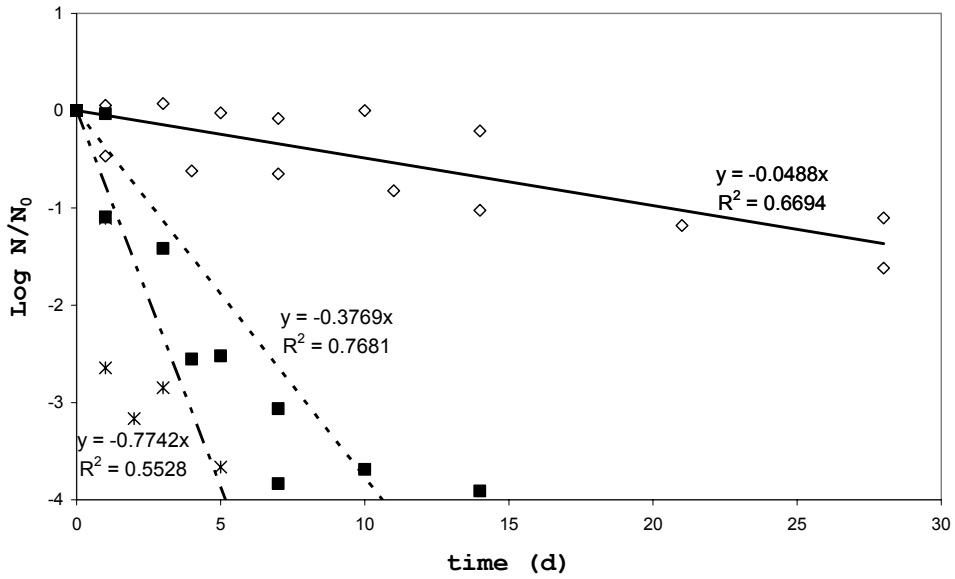
Enterococci, Bill Evers reservoir, pateurized, 30° C

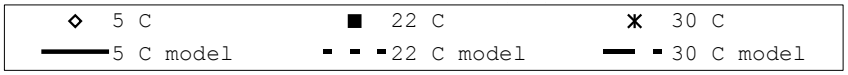
F. Enterococci Bradenton site raw water plots and fitted first-order regression models

Avon Park aquifer, raw, combined enterococci

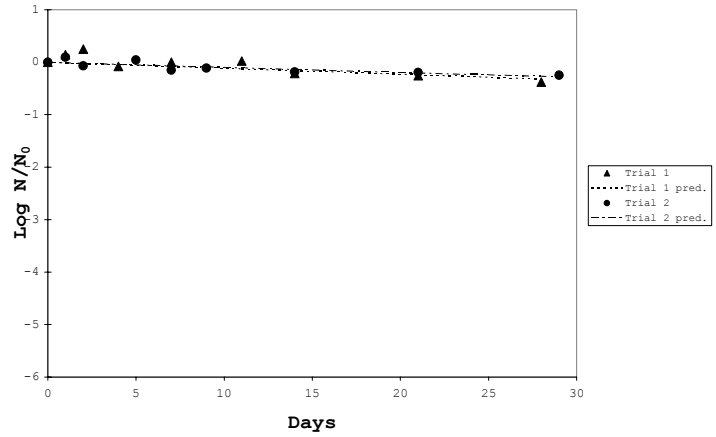


Bill Evers reservoir, raw, combined enterococci

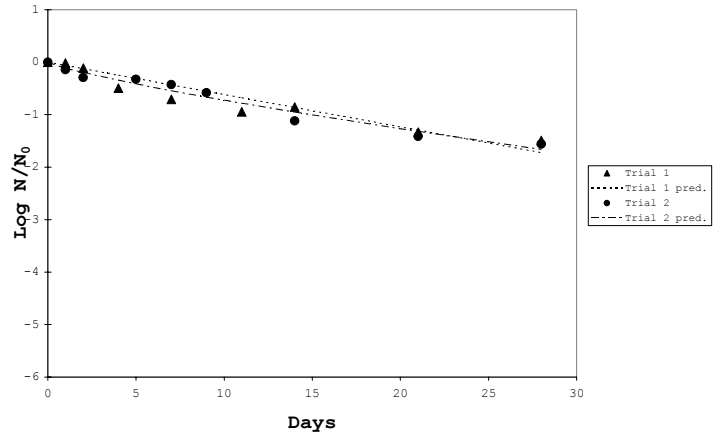




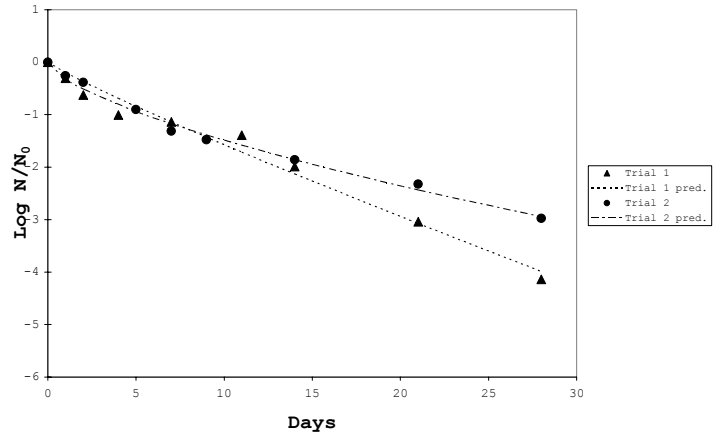
G. Enterococci West Palm Beach natural water experiments



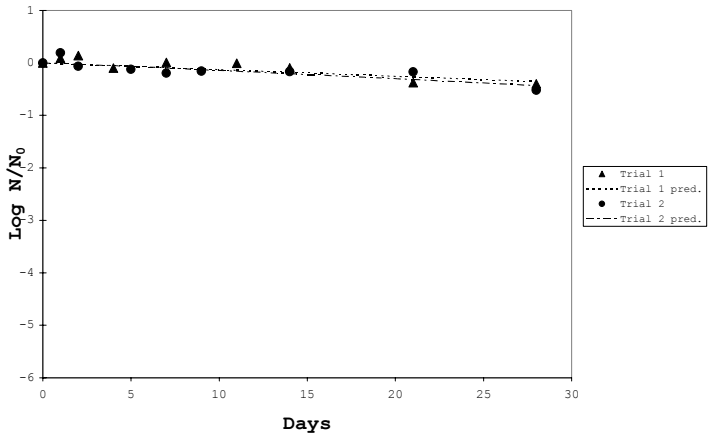
Enterococci, Lake Lytal aquifer, raw, 5° C



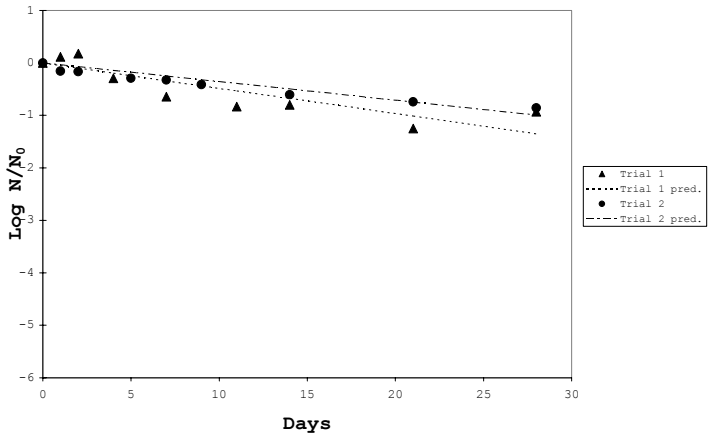
Enterococci, Lake Lytal aquifer, raw, 22° C



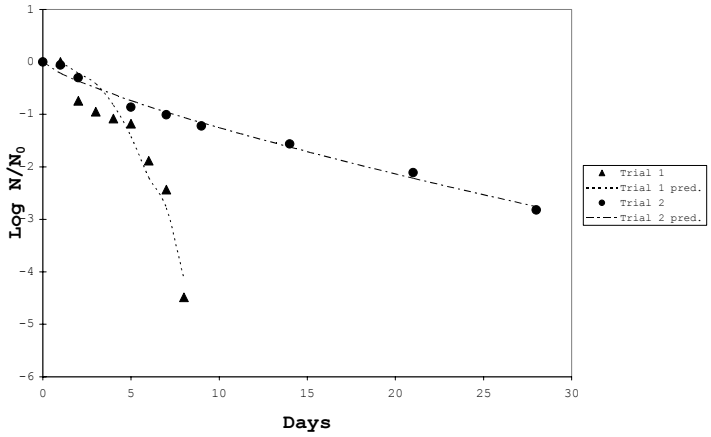
Enterococci, Lake Lytal aquifer, raw, 30° C



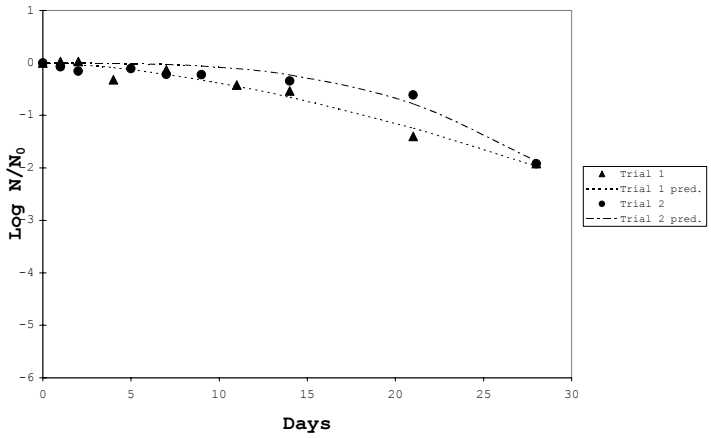
Enterococci, Lake Lytal aquifer, pasteurized, 5° C



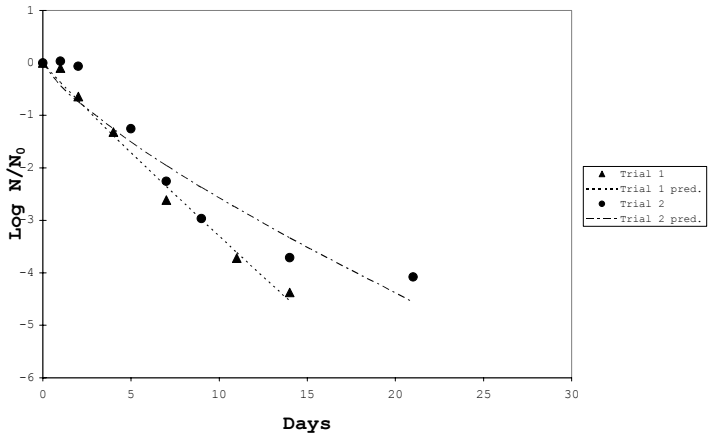
Enterococci, Lake Lytal aquifer, pasteurized, 22° C



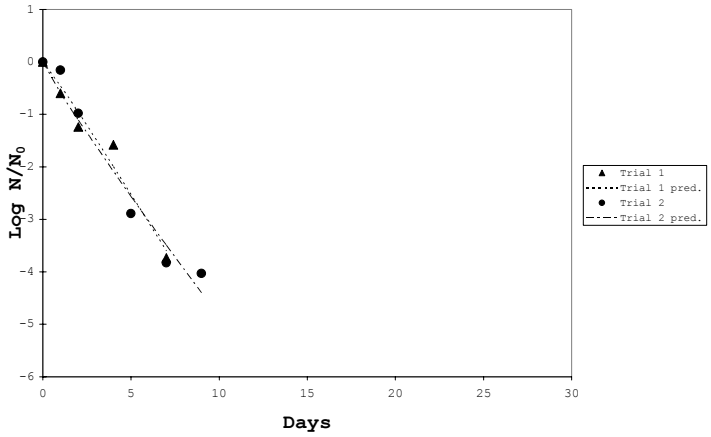
Enterococci, Lake Lytal aquifer, pasteurized, 30° C



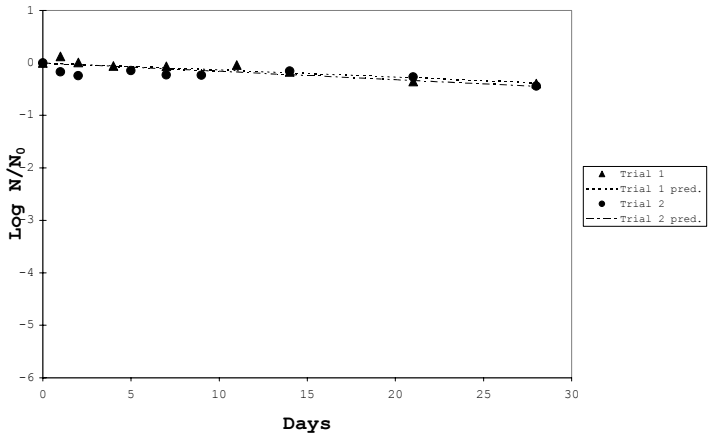
Enterococci, Clear Lake reservoir, raw, 5° C



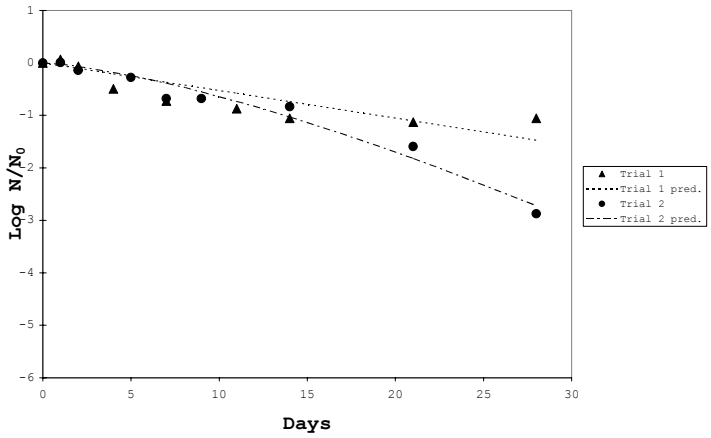
Enterococci, Clear Lake reservoir, raw, 22° C



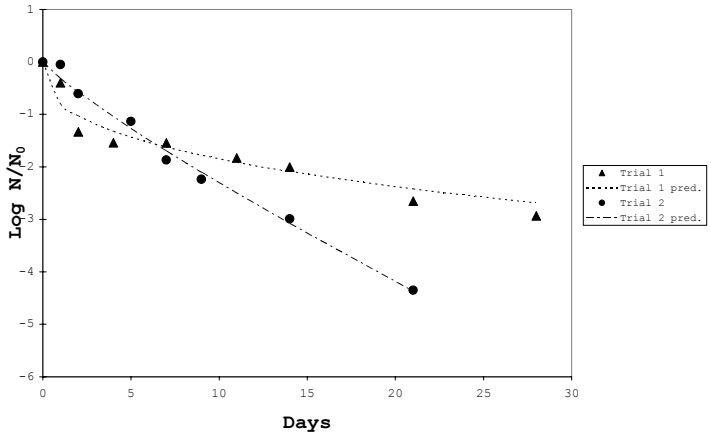
Enterococci, Clear Lake reservoir, raw, 30° C



Enterococci, Clear Lake reservoir, pasteurized, 5° C



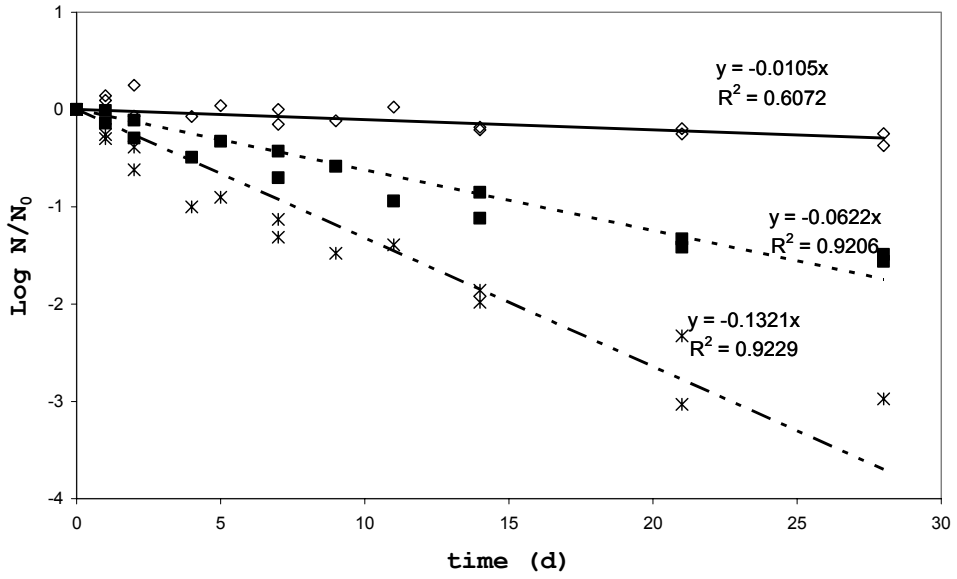
Enterococci, Clear Lake reservoir, pasteurized, 22° C



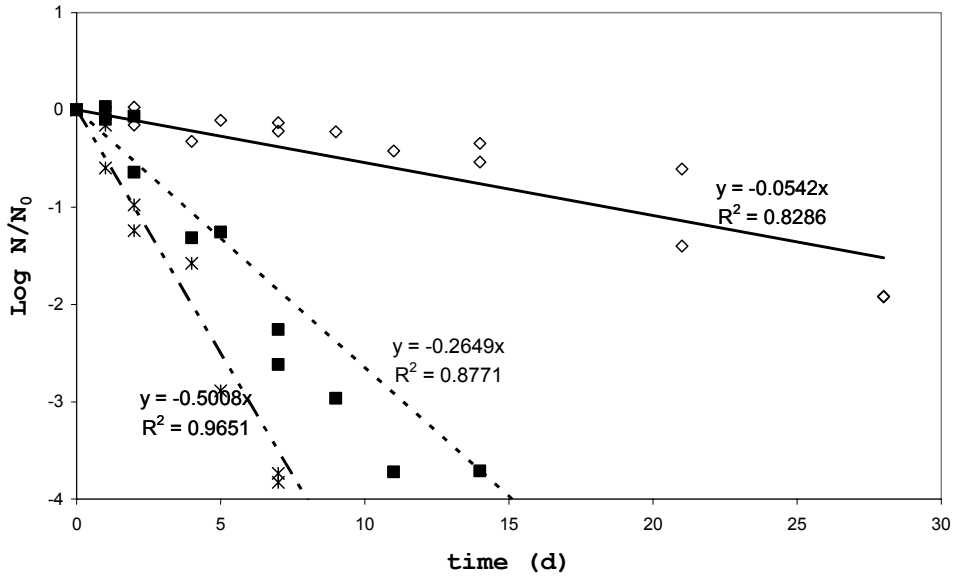
Enterococci, Clear Lake reservoir, pasteurized, 30° C

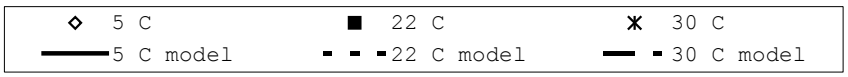
H. Enterococci West Palm Beach site raw water plots and fitted first-order regression models

Lake Lytal Park aquifer, raw, combined enterococci



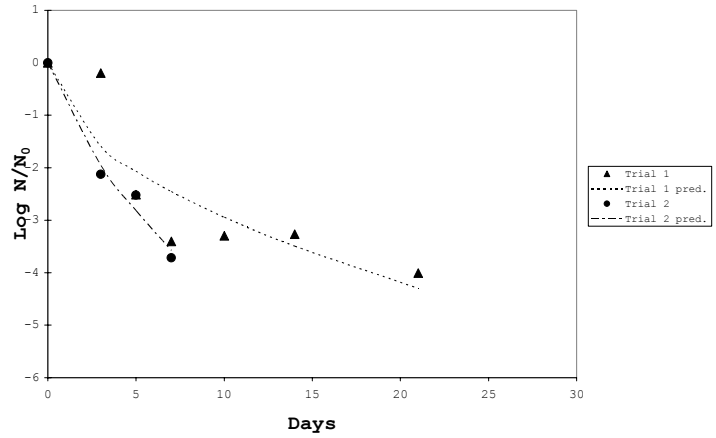
Clear Lake reservoir, raw, combined enterococci



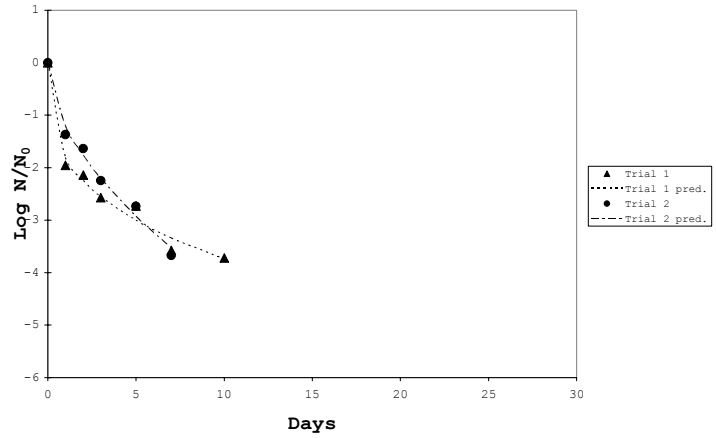


APPENDIX 3. Observed data plots and fitted model curves for aquifer and reservoir water sample studies with bacteriophage and intestinal parasites

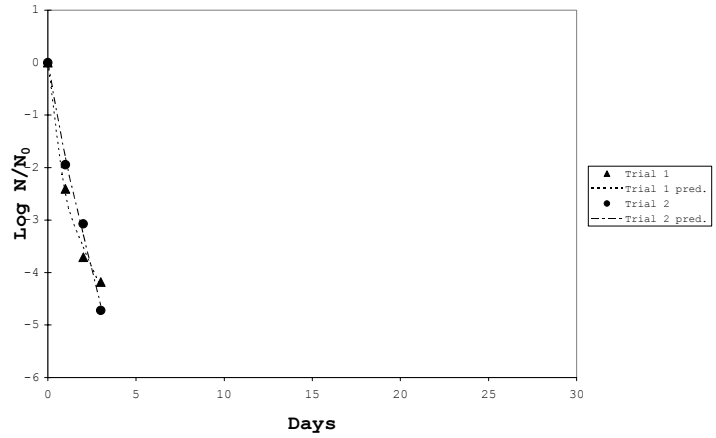
A. F+ RNA coliphage Bradenton site natural water experiments



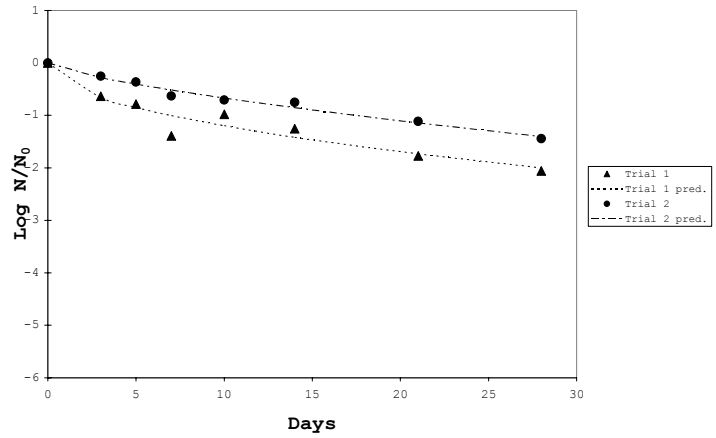
F+ RNA coliphage, Avon Park aquifer, raw, 5° C



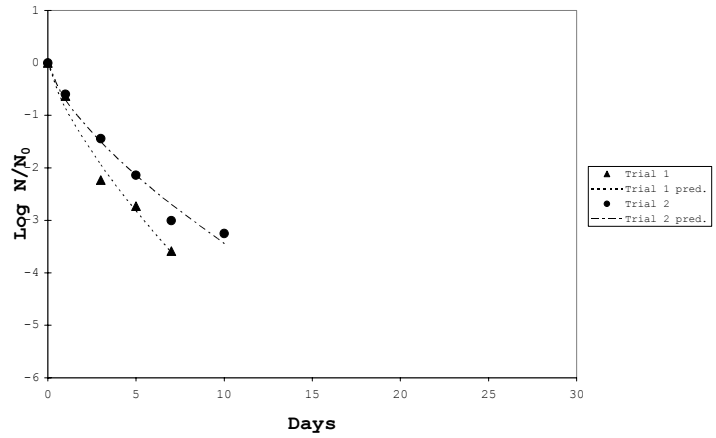
F+ RNA coliphage, Avon Park aquifer, raw, 22° C



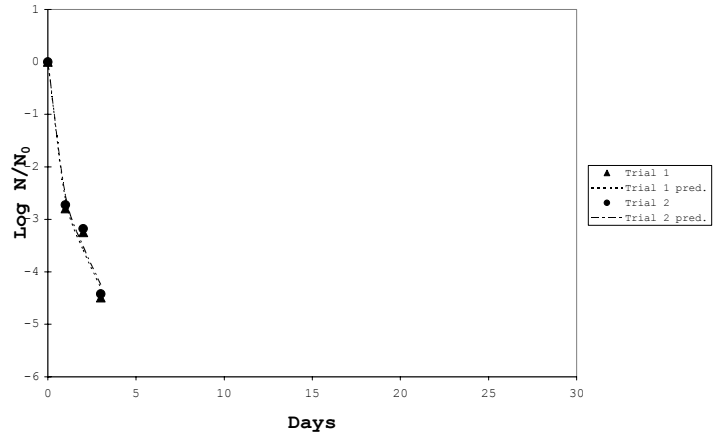
F+ RNA coliphage, Avon Park aquifer, raw, 30° C



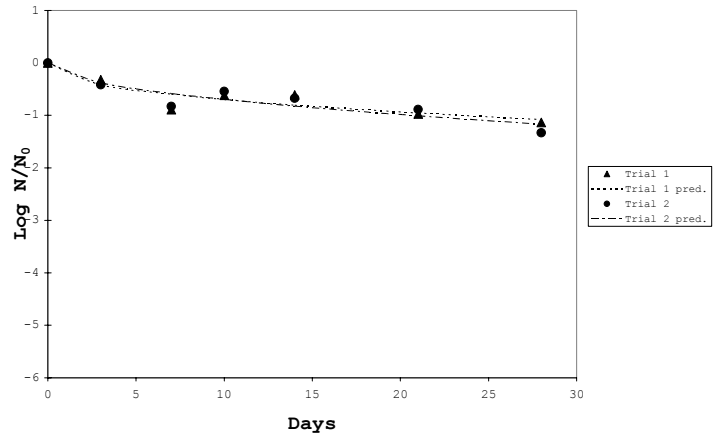
F+ RNA coliphage, Avon Park aquifer, pasteurized, 5° C



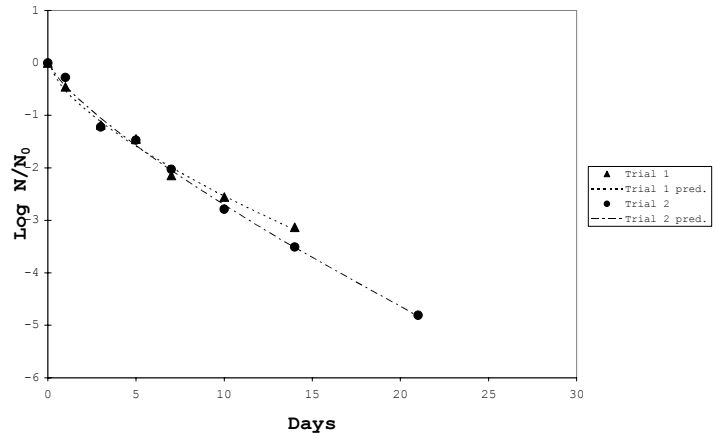
F+ RNA coliphage, Avon Park aquifer, pasteurized, 22° C



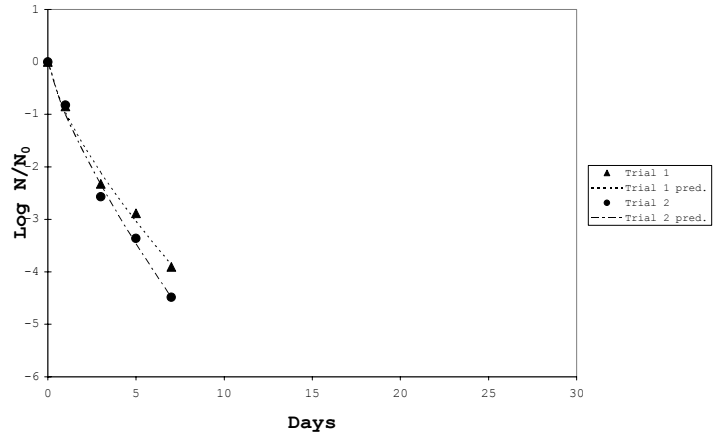
F+ RNA coliphage, Avon Park aquifer, pasteurized, 30° C



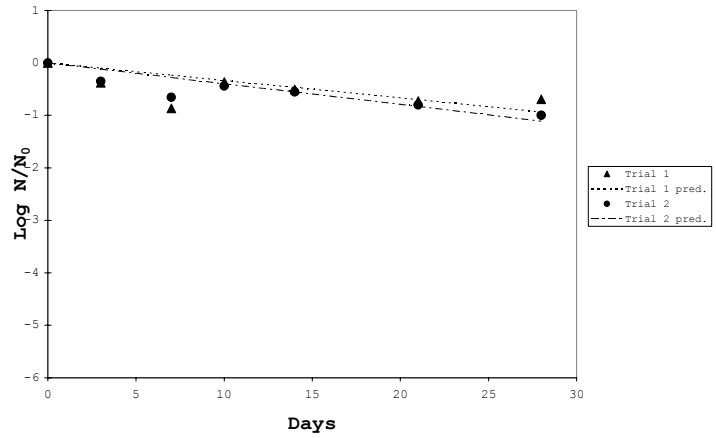
F+ RNA coliphage, Bill Evers reservoir, raw, 5° C



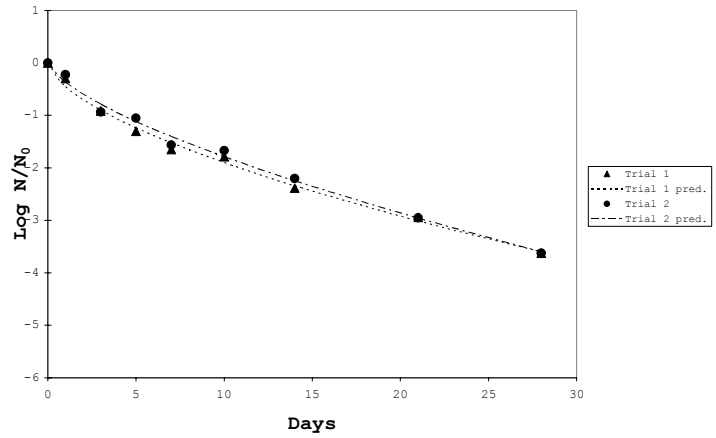
F+ RNA coliphage, Bill Evers reservoir, raw, 22° C



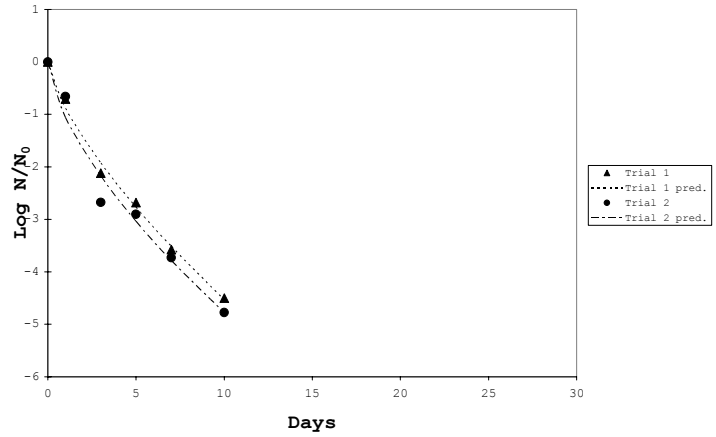
F+ RNA coliphage, Bill Evers reservoir, raw, 30° C



F+ RNA coliphage, Bill Evers reservoir, pasteurized, 5° C



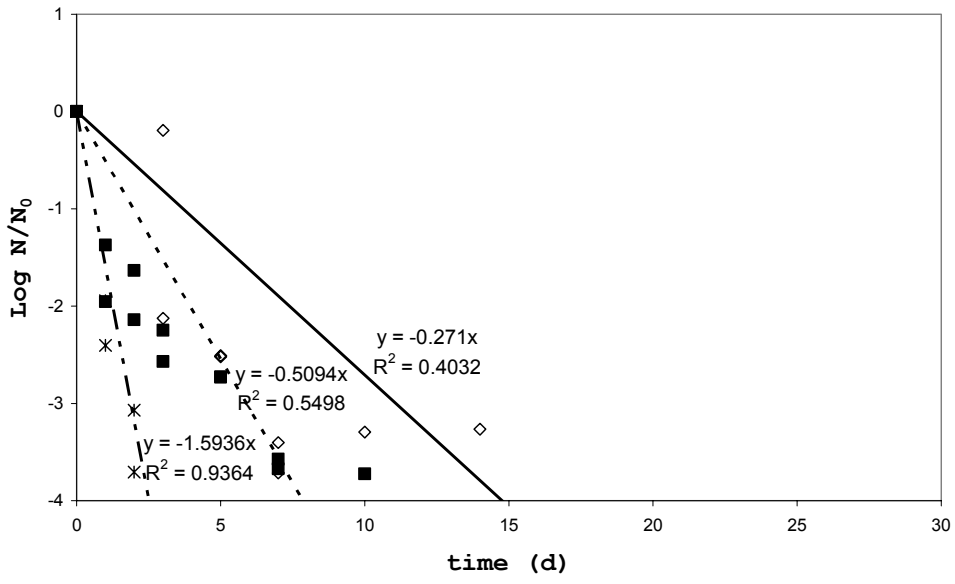
F+ RNA coliphage, Bill Evers reservoir, pasteurized, 22° C



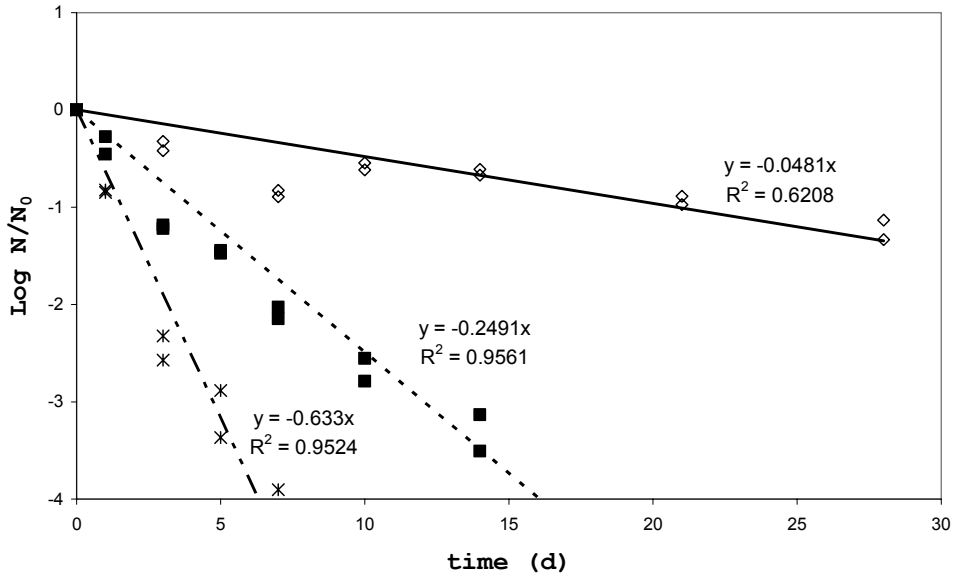
F+ RNA coliphage, Bill Evers reservoir, pasteurized, 30° C

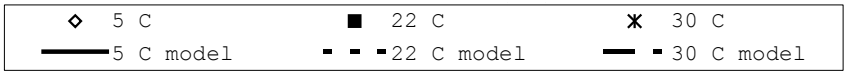
B. F+ RNA coliphage Bradenton site raw water plots and fitted first-order regression models

Avon Park aquifer, raw, combined F+ RNA coliphage

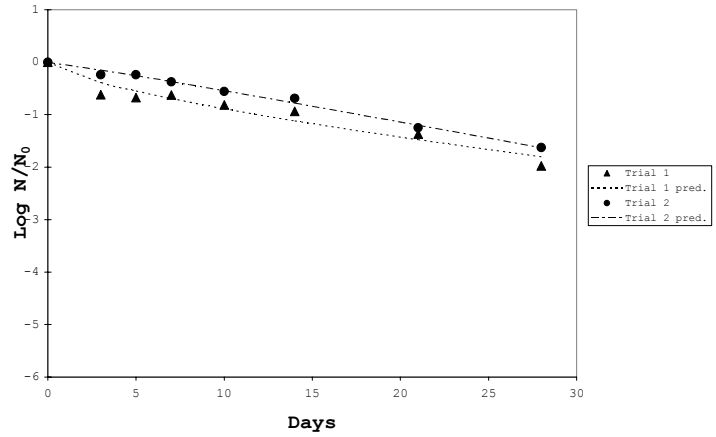


Bill Evers reservoir, raw, combined F+ RNA coliphage

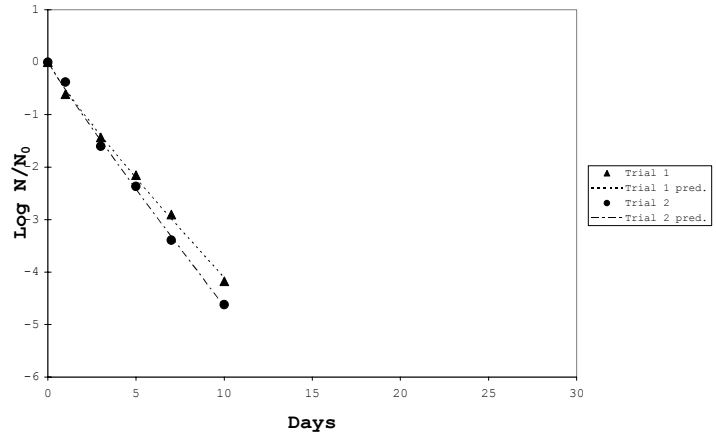




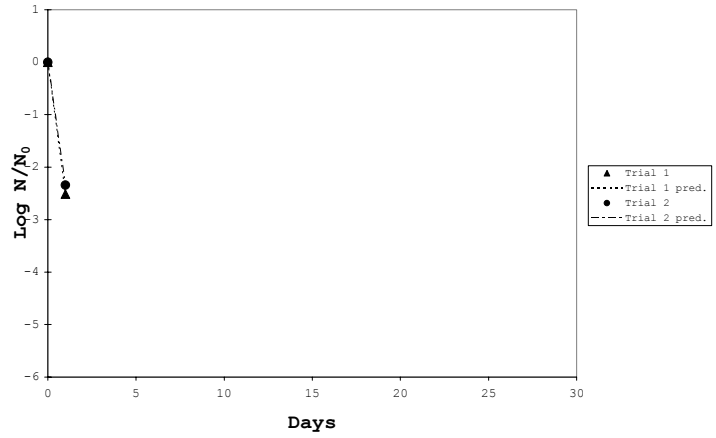
C. F+ RNA coliphage West Palm Beach natural water experiments



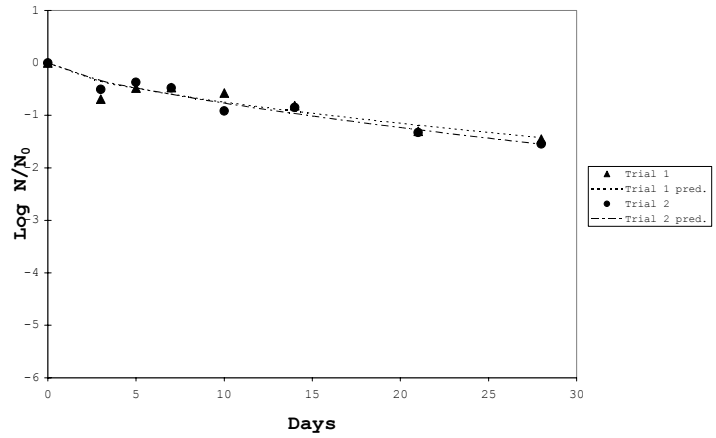
F+ RNA coliphage, Lake Lytal aquifer, raw, 5° C



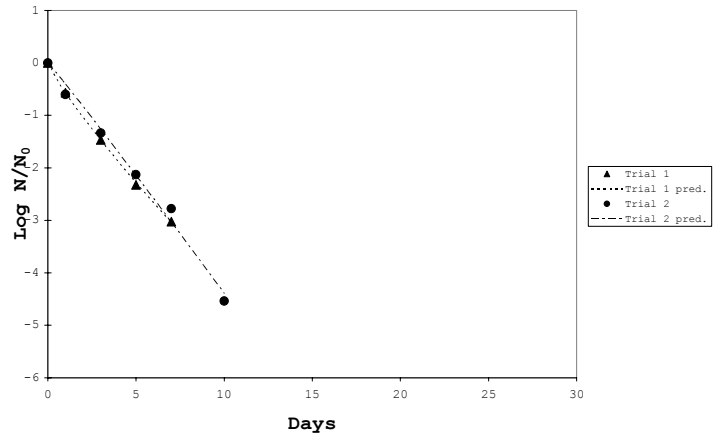
F+ RNA coliphage, Lake Lytal aquifer, raw, 22° C



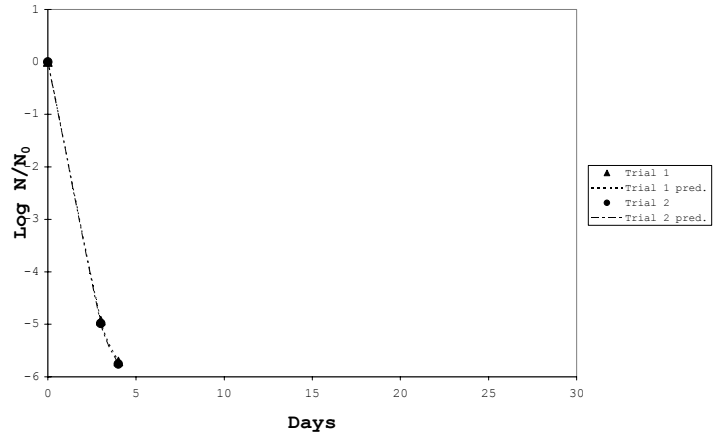
F+ RNA coliphage, Lake Lytal aquifer, raw, 30° C



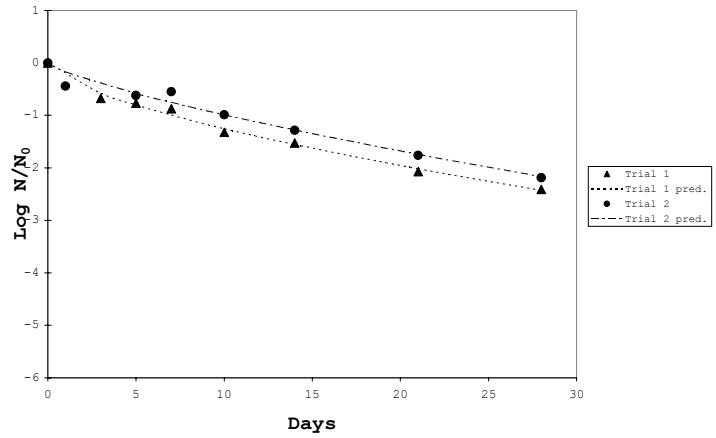
F+ RNA coliphage, Lake Lytal aquifer, pateurized, 5° C



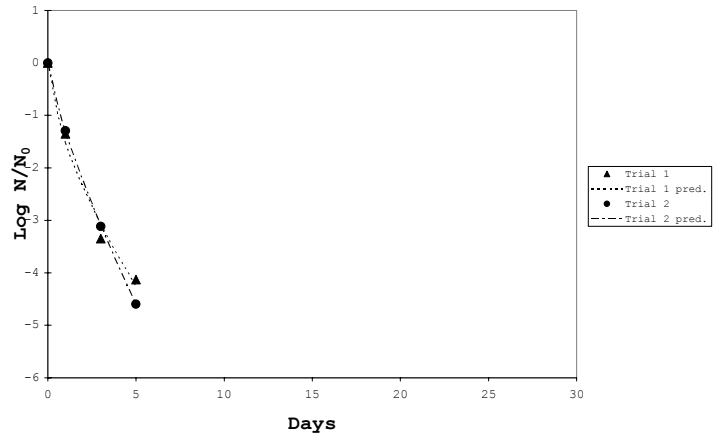
F+ RNA coliphage, Lake Lytal aquifer, pasteurized, 22° C



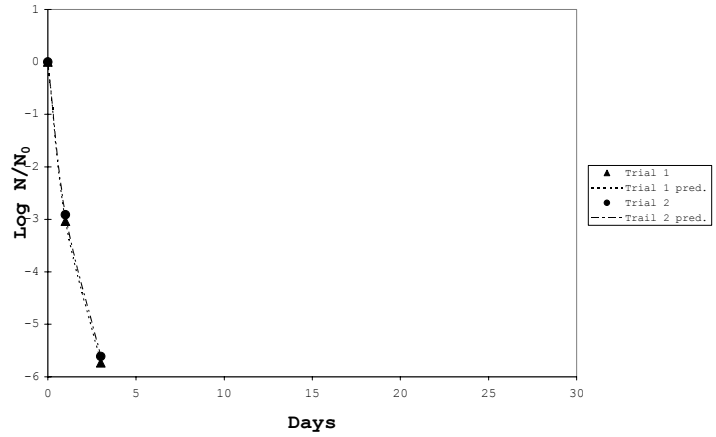
F+ RNA coliphage, Lake Lytal aquifer, pasteurized, 30° C



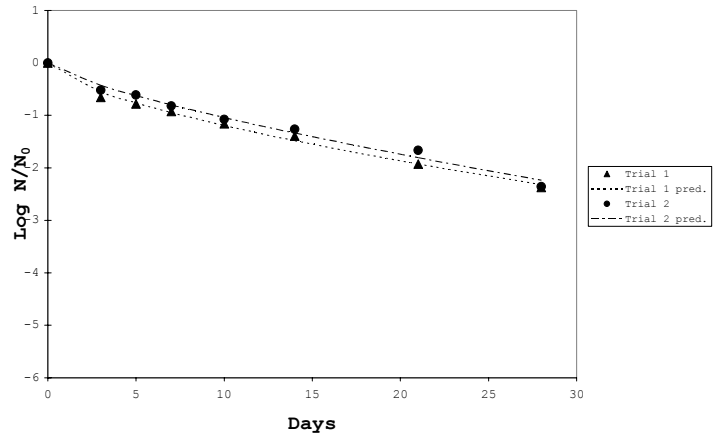
F+ RNA coliphage, Clear Lake reservoir, raw, 5° C



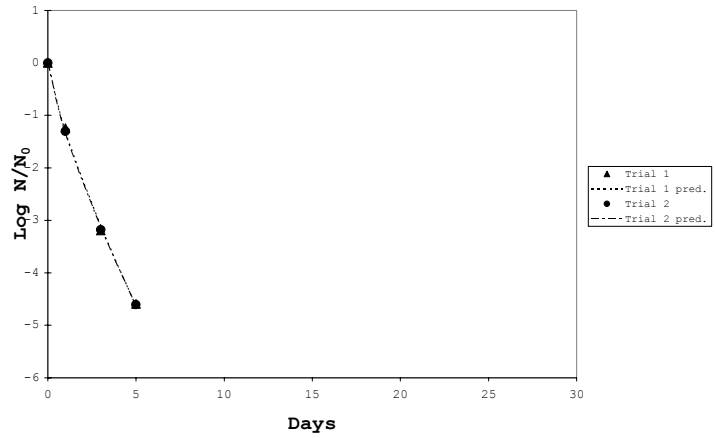
F+ RNA coliphage, Clear Lake reservoir, raw, 22° C



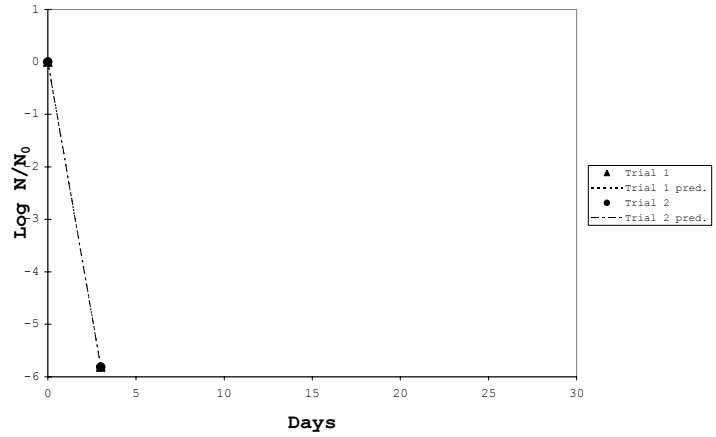
F+ RNA coliphage, Clear Lake reservoir, raw, 30° C



F+ RNA coliphage, Clear Lake reservoir, pasteurized, 5° C



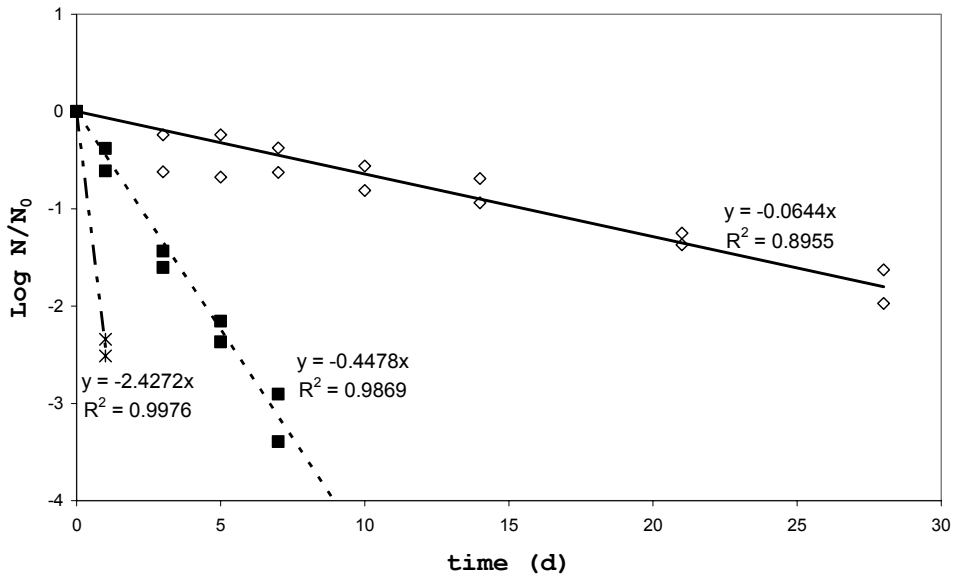
F+ RNA coliphage, Clear Lake reservoir, pasteurized, 22° C



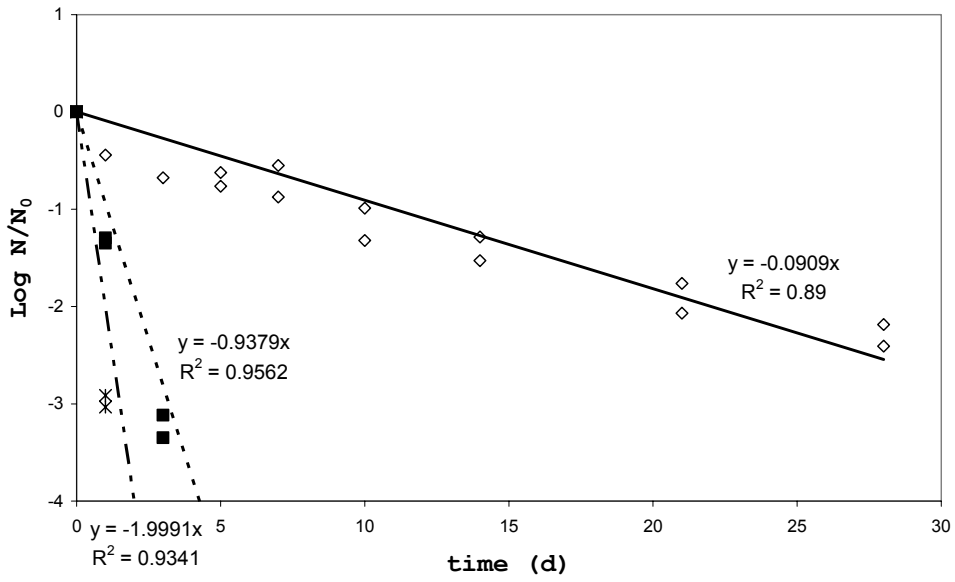
F+ RNA coliphage, Clear Lake reservoir, pasteurized, 30° C

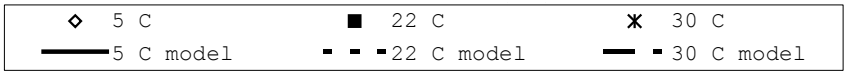
D. F+ RNA coliphage West Palm Beach site raw water plots and fitted first-order regression models

Lake Lytal Park aquifer, raw, combined F+ RNA coliphage

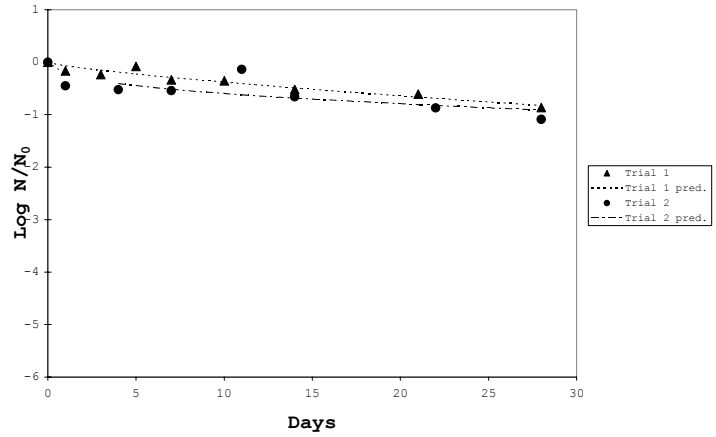


Clear Lake reservoir, raw, combined F+ RNA coliphage

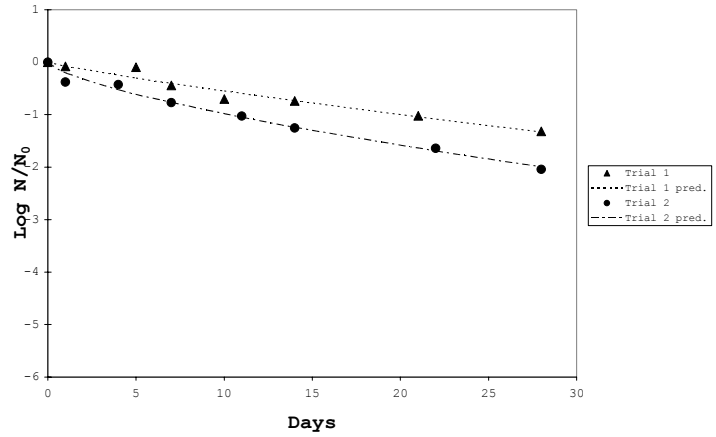




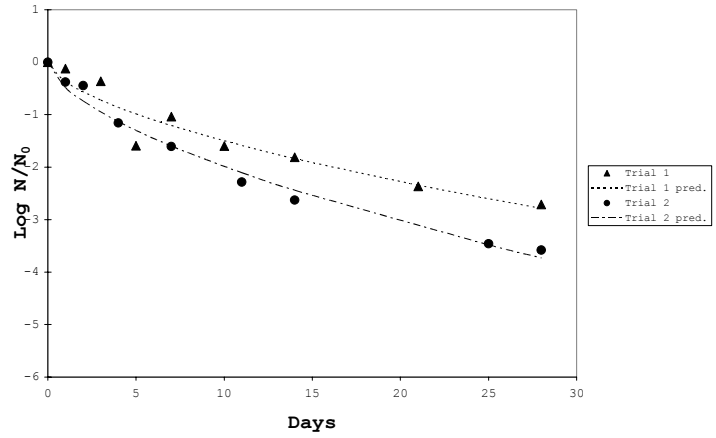
E. DNA coliphage Bradenton site natural water experiments



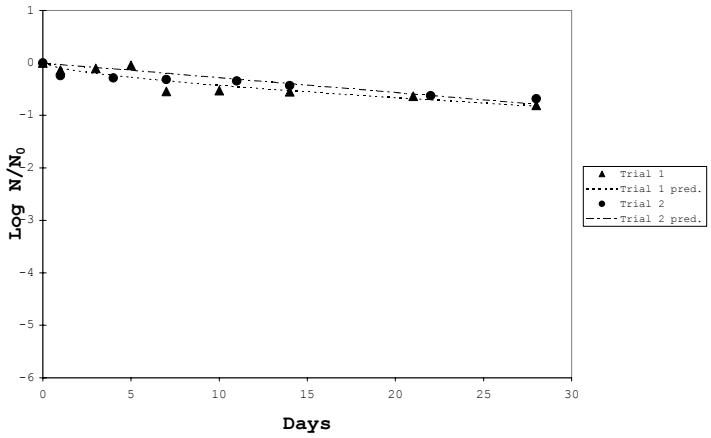
DNA coliphage, Avon Park aquifer, raw, 5° C



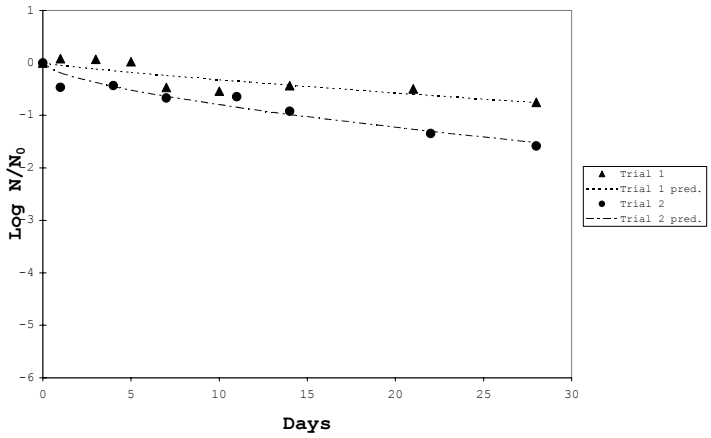
DNA coliphage, Avon Park aquifer, raw, 22° C



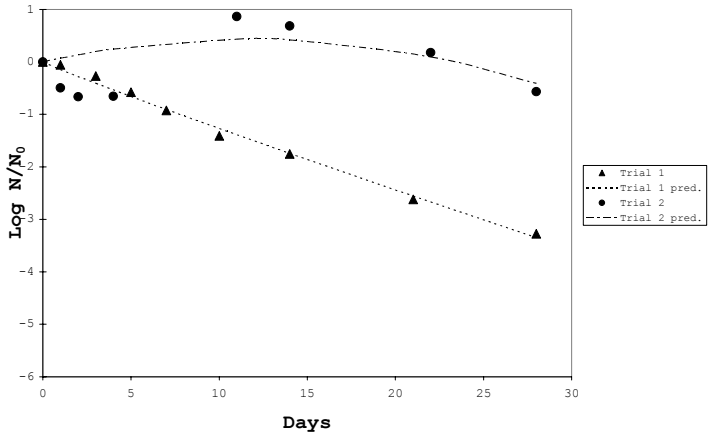
DNA coliphage, Avon Park aquifer, raw, 30° C



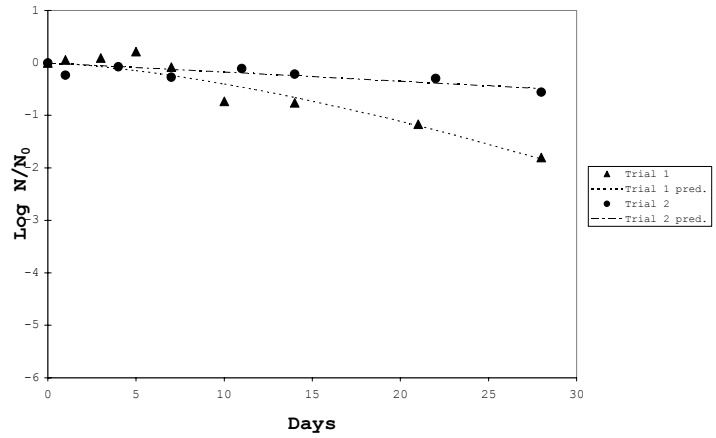
DNA coliphage, Avon Park aquifer, pasteurized, 5° C



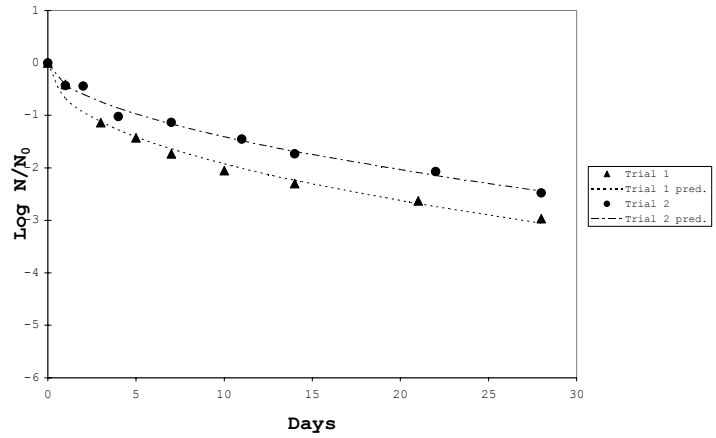
DNA coliphage, Avon Park aquifer, pasteurized, 22° C



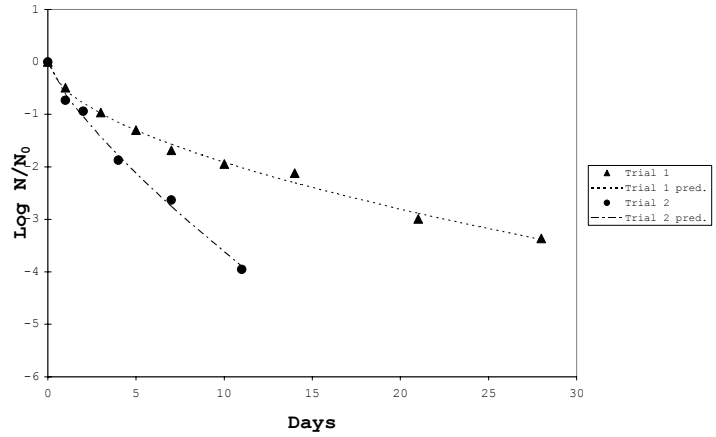
DNA coliphage, Avon Park aquifer, pasteurized, 30° C



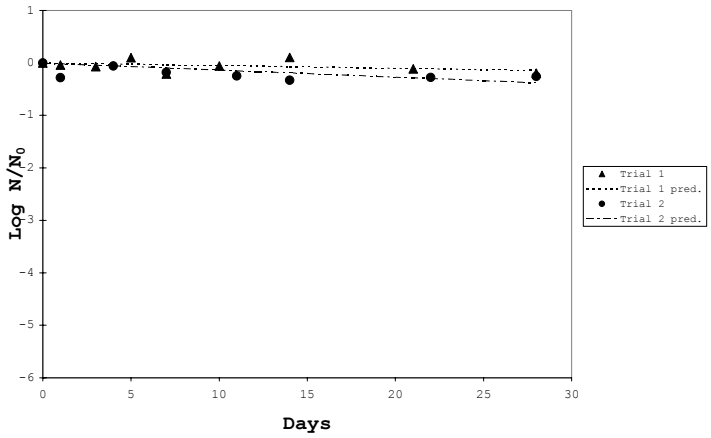
DNA coliphage, Bill Evers reservoir, raw, 5° C



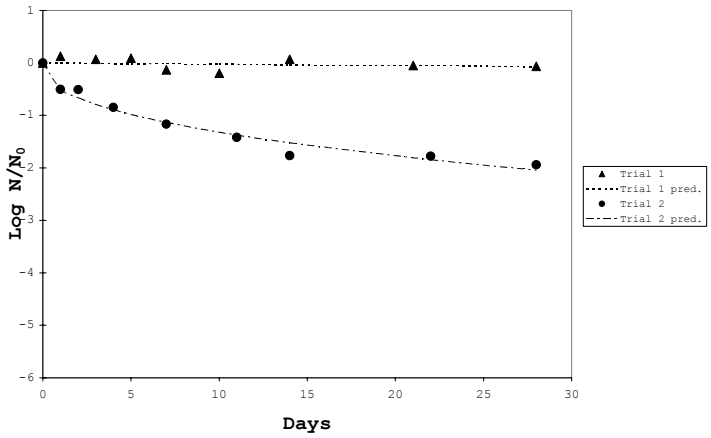
DNA coliphage, Bill Evers reservoir, raw, 22° C



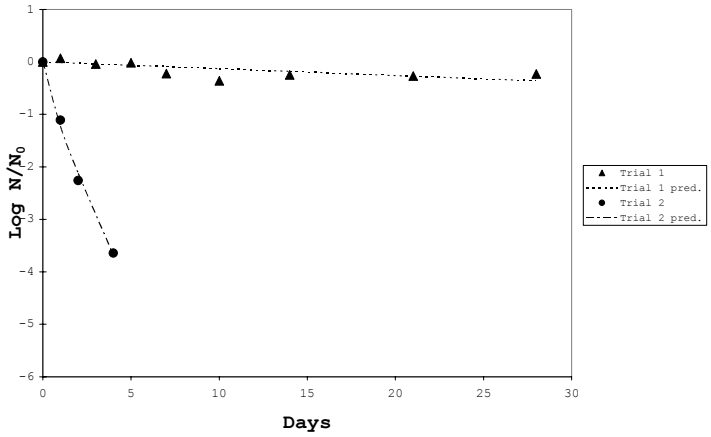
DNA coliphage, Bill Evers reservoir, raw, 30° C



DNA coliphage, Bill Evers reservoir, pasteurized, 5° C



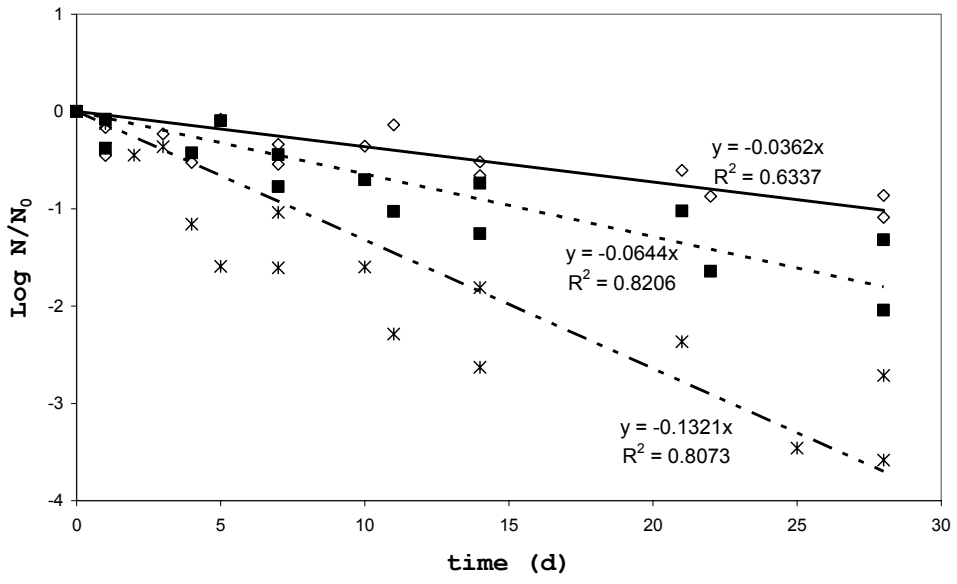
DNA coliphage, Bill Evers reservoir, pasteurized, 22° C



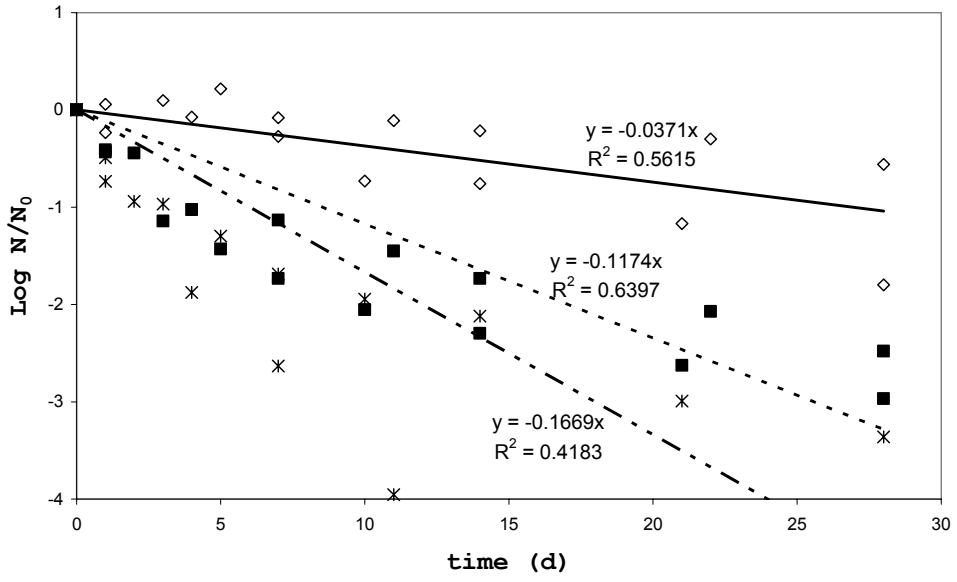
DNA coliphage, Bill Evers reservoir, pasteurized, 30° C

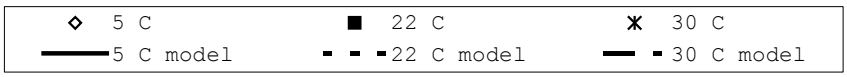
F. DNA coliphage Bradenton site raw water plots and fitted first-order regression models

Avon Park aquifer, raw, combined DNA coliphage

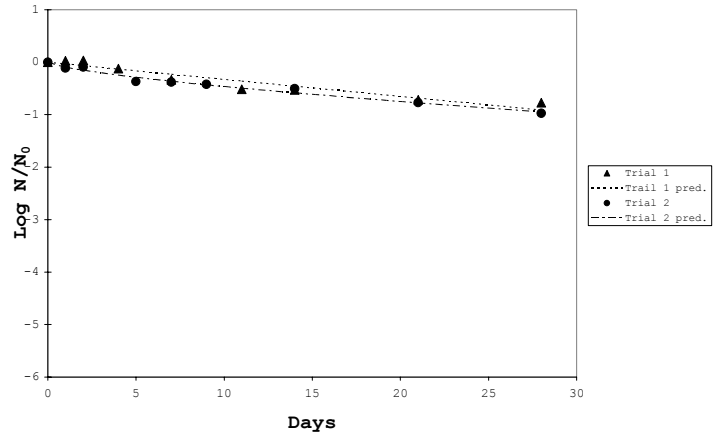


Bill Evers reservoir, raw, combined DNA coliphage

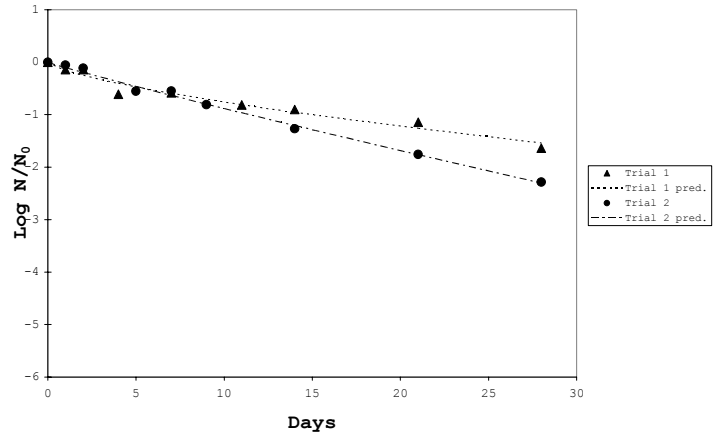




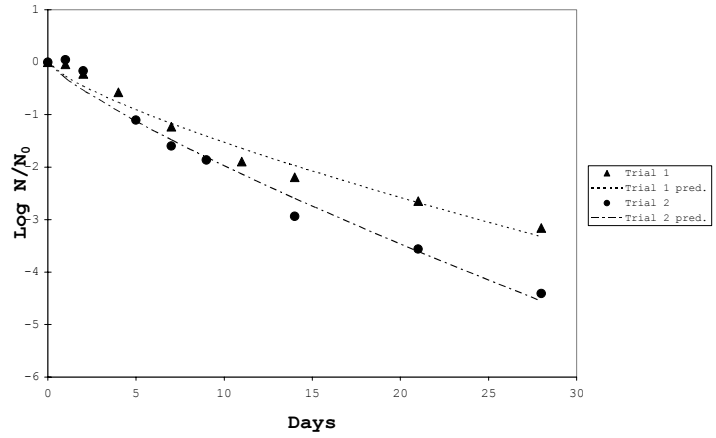
G. DNA coliphage West Palm Beach site natural water experiments



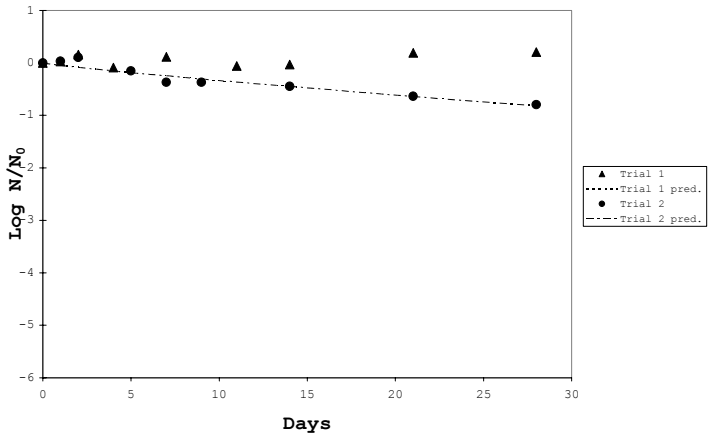
DNA coliphage, Lake Lytal aquifer, raw, 5° C



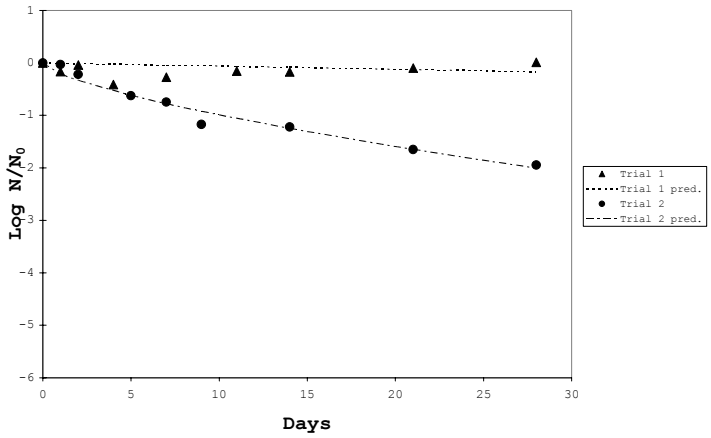
DNA coliphage, Lake Lytal aquifer, raw, 22° C



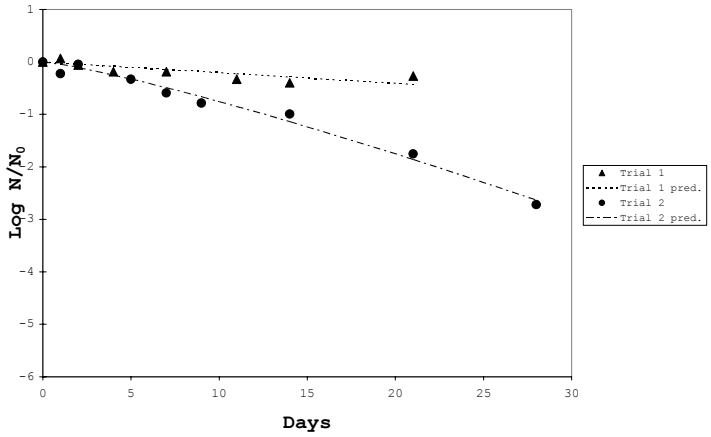
DNA coliphage, Lake Lytal aquifer, raw, 30° C



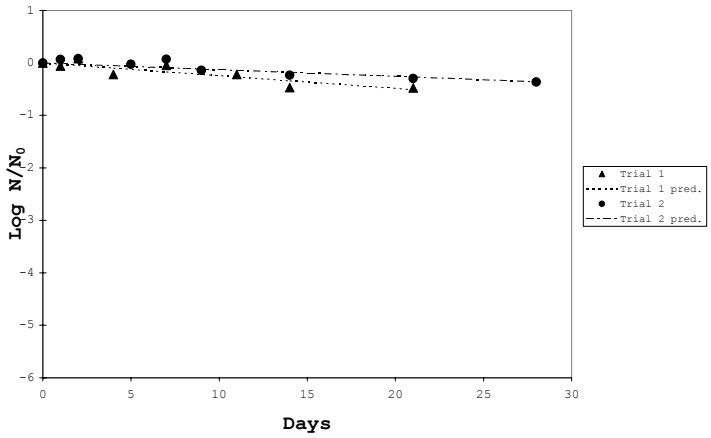
DNA coliphage, Lake Lytal aquifer, pasteurized, 5° C



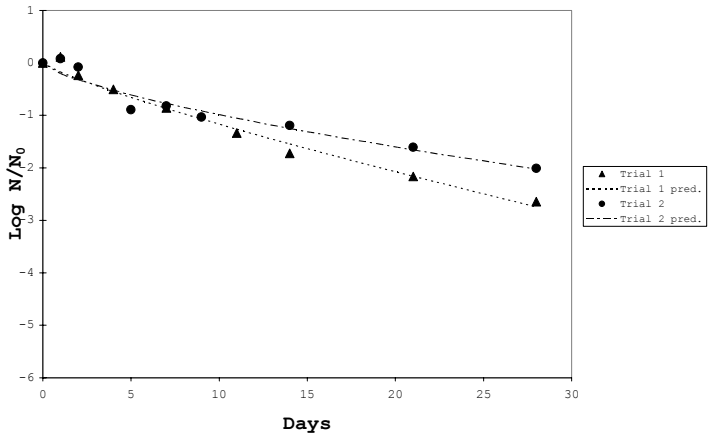
DNA coliphage, Lake Lytal aquifer, pasteurized, 22° C



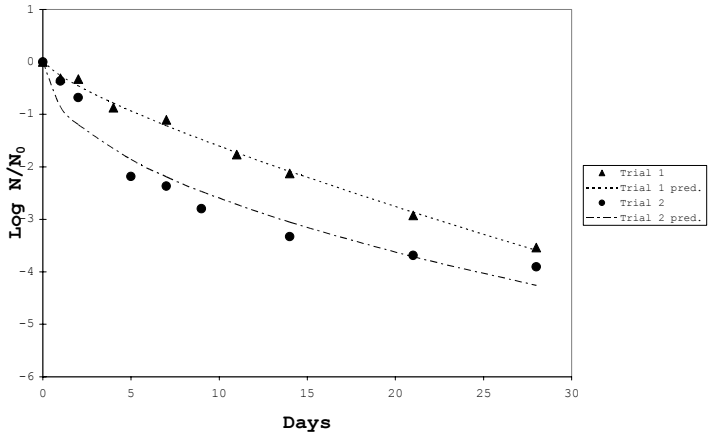
DNA coliphage, Lake Lytal aquifer, pasteurized, 30° C



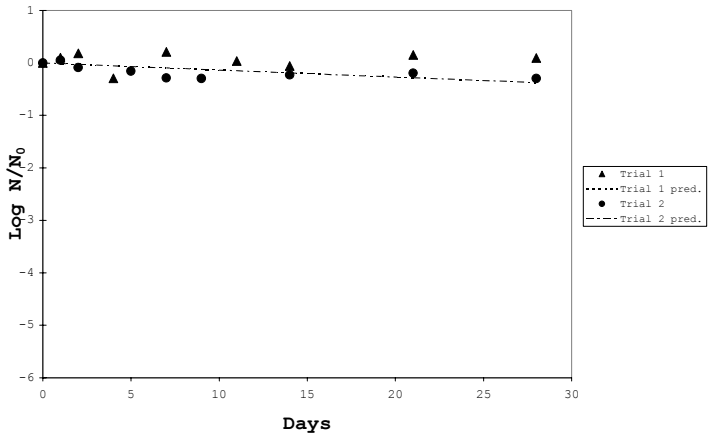
DNA coliphage, Clear Lake reservoir, raw, 5° C



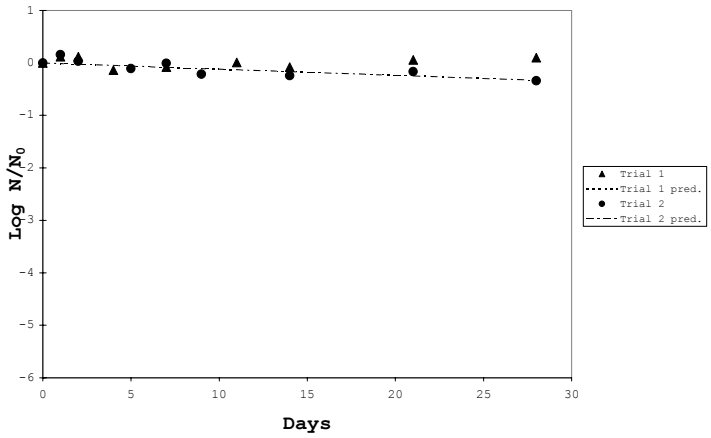
DNA coliphage, Clear Lake reservoir, raw, 22° C



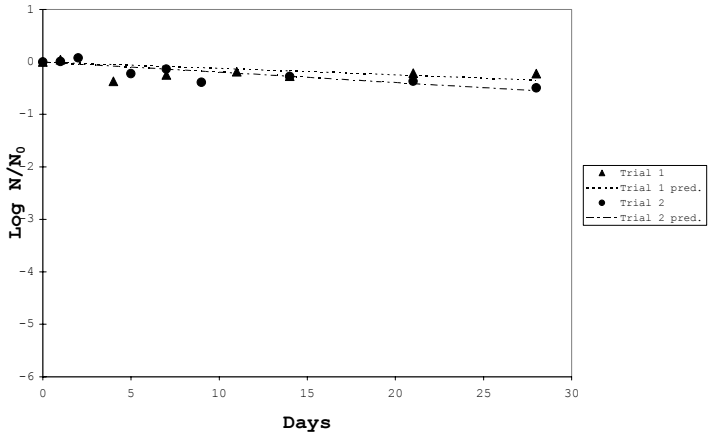
DNA coliphage, Clear Lake reservoir, raw, 30° C



DNA coliphage, Clear Lake reservoir, pasteurized, 5° C



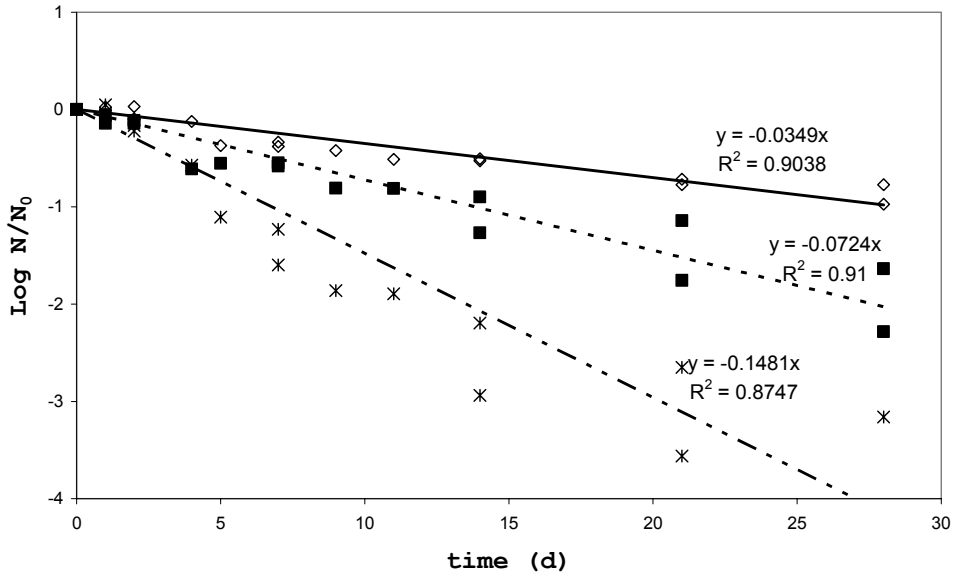
DNA coliphage, Clear Lake reservoir, pasteurized, 22° C



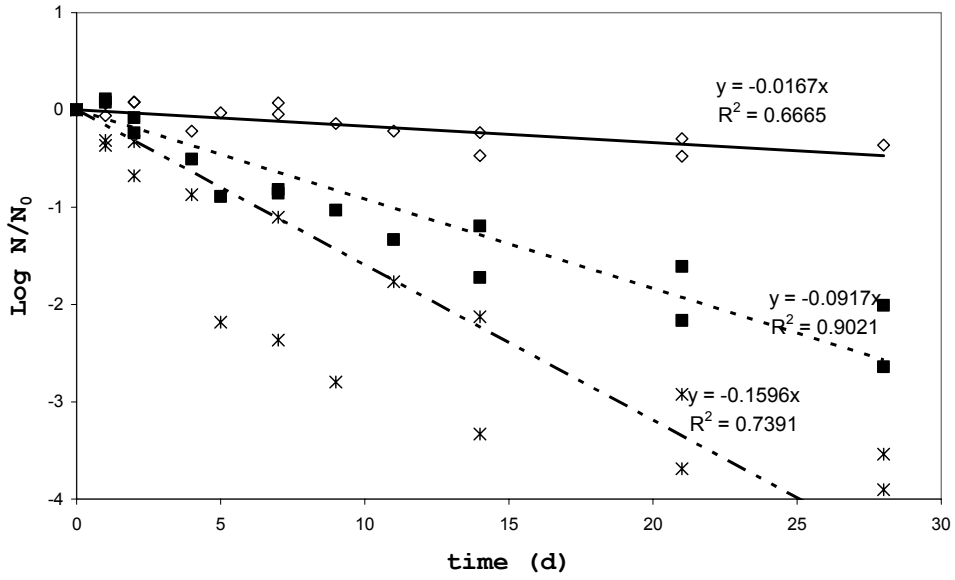
DNA coliphage, Clear Lake reservoir, pasteurized, 30° C

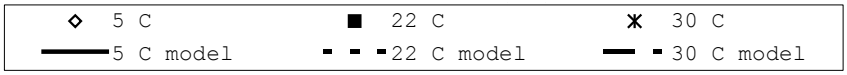
H. DNA coliphage West Palm Beach site raw water plots and fitted first-order regression models

Lake Lytal Park aquifer, raw, combined DNA coliphage

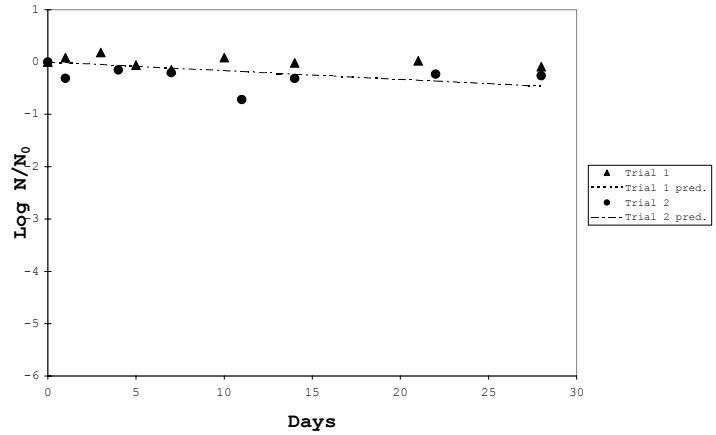


Clear Lake reservoir, raw, combined DNA coliphage

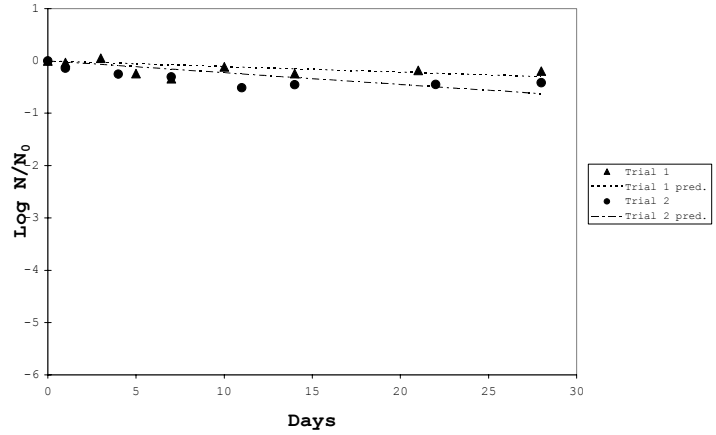




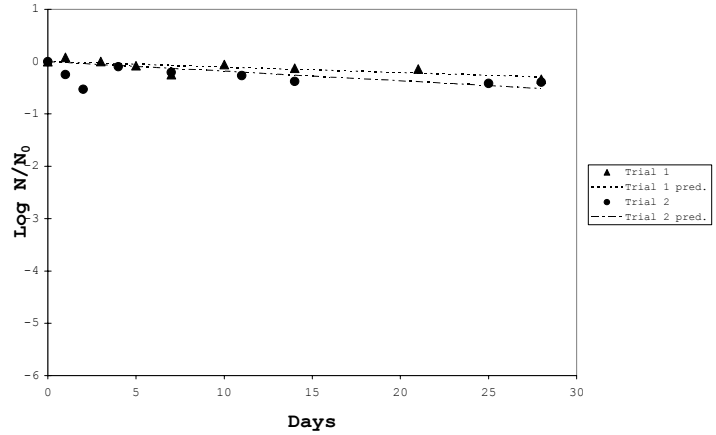
I. PRD-1 Bradenton site natural water experiments



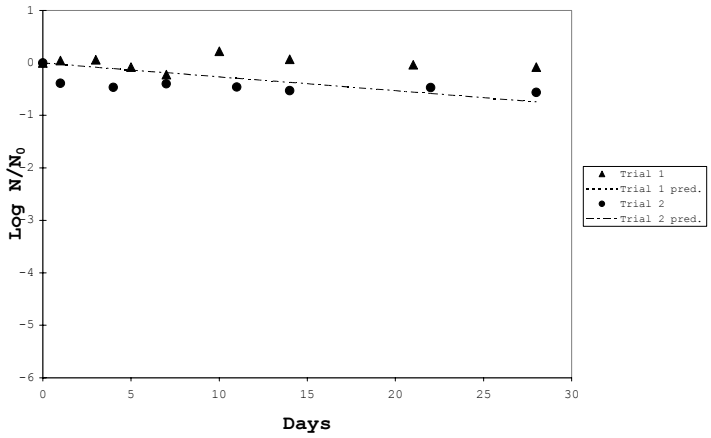
PRD-1, Avon Park aquifer, raw, 5° C



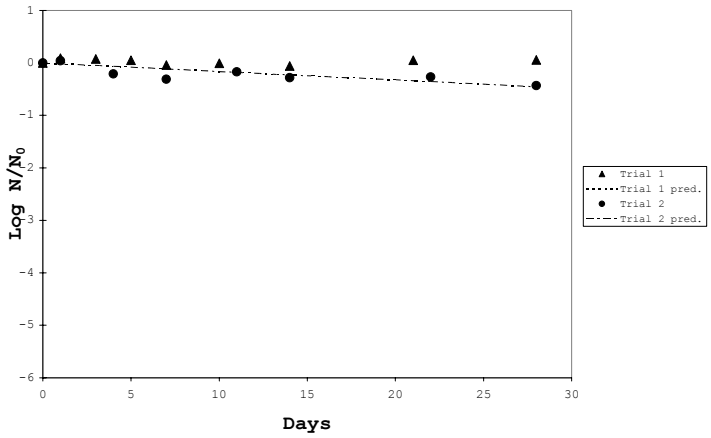
PRD-1, Avon Park aquifer, raw, 22° C



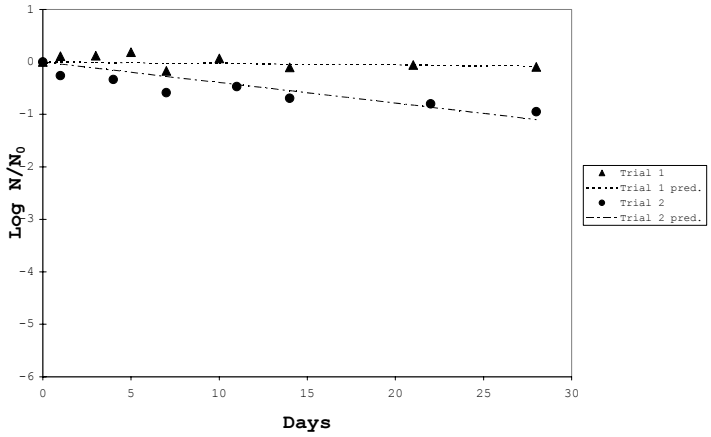
PRD-1, Avon Park aquifer, raw, 30° C



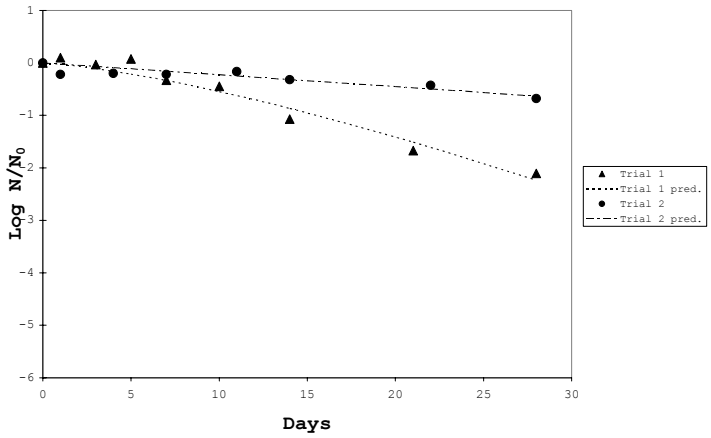
PRD-1, Avon Park aquifer, pasteurized, 5° C



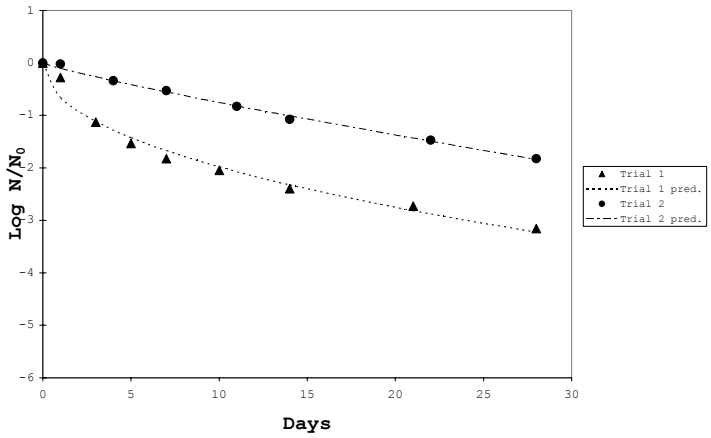
PRD-1, Avon Park aquifer, pasteurized, 22° C



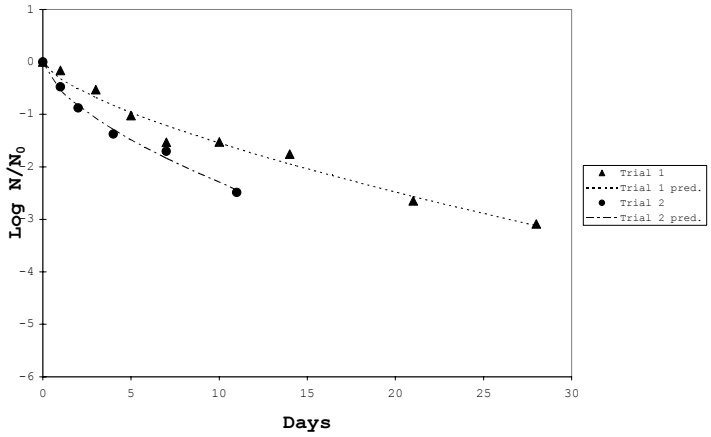
PRD-1, Avon Park aquifer, pasteurized, 30° C



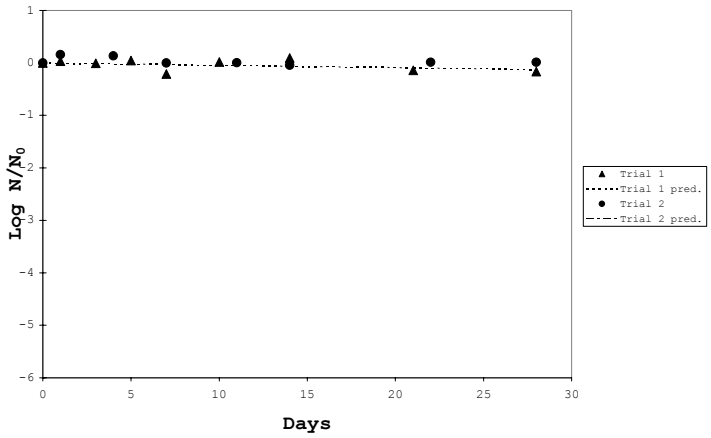
PRD-1, Bill Evers reservoir, raw, 5° C



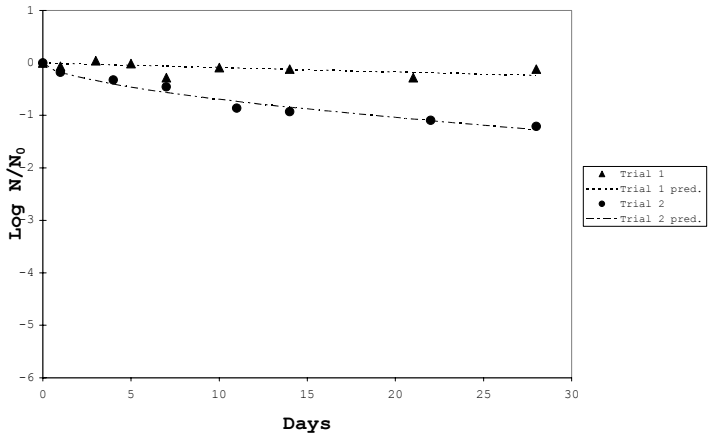
PRD-1, Bill Evers reservoir, raw, 22° C



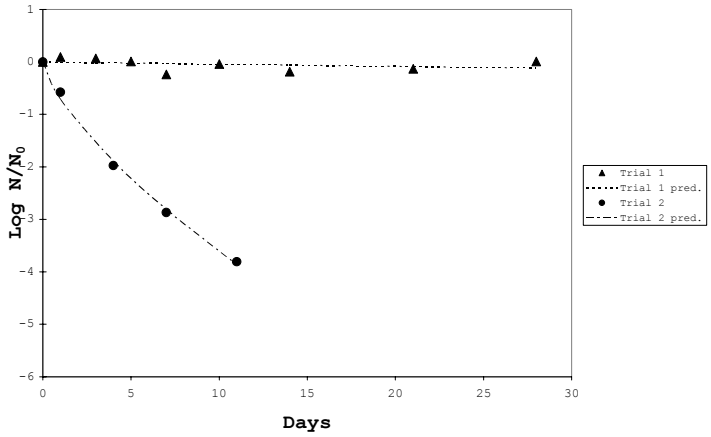
PRD-1, Bill Evers reservoir, raw, 30° C



PRD-1, Bill Evers reservoir, pasteurized, 5° C



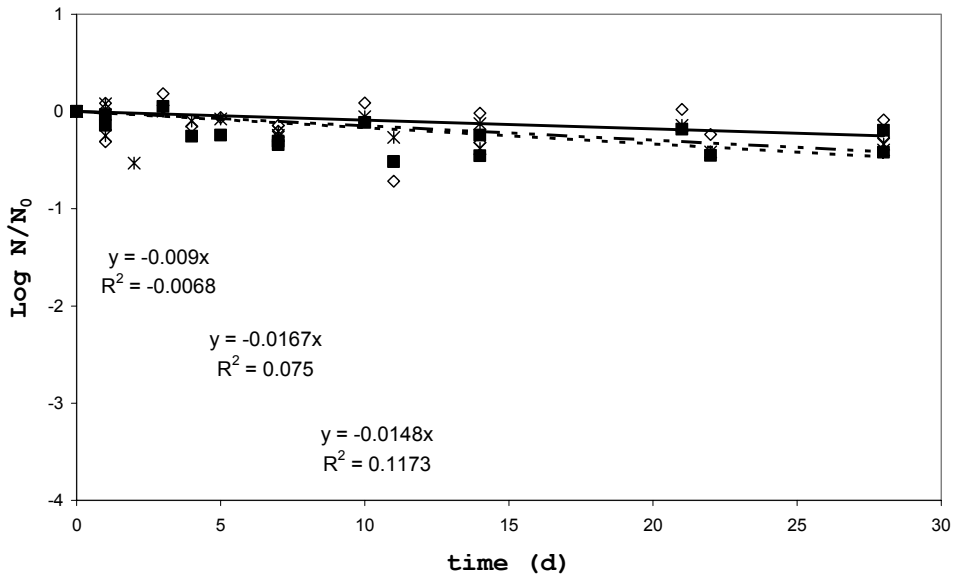
PRD-1, Bill Evers reservoir, pasteurized, 22° C



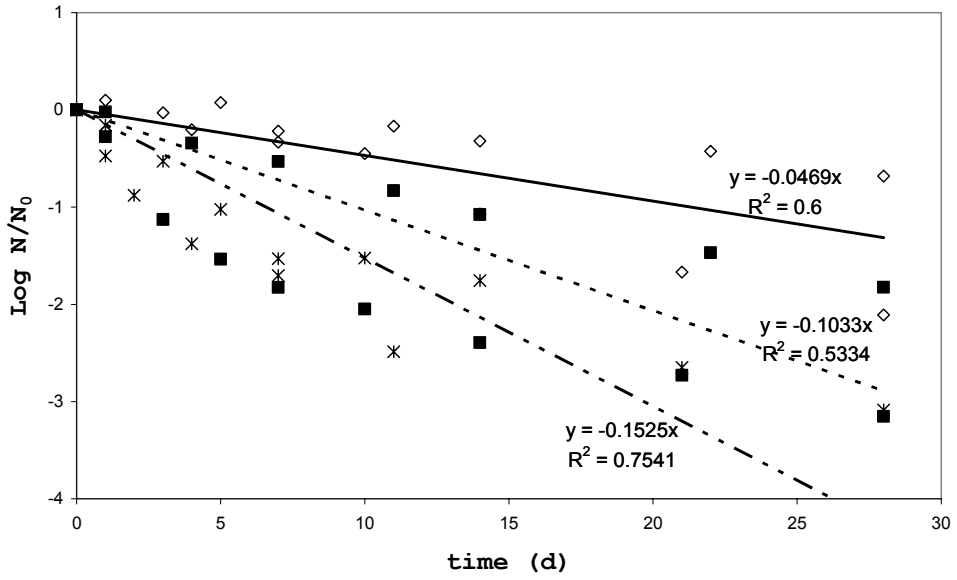
PRD-1, Bill Evers reservoir, pasteurized, 30° C

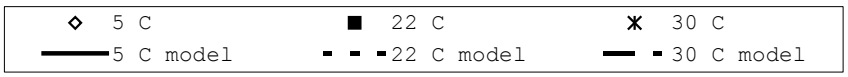
J. PRD-1 Bradenton site raw water plots and fitted first-order regression models

Avon Park aquifer, raw, combined PRD-1

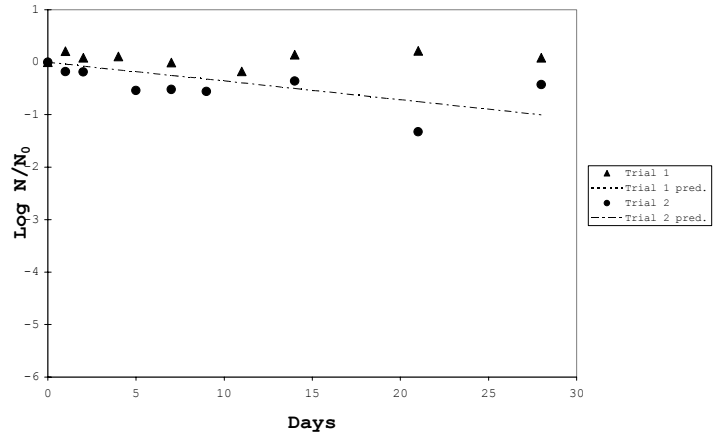


Bill Evers reservior, raw, combined PRD-1

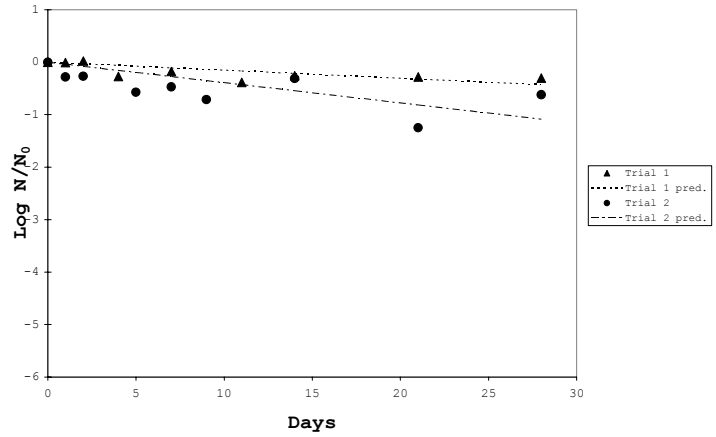




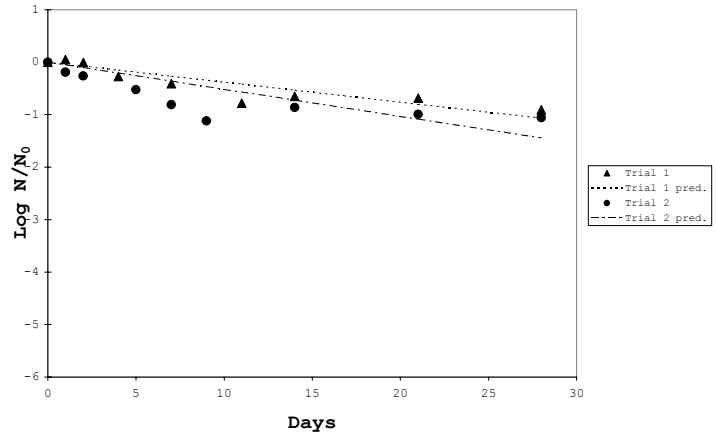
K. PRD-1 West Palm Beach natural water experiments



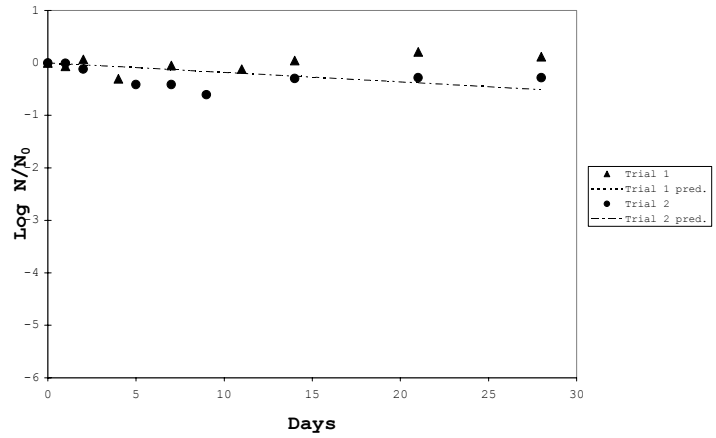
PRD-1, Lake Lytal aquifer, raw, 5° C



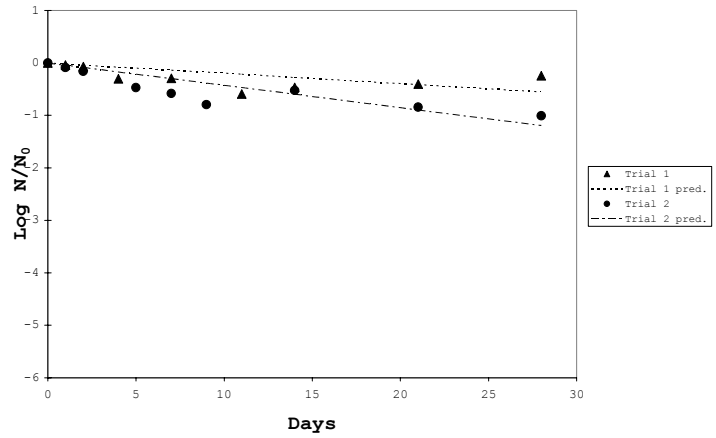
PRD-1, Lake Lytal aquifer, raw, 22° C



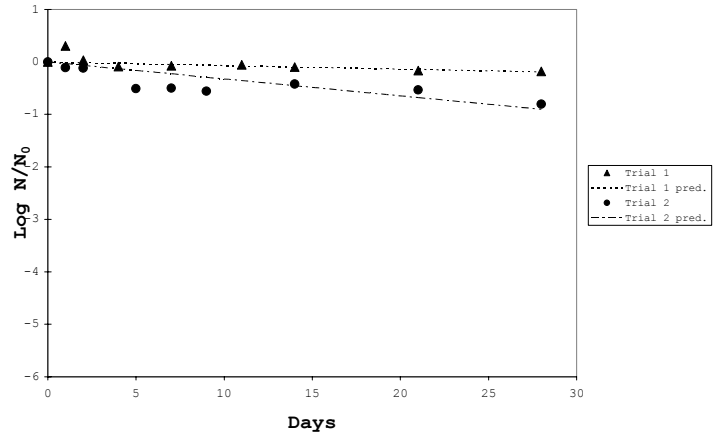
PRD-1, Lake Lytal aquifer, raw, 30° C



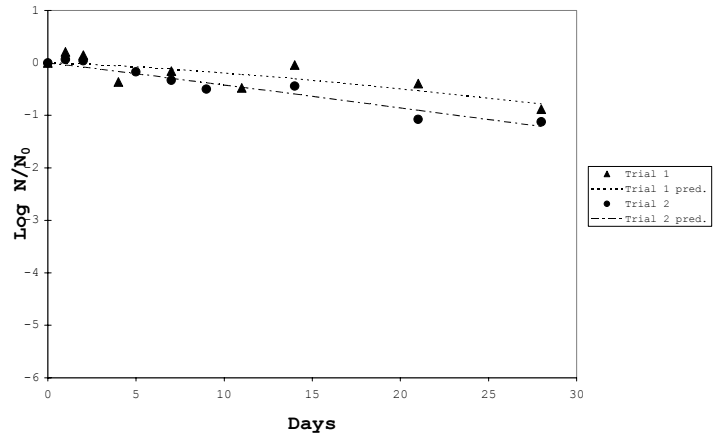
PRD-1, Lake Lytal aquifer, pasteurized, 5° C



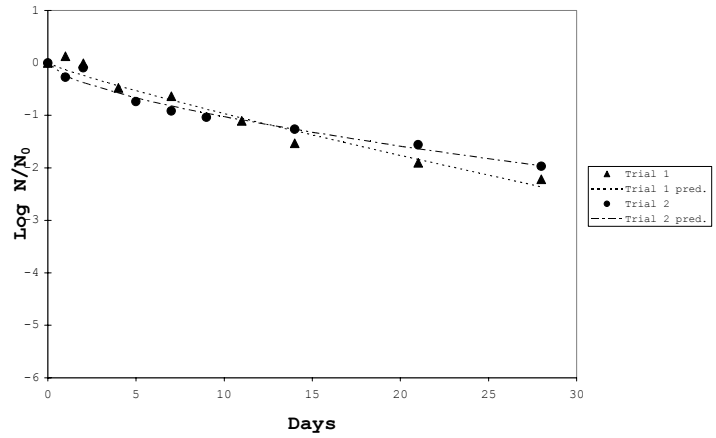
PRD-1, Lake Lytal aquifer, pasteurized, 22° C



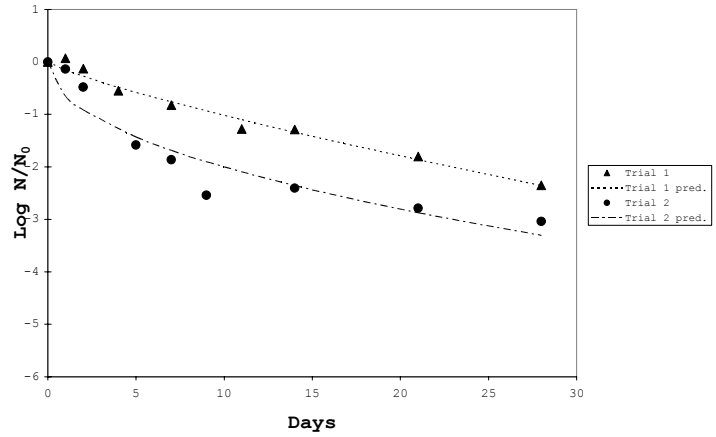
PRD-1, Lake Lytal aquifer, pasteurized, 30° C



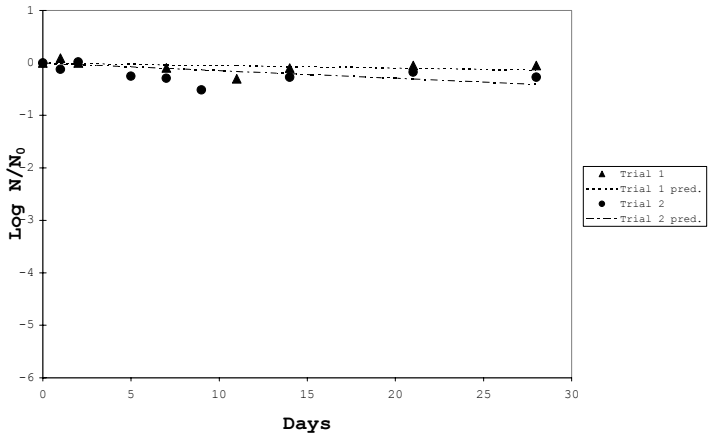
PRD-1, Clear Lake reservoir, raw, 5° C



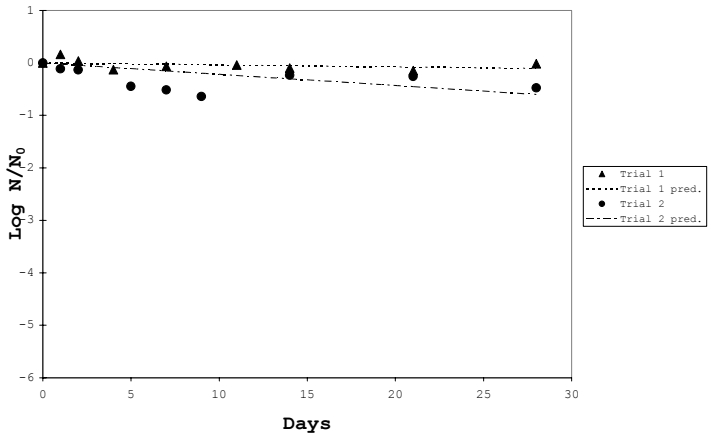
PRD-1, Clear Lake reservoir, raw, 22° C



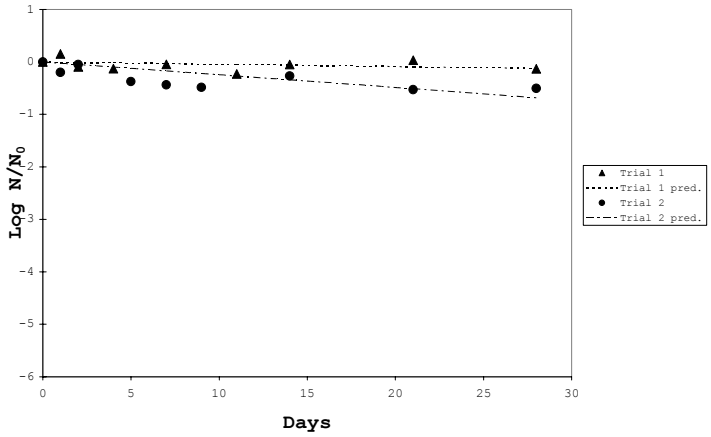
PRD-1, Clear Lake reservoir, raw, 30° C



PRD-1, Clear Lake reservoir, pasteurized, 5° C



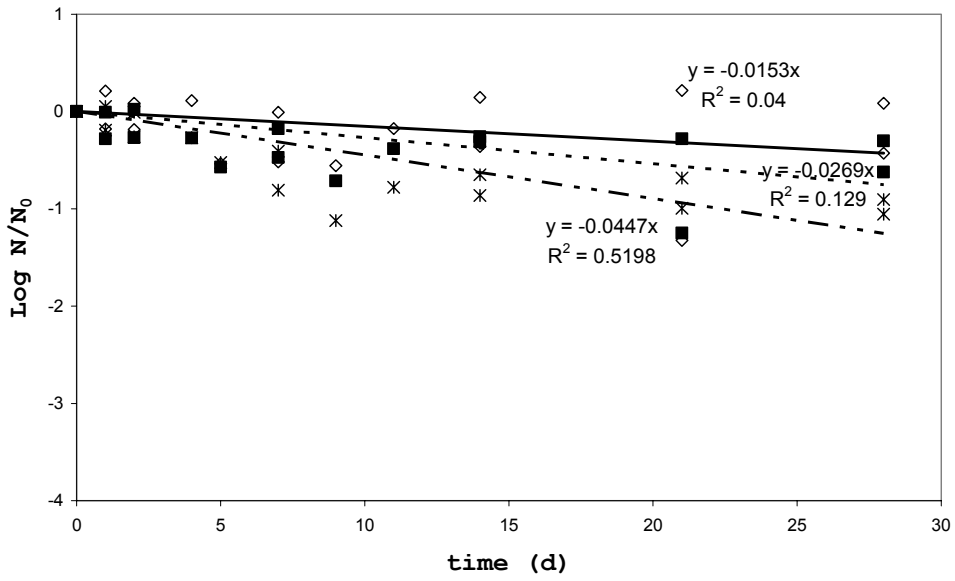
PRD-1, Clear Lake reservoir, pasteurized, 22° C



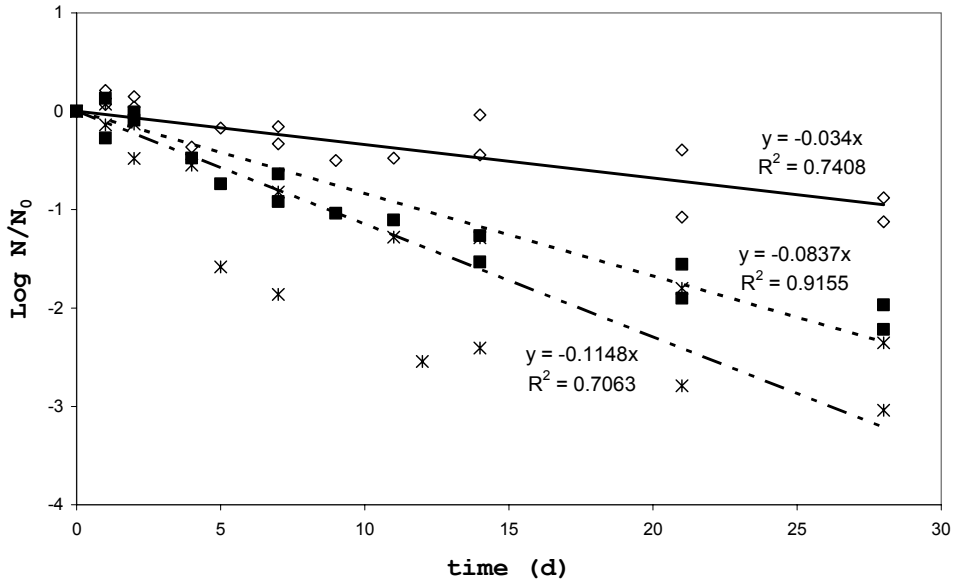
PRD-1, Clear Lake reservoir, pasteurized, 30° C

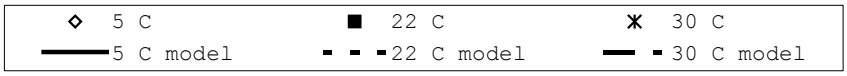
L. PRD-1 West Palm Beach site raw water plots and fitted first-order regression models

Lake Lytal Park aquifer, raw, combined PRD-1

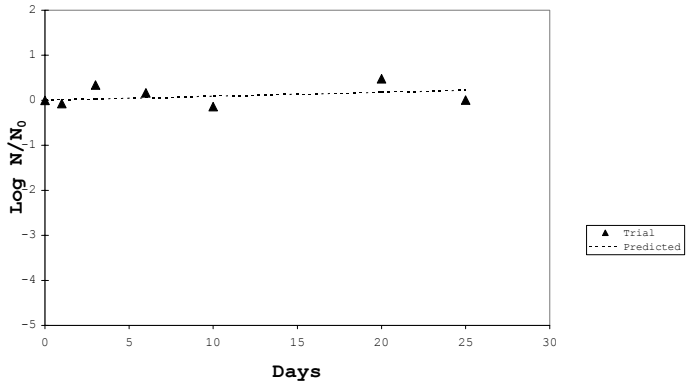


Clear Lake reservoir, raw, combined PRD-1

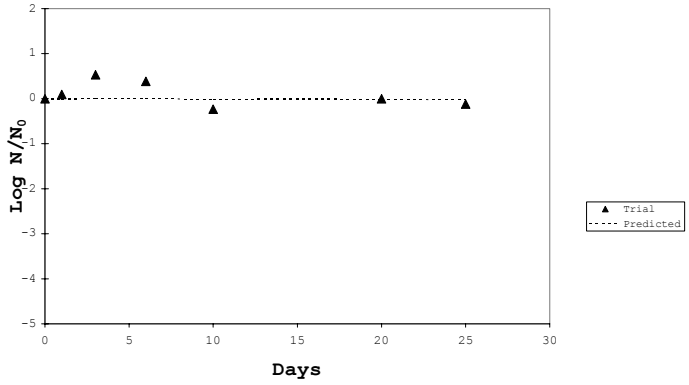




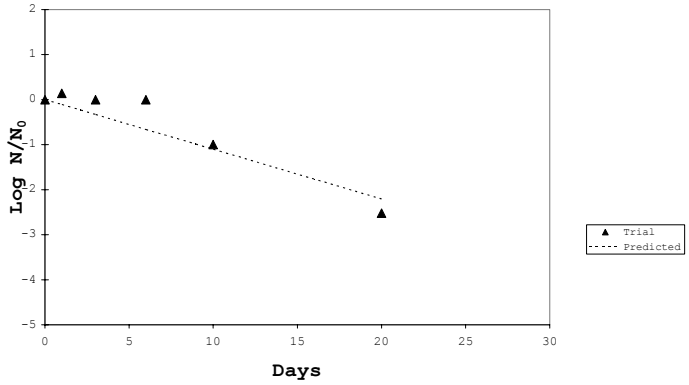
M. *Cryptosporidium parvum* natural water observed infectivity results and model curves, both sites



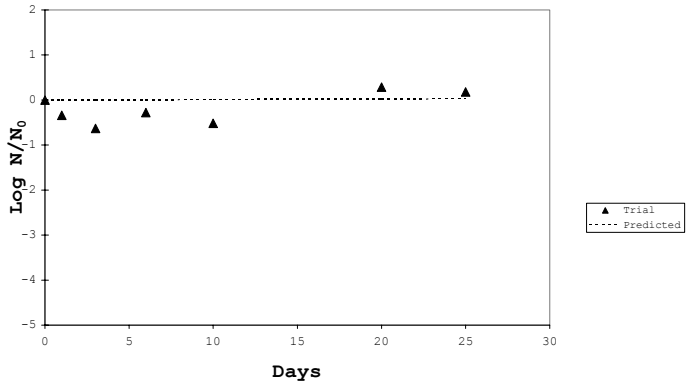
***Cryptosporidium parvum*, Avon Park, 5° C**



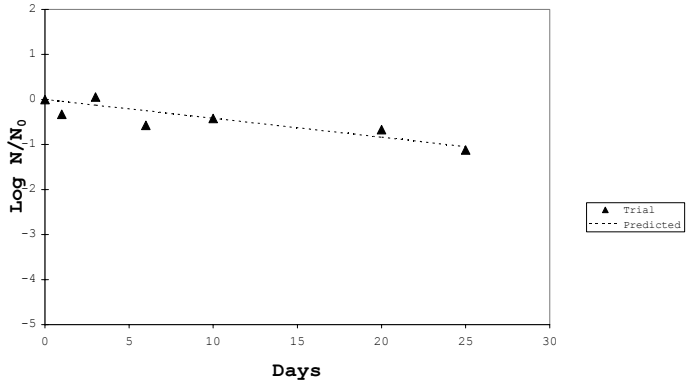
***Cryptosporidium parvum*, Avon Park, 22° C**



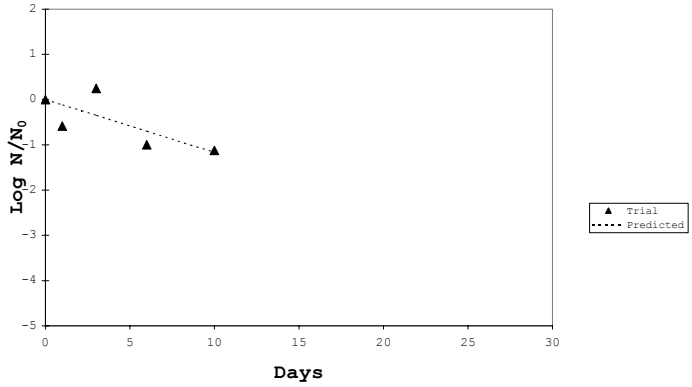
***Cryptosporidium parvum*, Avon Park, 30° C**



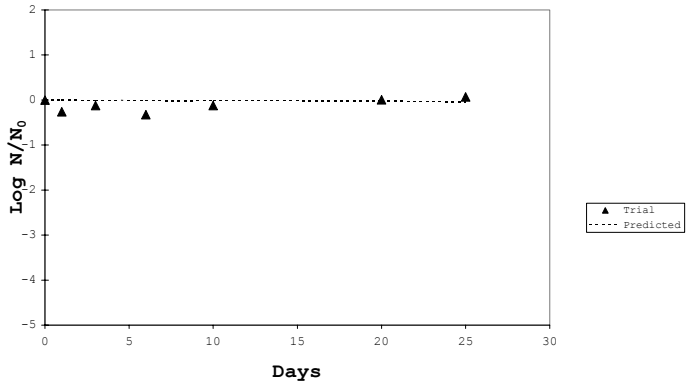
***Cryptosporidium parvum*, Lake Lytal Well, 5° C**



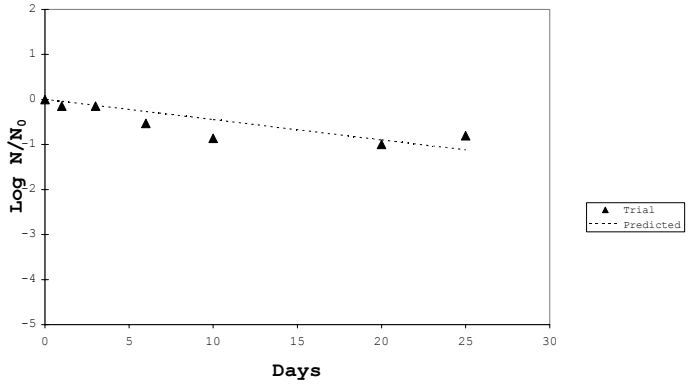
***Cryptosporidium parvum*, Lake Lytal Well, 22° C**



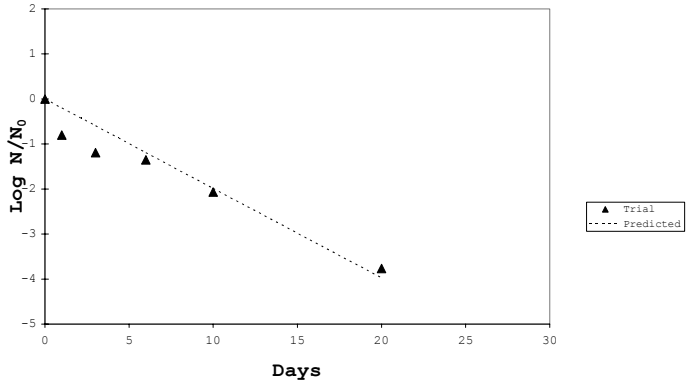
***Cryptosporidium parvum*, Lake Lytal Well, 30° C**



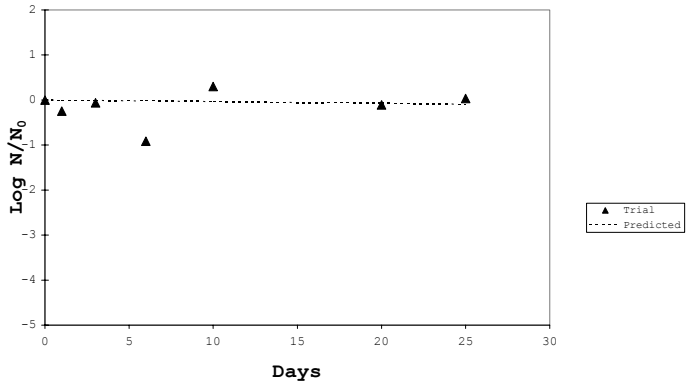
Cryptosporidium parvum, Bill Evers Reservoir, 5° C



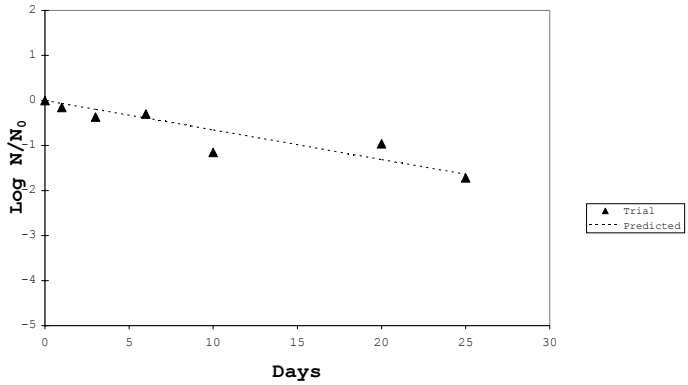
Cryptosporidium parvum, Bill Evers Reservoir, 22° C



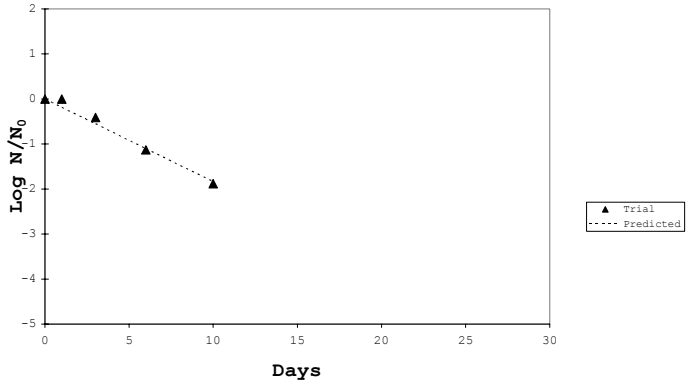
Cryptosporidium parvum, Bill Evers Reservoir, 30° C



***Cryptosporidium parvum*, Clear Lake Reservoir, 5° C**

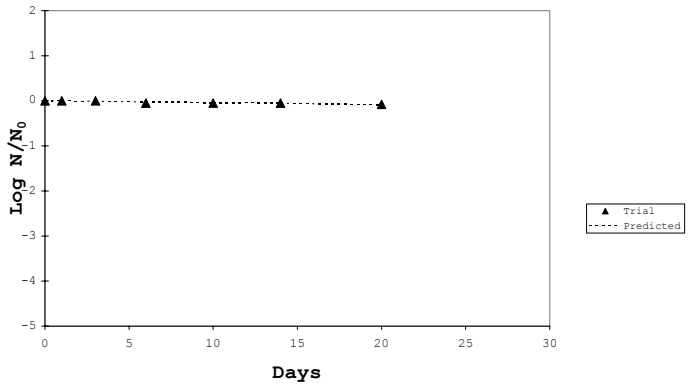


***Cryptosporidium parvum*, Clear Lake Reservoir, 22° C**

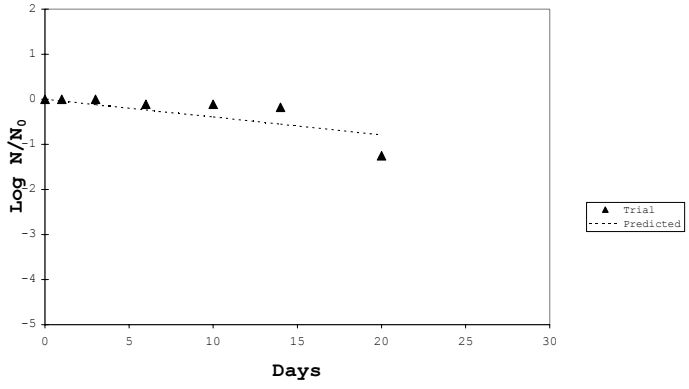


***Cryptosporidium parvum*, Clear Lake Reservoir, 30° C**

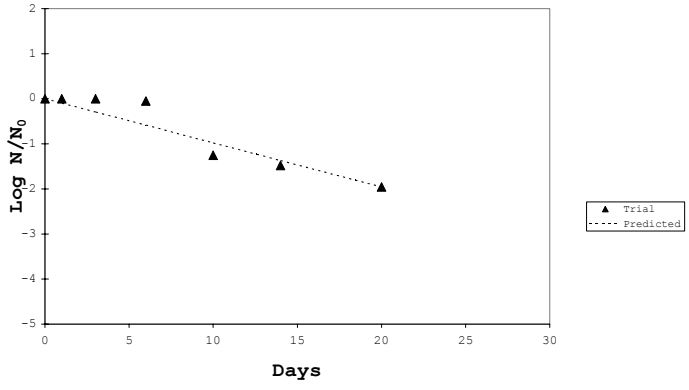
N. *Giardia lamblia* natural water observed viability results and model curves, both sites



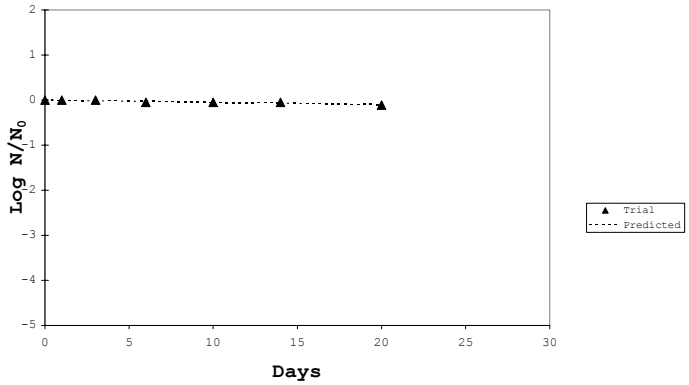
***Giardia lamblia*, Avon Park, 5° C**



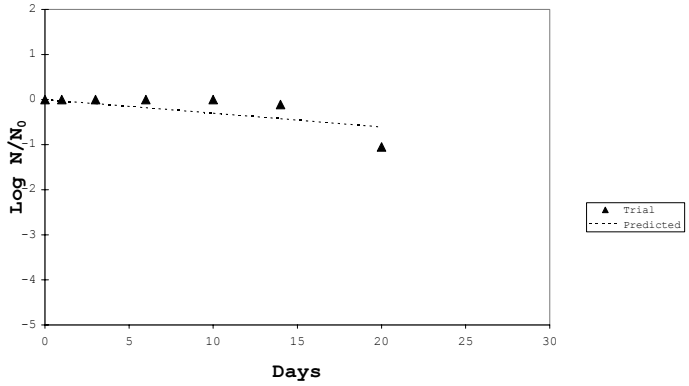
***Giardia lamblia*, Avon Park, 22° C**



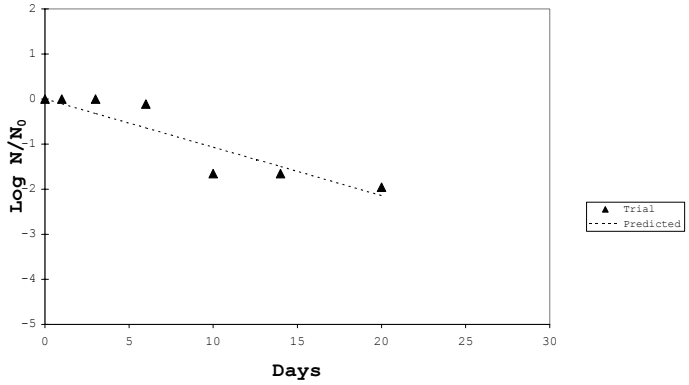
***Giardia lamblia*, Avon Park, 30° C**



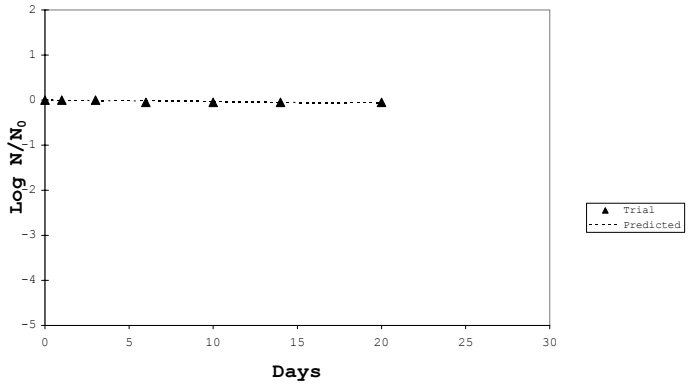
***Giardia lamblia*, Lake Lytal Well, 5° C**



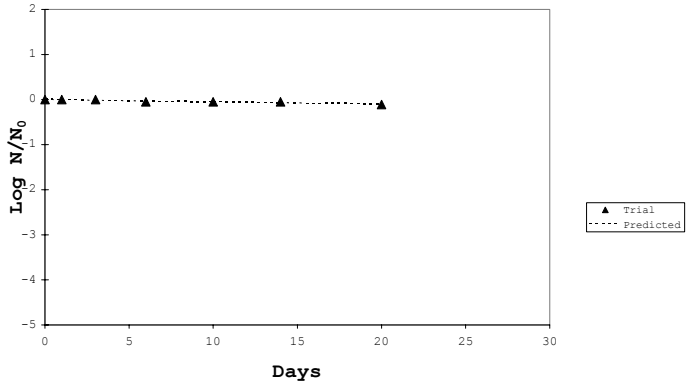
***Giardia lamblia*, Lake Lytal, 22° C**



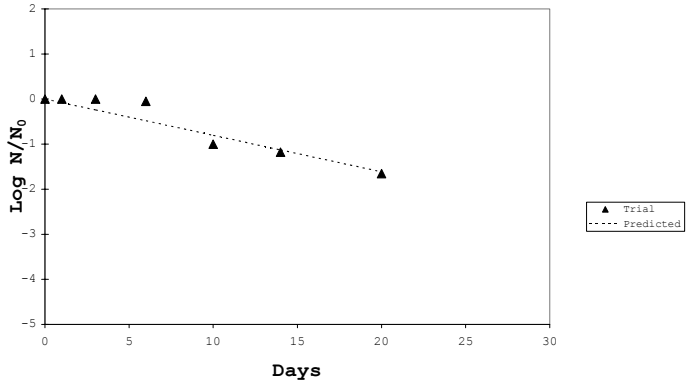
***Giardia lamblia*, Lake Lytal Well, 30° C**



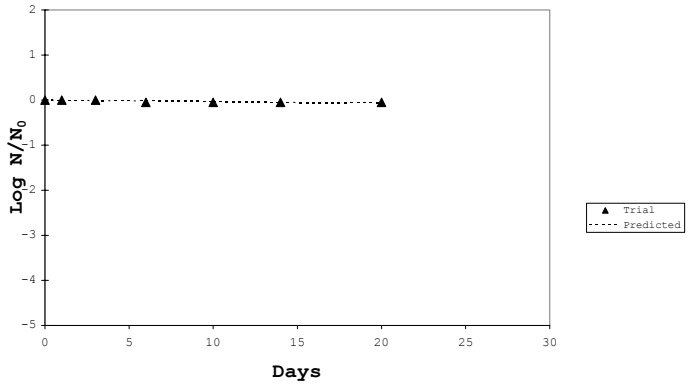
***Giardia lamblia*, Bill Evers Reservoir, 5° C**



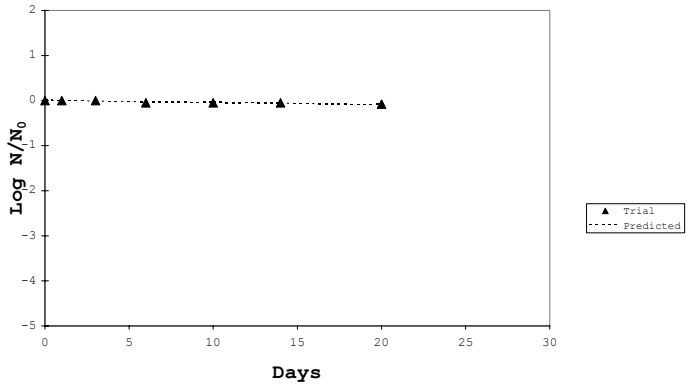
***Giardia lamblia*, Bill Evers Reservoir, 22° C**



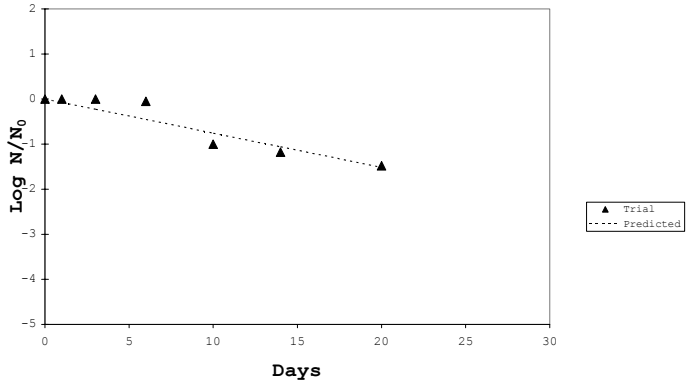
***Giardia lamblia*, Bill Evers Reservoir, 30° C**



***Giardia lamblia*, Clear Lake Reservoir, 5° C**



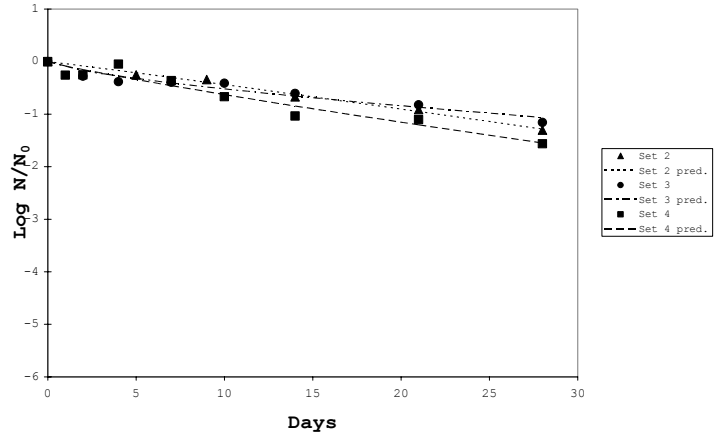
***Giardia lamblia*, Clear Lake Reservoir, 22° C**



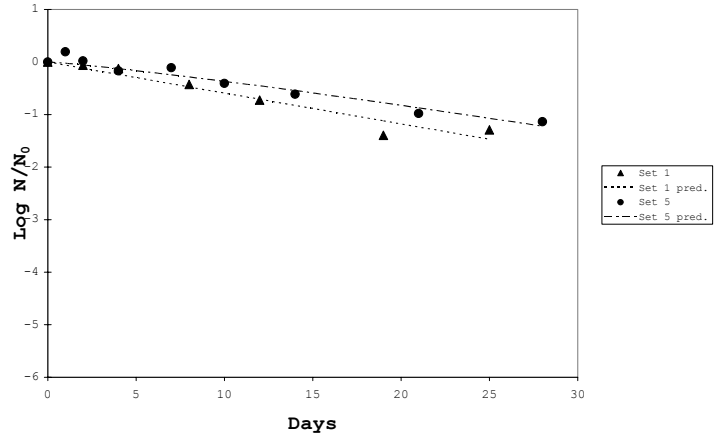
***Giardia lamblia*, Clear Lake Reservoir, 30° C**

APPENDIX 4. Observed data plots, fitted model curves and statistics for PBS control trials, indicator organisms

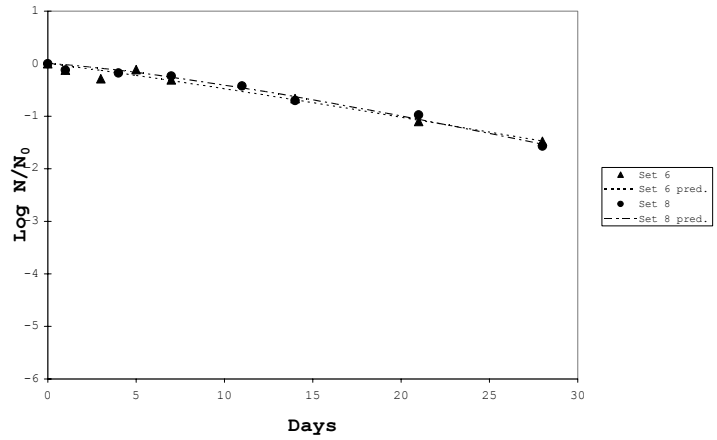
A. Fecal coliform PBS results



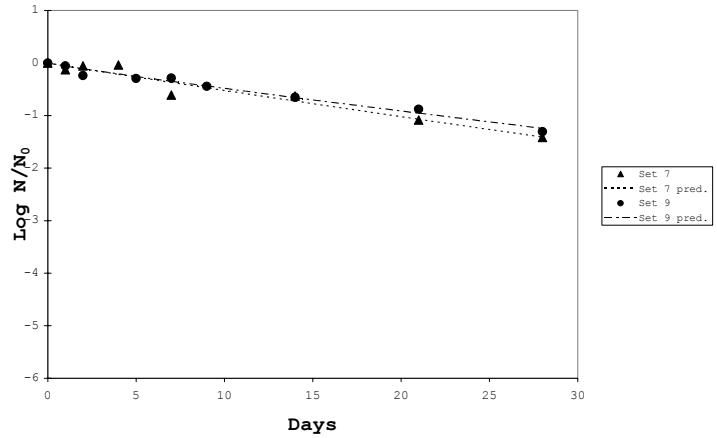
Fecal coliform, PBS, 5° C, Sets 2, 3, & 4 (ASW trials)



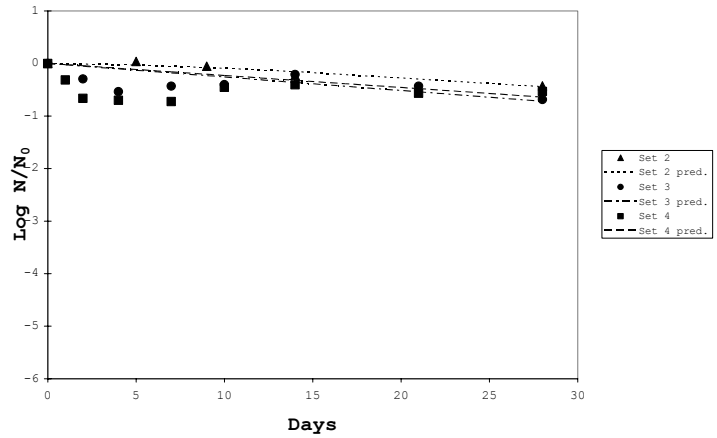
Fecal coliform, PBS, 5° C, Sets 1 & 5 (ASW trials)



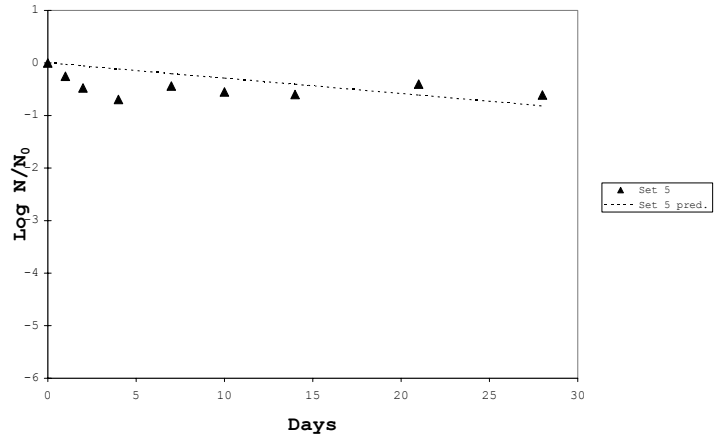
Fecal coliform, PBS, 5° C, Sets 6 & 8 (Bradenton water)



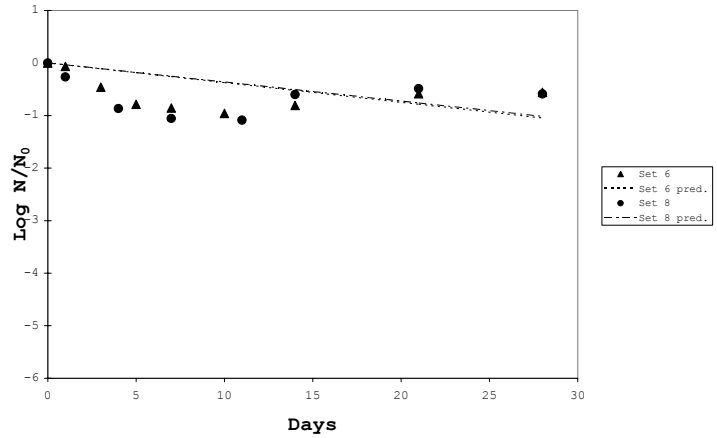
Fecal coliform, PBS, 5° C, Sets 7 & 9 (West Palm Bch. water)



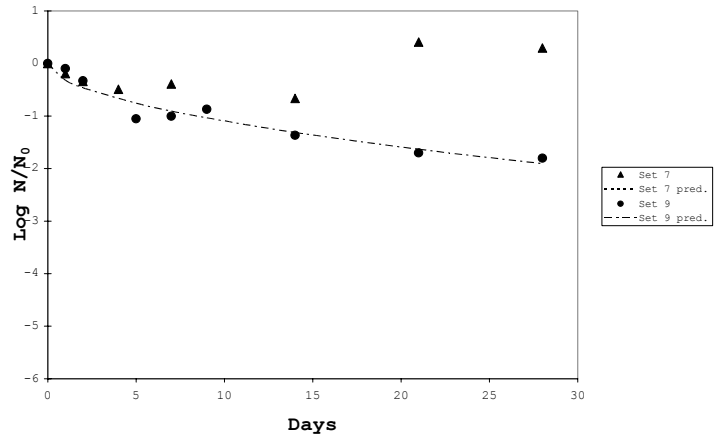
Fecal coliform, PBS, 22° C, Sets 2, 3, & 4 (ASW trials)



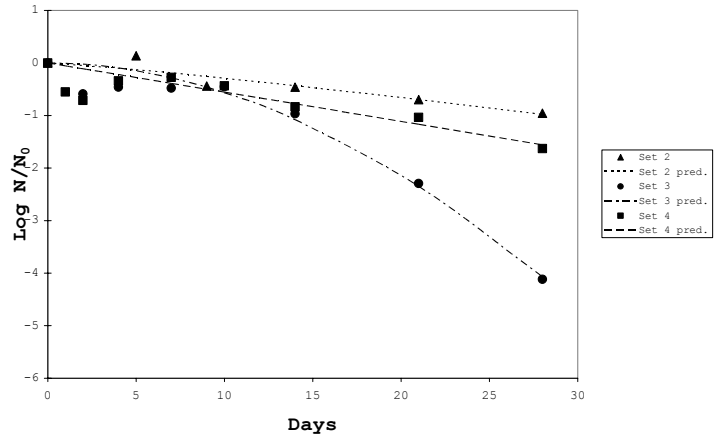
Fecal coliform, PBS, 22° C, Set 5 (ASW trials)



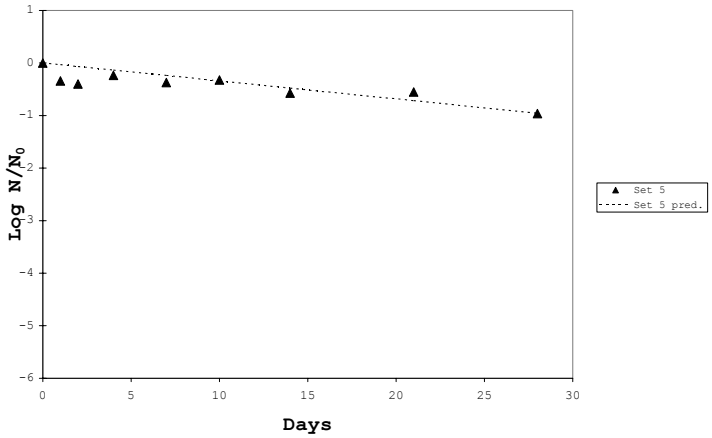
Fecal coliform, PBS, 22° C, Sets 6 & 8 (Bradenton water)



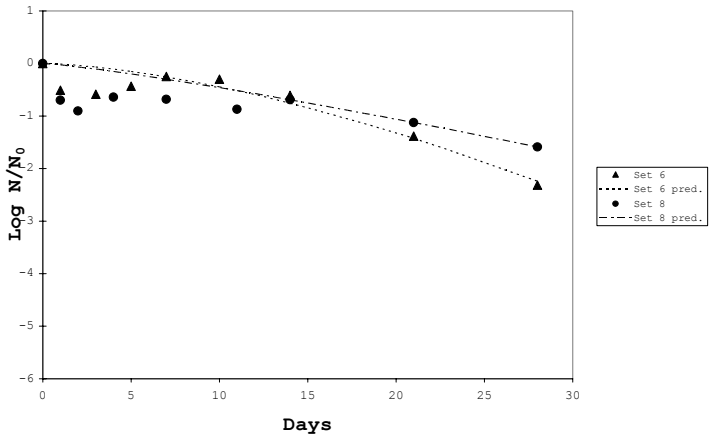
Fecal coliform, PBS, 22° C, Sets 7 & 9 (West Palm Bch. water)



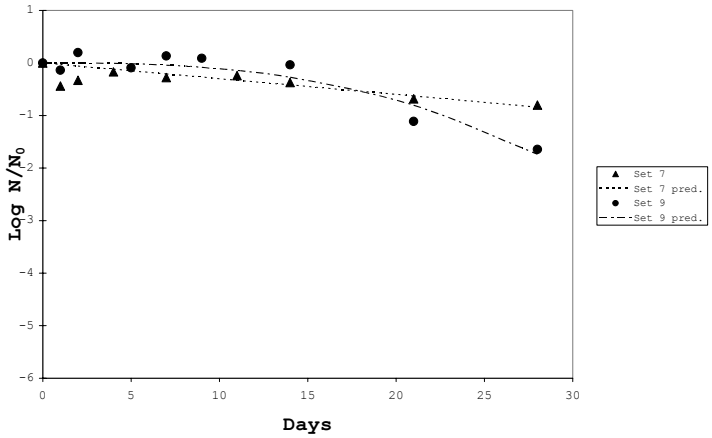
Fecal coliform, PBS, 30° C, Sets 2, 3, & 4 (ASW trials)



Fecal coliform, PBS, 30° C, Set 5 (ASW trials)



Fecal coliform, PBS, 30° C, Sets 6 & 8 (Bradenton water)

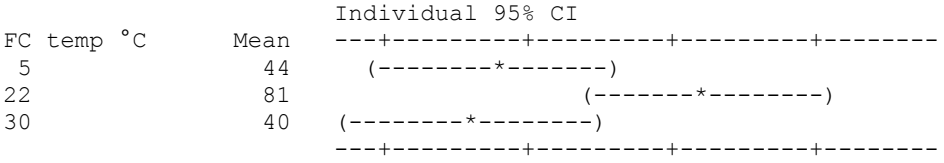


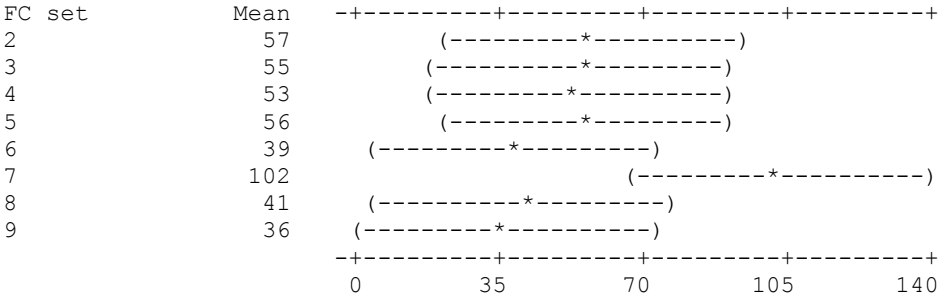
Fecal coliform, PBS, 30° C, Sets 7 & 9 (West Palm Bch. water)

Fecal coliform, 2-way ANOVA, PBS

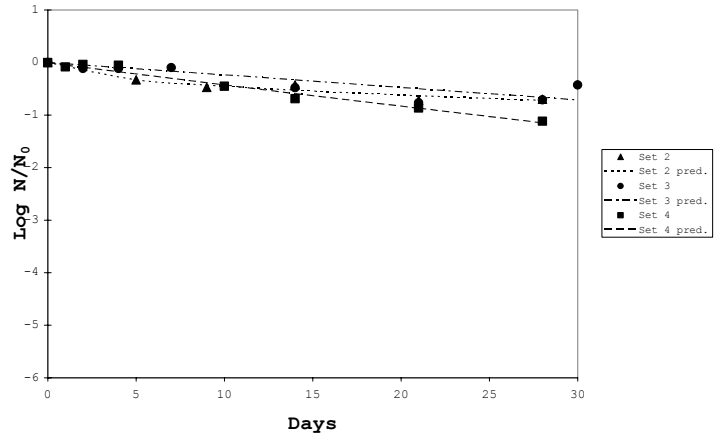
Analysis of Variance for FC days

Source	DF	SS	MS	F	P
FC temp	2	8191	4095	4.79	0.026
FC set	7	9195	1314	1.54	0.234
Error	14	11972	855		
Total	23	29358			

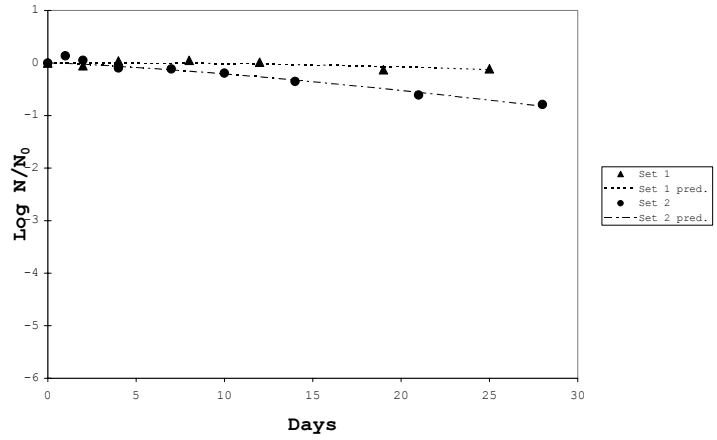




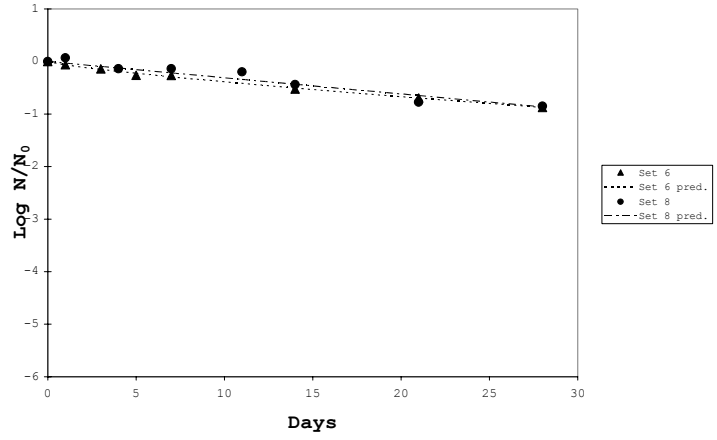
B. Enterococci PBS results



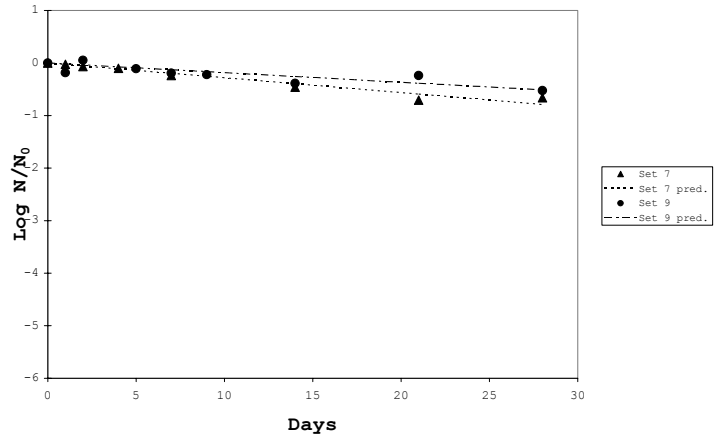
Enterococci, PBS, 5° C, Sets 2, 3, & 4 (ASW trials)



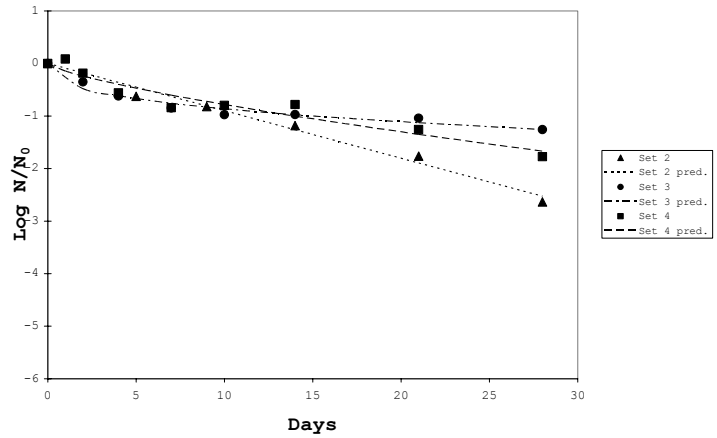
Enterococci, PBS, 5° C, Sets 1 & 5 (ASW trials)



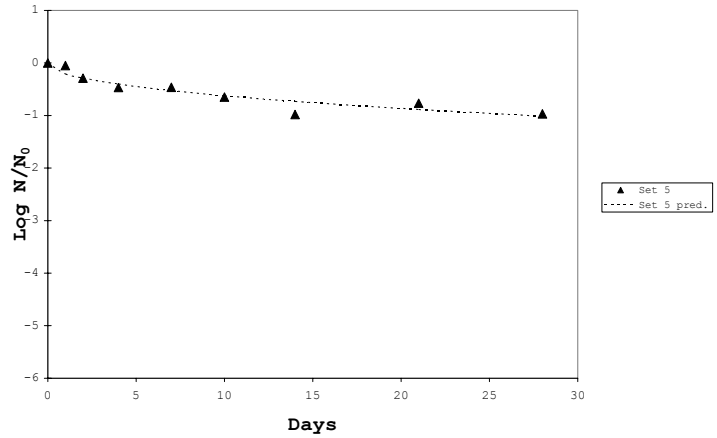
Enterococci, PBS, 5° C, Sets 6 & 8 (Bradenton water)



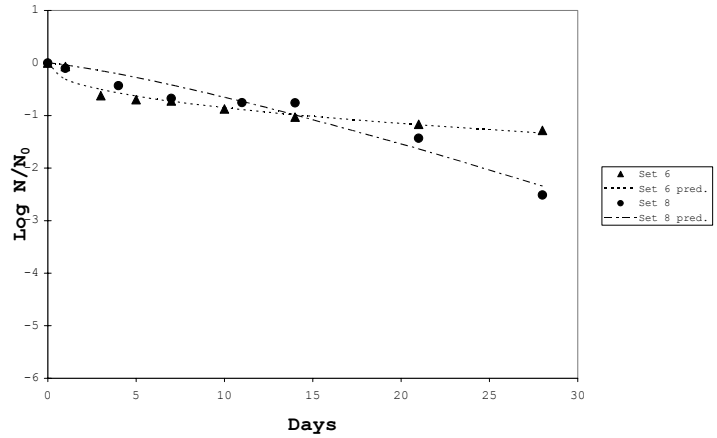
Enterococci, PBS, 5° C, Sets 7 & 9 (West Palm Bch. water)



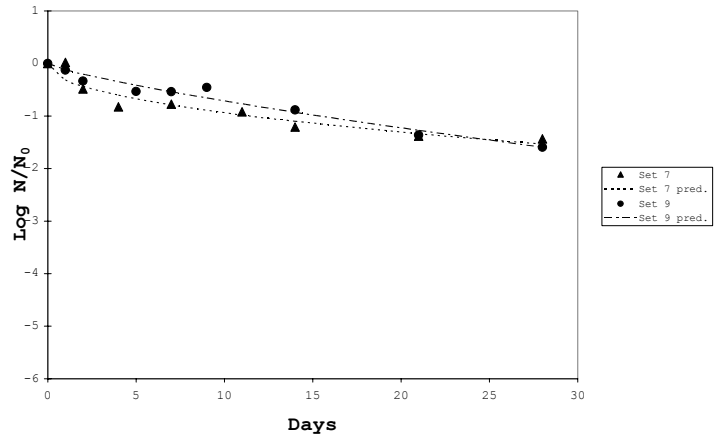
Enterococci, PBS, 22° C, Sets 2, 3, & 4 (ASW trials)



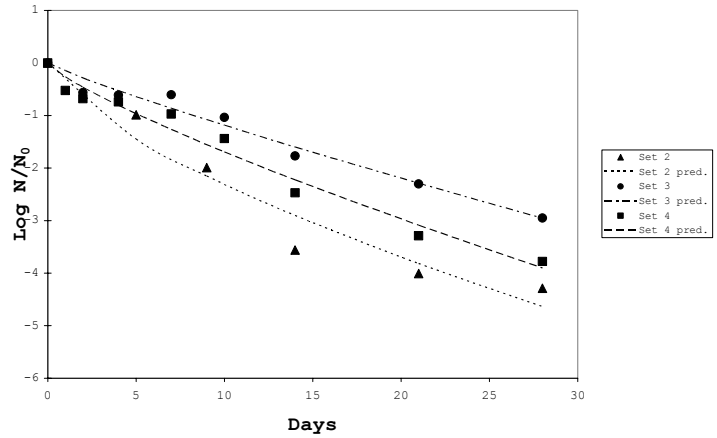
Enterococci, PBS, 22° C, Set 5 (ASW trials)



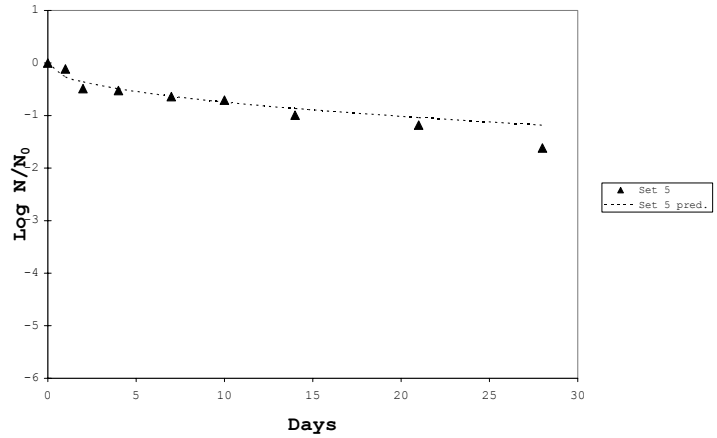
Enterococci, PBS, 22° C, Sets 6 & 8 (Bradenton water)



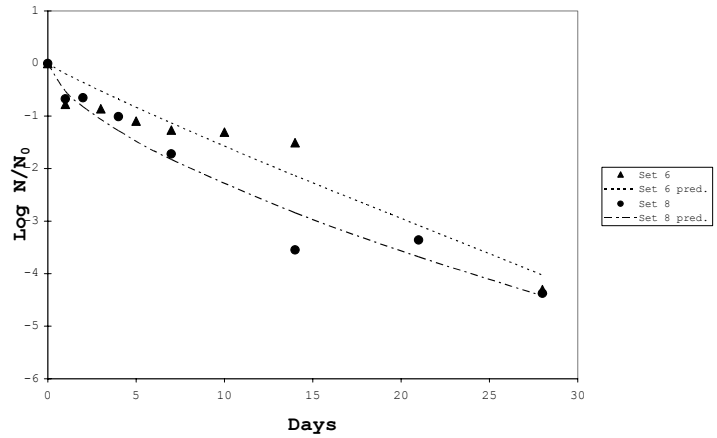
Enterococci, PBS, 22° C, Sets 7 & 9 (West Palm Bch. water)



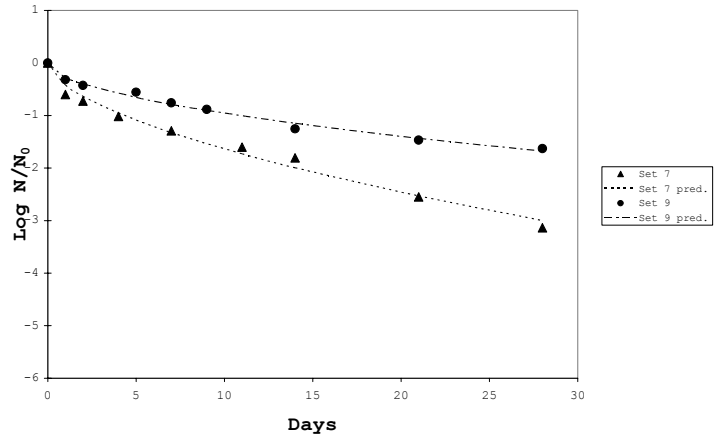
Enterococci, PBS, 30° C, Sets 2, 3, & 4 (ASW trials)



Enterococci, PBS, 30° C, Set 5 (ASW trials)



Enterococci, PBS, 30° C, Sets 6 & 8 (Bradenton water)

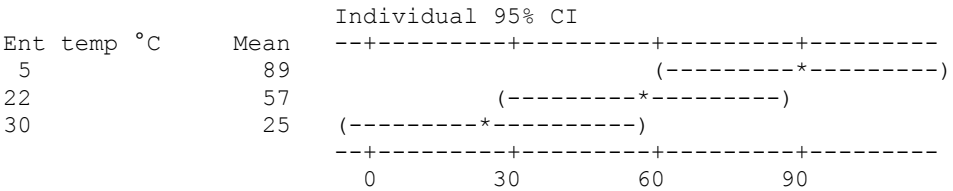


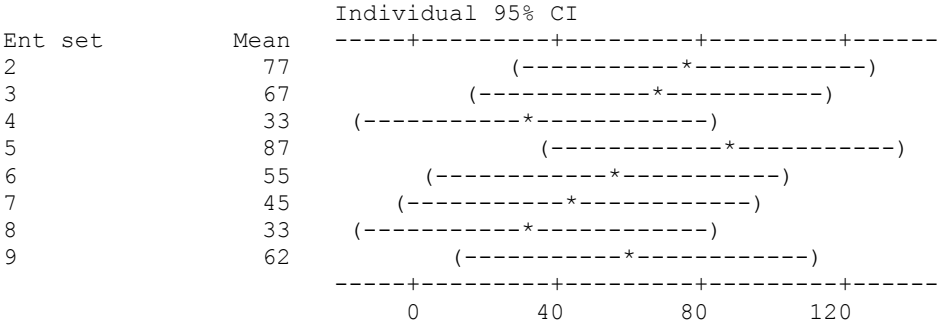
Enterococci, PBS, 30° C, Sets 7 & 9 (West Palm Bch. water)

Enterococci, 2-way ANOVA, PBS

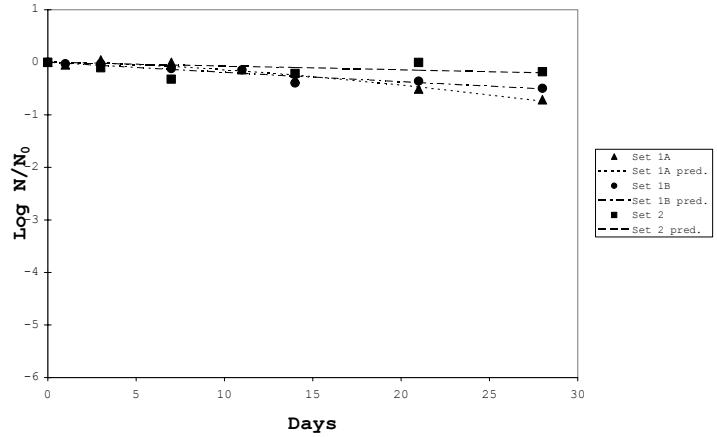
Analysis of Variance for Ent days

Source	DF	SS	MS	F	P
Ent temp	2	16448	8224	5.15	0.021
Ent set	7	8326	1189	0.75	0.639
Error	14	22340	1596		
Total	23	47114			

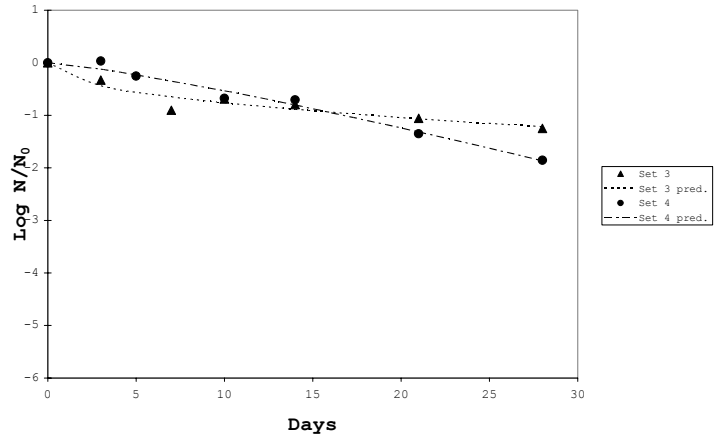




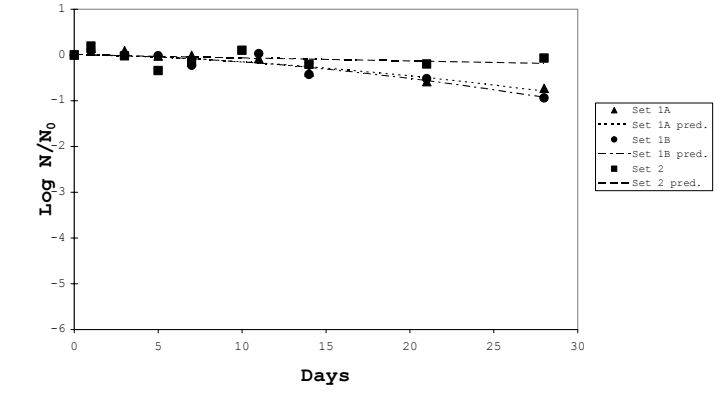
C. F+ RNA PBS results



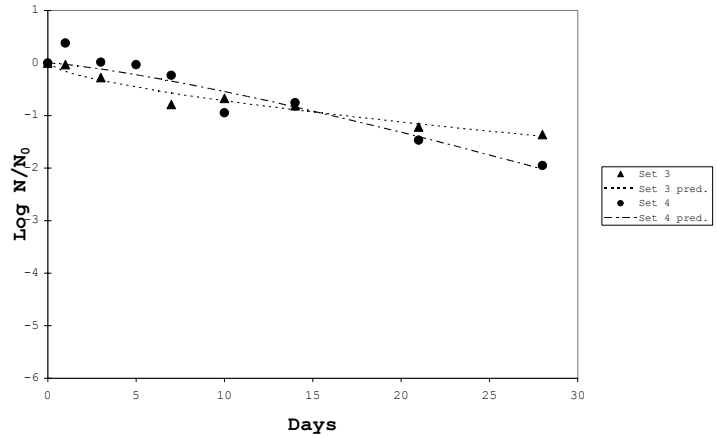
F+ RNA coliphage, PBS, 5° C, Sets 1A, 1B & 2 (ASW trials)



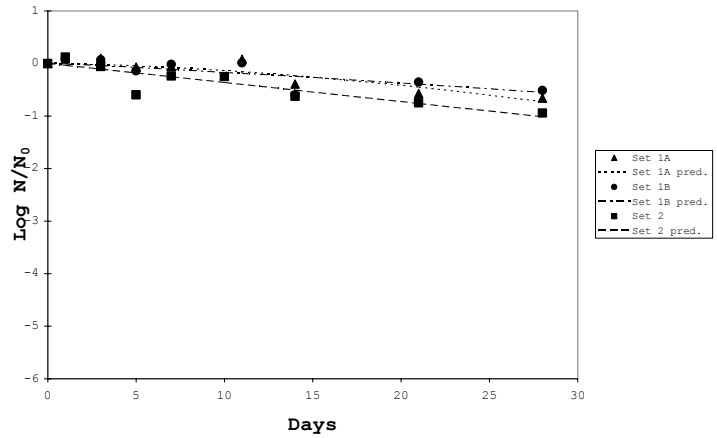
F+ RNA coliphage, PBS, 5° C, Sets 3 & 4 (natural water trials)



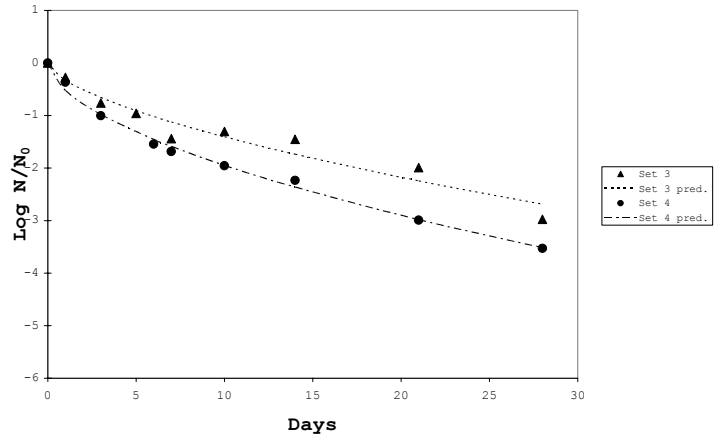
F+ RNA coliphage, PBS, 22° C, Sets 1A, 1B & 2 (ASW trials)



F+ RNA coliphage, PBS, 22° C, Sets 3 & 4 (natural water trials)



F+ RNA coliphage, PBS, 30° C, Sets 1A, 1B & 2 (ASW trials)

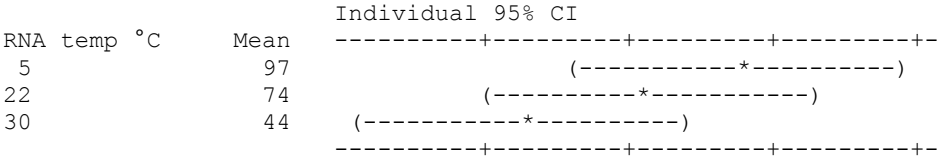


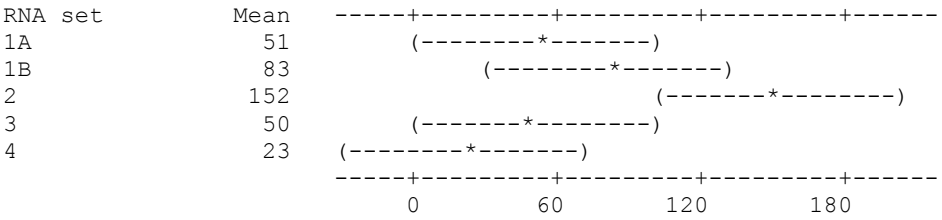
F+ RNA coliphage, PBS, 30° C, Sets 3 & 4 (natural water trials)

F+ RNA coliphage, 2-way ANOVA, PBS

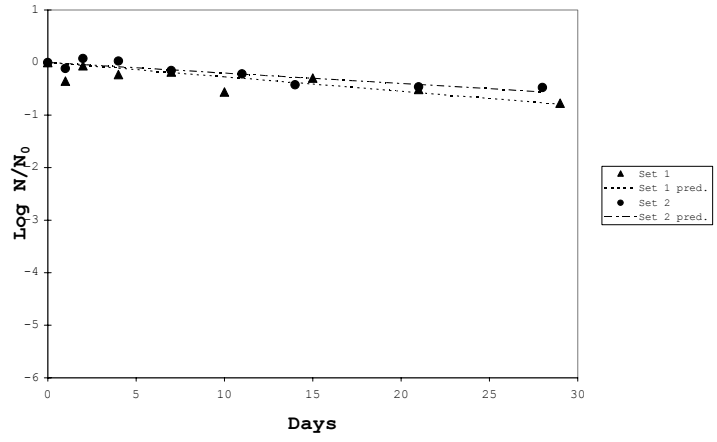
Analysis of Variance for RNA days

Source	DF	SS	MS	F	P
RNA temp	2	7070	3535	2.29	0.163
RNA set	4	29468	7367	4.78	0.029
Error	8	12323	1540		
Total	14	48861			

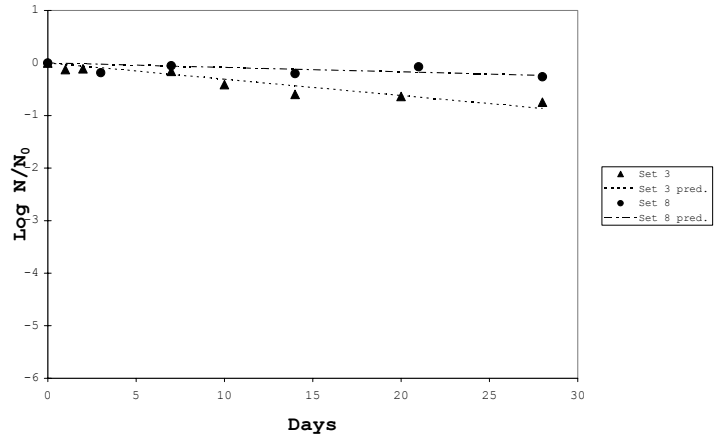




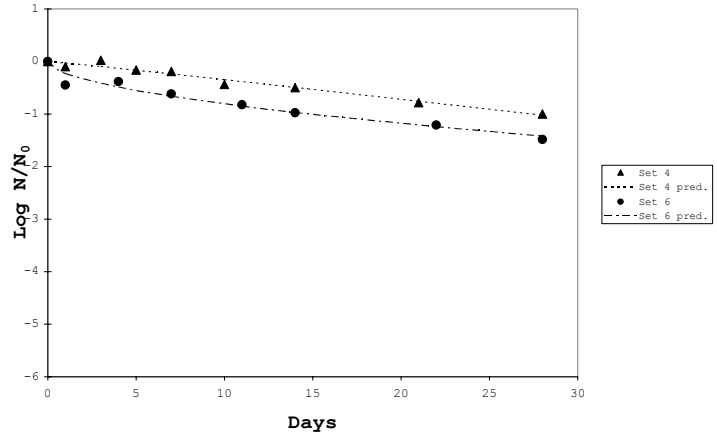
D. DNA coliphage PBS results



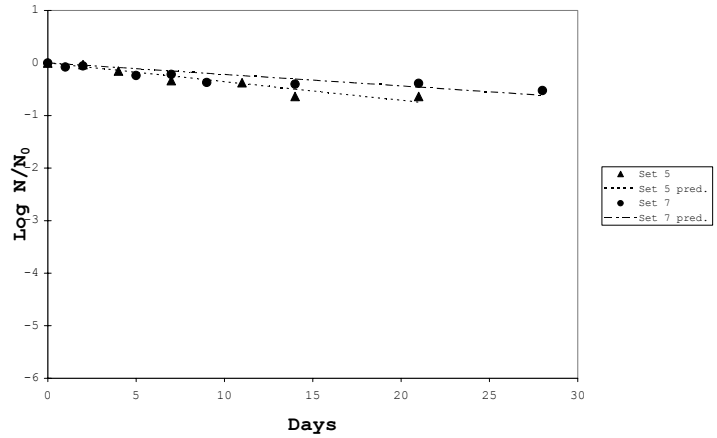
DNA coliphage, PBS, 5° C, Sets 1 & 2 (ASW trials)



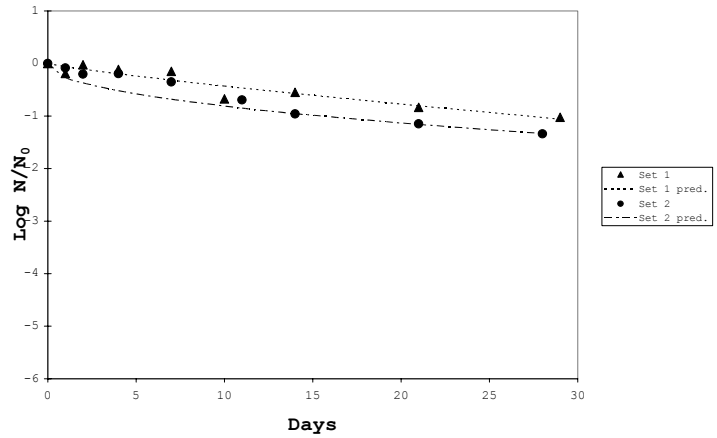
DNA coliphage, PBS, 5° C, Sets 3 & 8 (ASW trials)



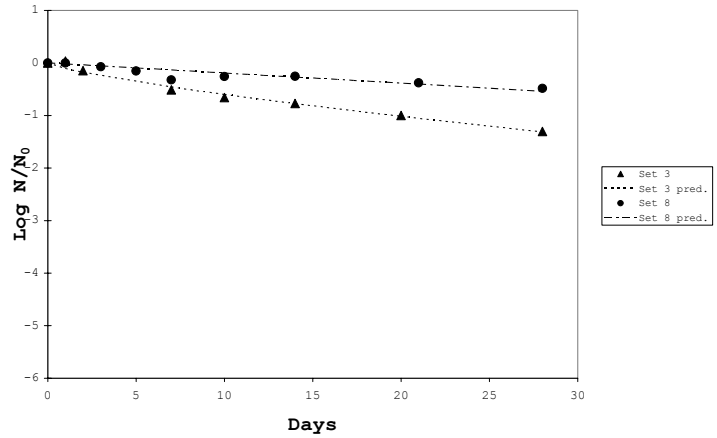
DNA coliphage, PBS, 5° C, Sets 4 & 6 (Bradenton water)



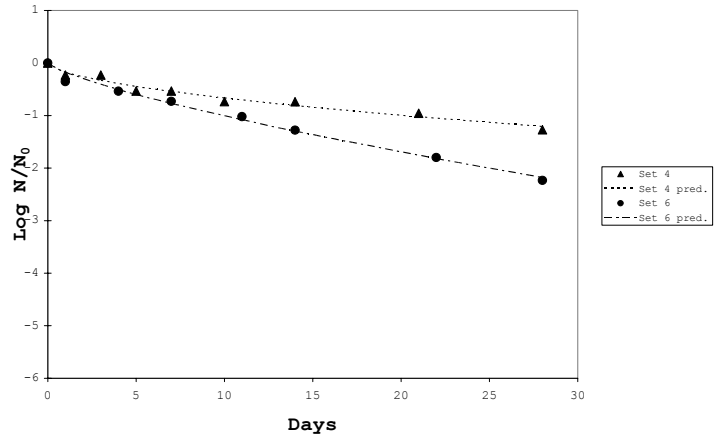
DNA coliphage, PBS, 5° C, Sets 5 & 7 (West Palm Bch. water)



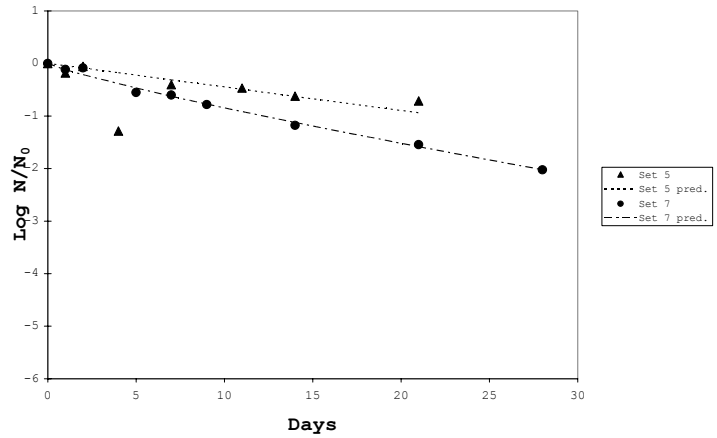
DNA coliphage, PBS, 22° C, Sets 1 & 2 (ASW trials)



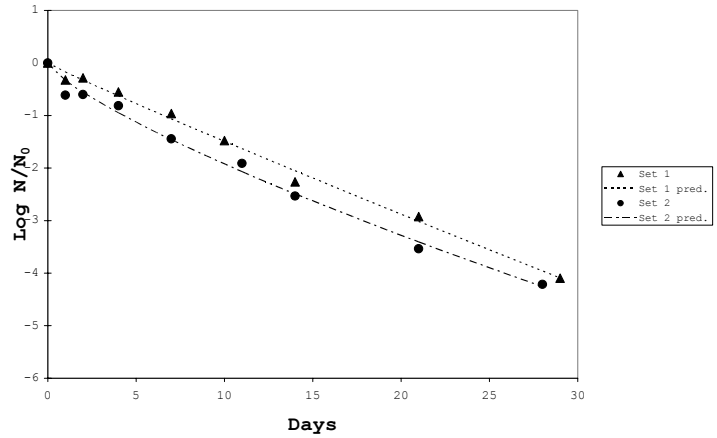
DNA coliphage, PBS, 22° C, Sets 3 & 8 (ASW trials)



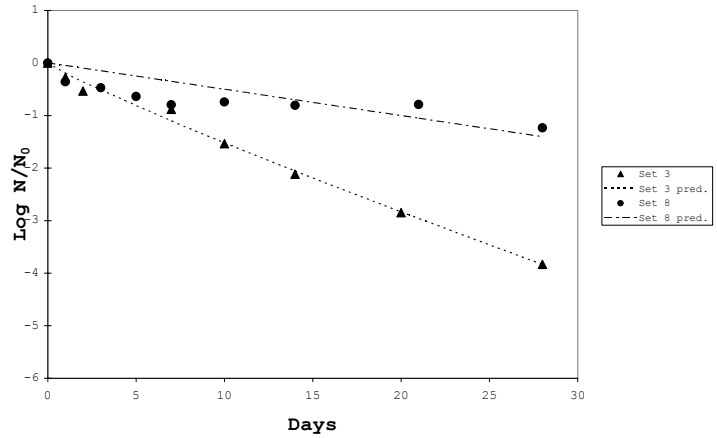
DNA coliphage, PBS, 22° C, Sets 4 & 6 (Bradenton water)



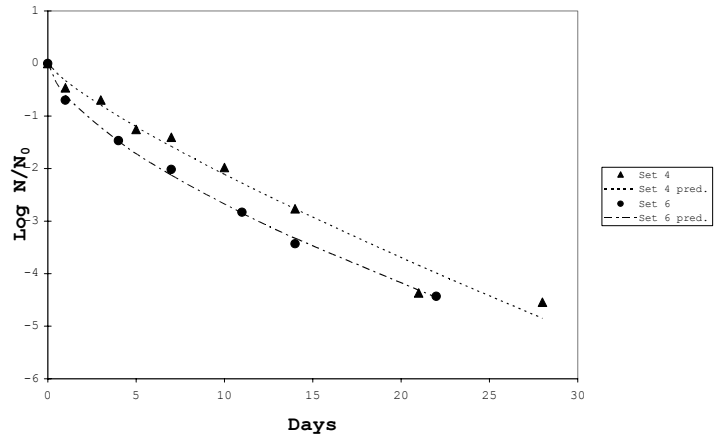
DNA coliphage, PBS, 22° C, Sets 5 & 7 (West Palm Bch. water)



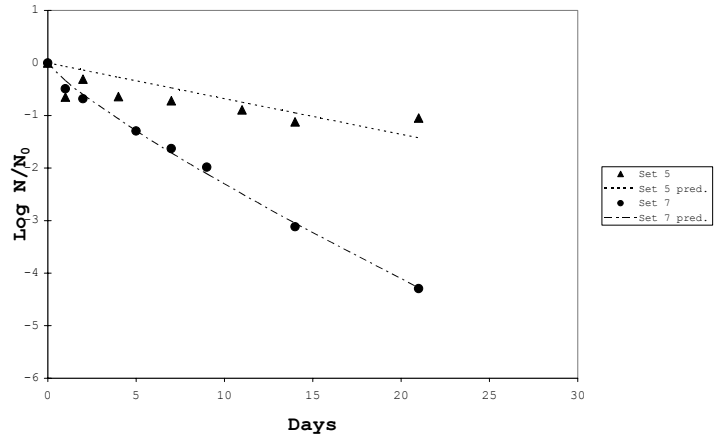
DNA coliphage, PBS, 30° C, Sets 1 & 2 (ASW trials)



DNA coliphage, PBS, 30° C, Sets 3 & 8 (ASW trials)



DNA coliphage, PBS, 30° C, Sets 4 & 6 (Bradenton water)

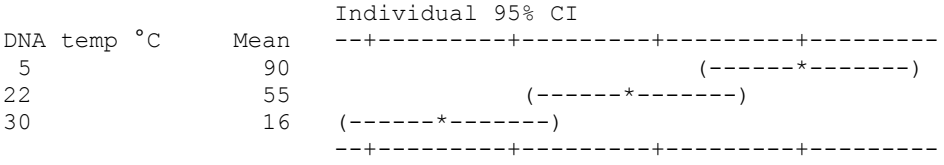


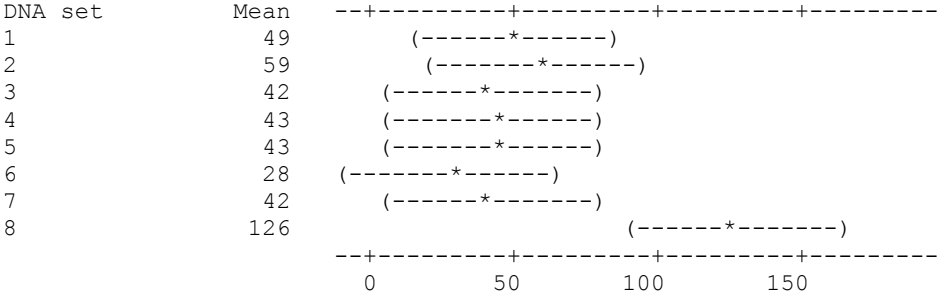
DNA coliphage, PBS, 30° C, Sets 5 & 7 (West Palm Bch. water)

DNA coliphage, 2-way ANOVA, PBS

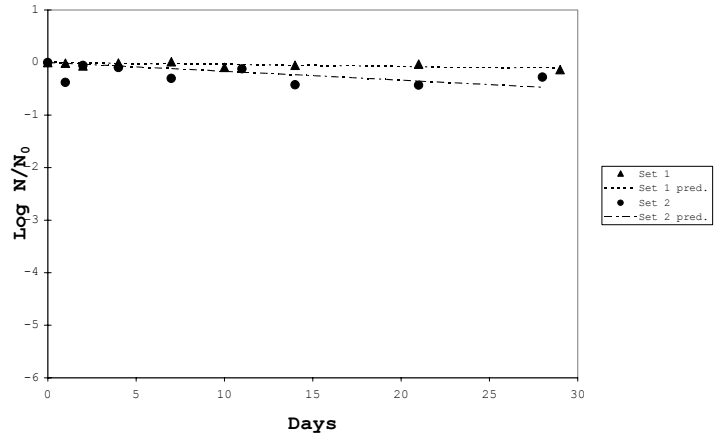
Analysis of Variance for DNA days

Source	DF	SS	MS	F	P
DNA temp	2	21998	10999	12.77	0.001
DNA set	7	19452	2779	3.23	0.030
Error	14	12054	861		
Total	23	53504			

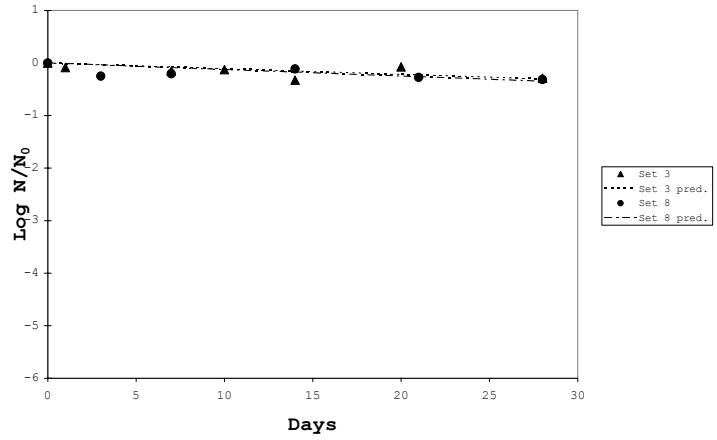




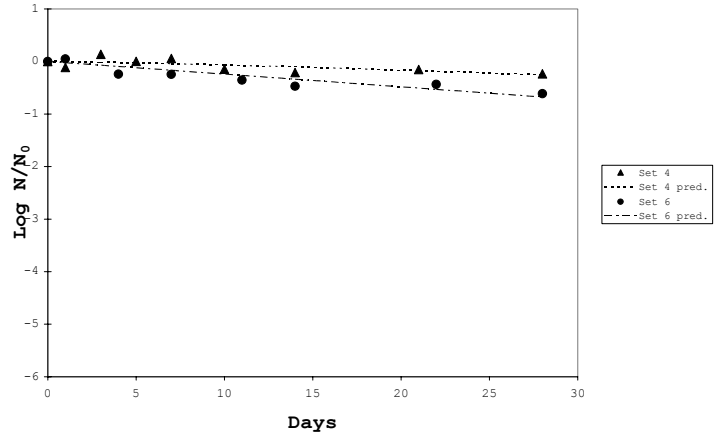
E. PRD-1 PBS results



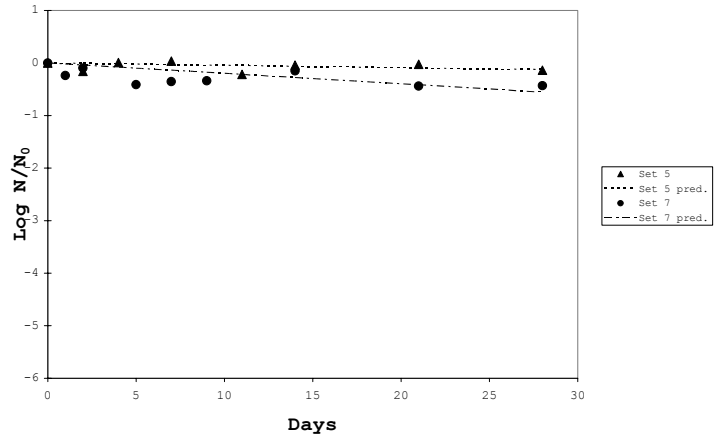
PRD-1, PBS, 5° C, Sets 1 & 2 (ASW trials)



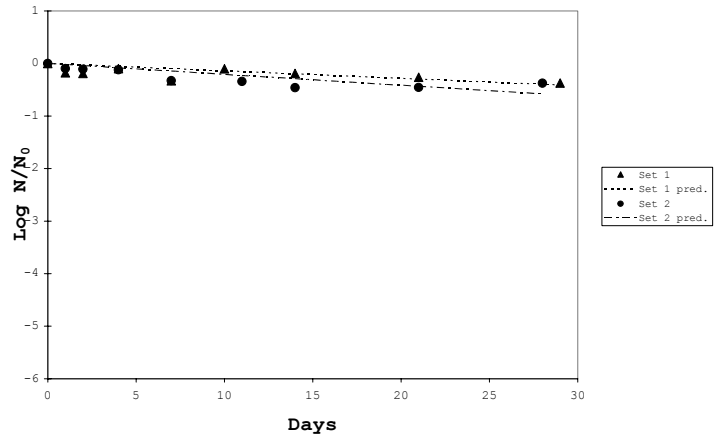
PRD-1, PBS, 5° C, Sets 3 & 8 (ASW trials)



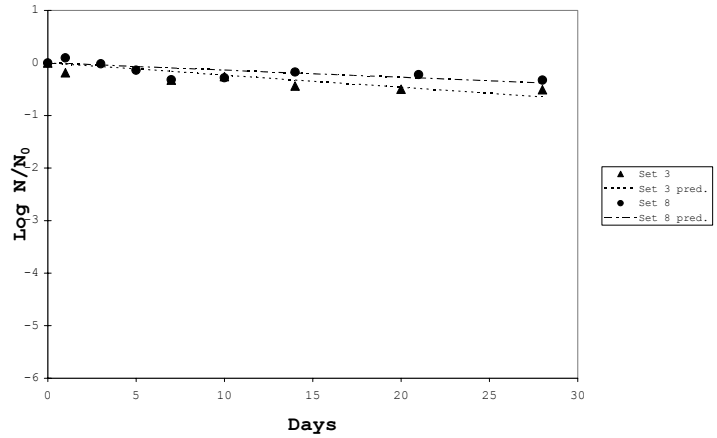
PRD-1, PBS, 5° C, Sets 4 & 6 (Bradenton water)



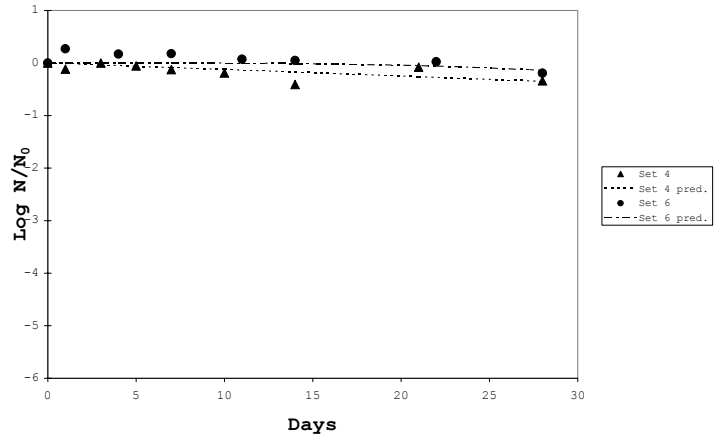
PRD-1, PBS, 5° C, Sets 5 & 7 (West Palm Bch. water)



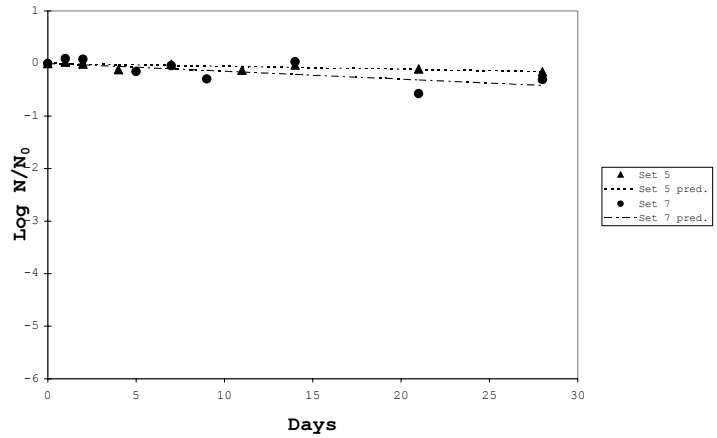
PRD-1, PBS, 22° C, Sets 1 & 2 (ASW trials)



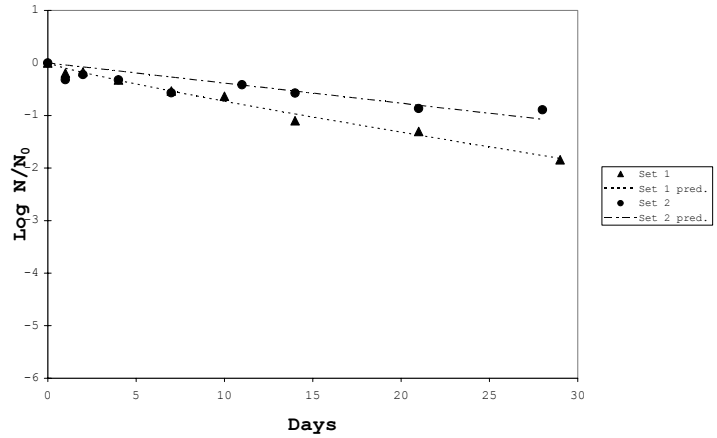
PRD-1, PBS, 22° C, Sets 3 & 8 (ASW trials)



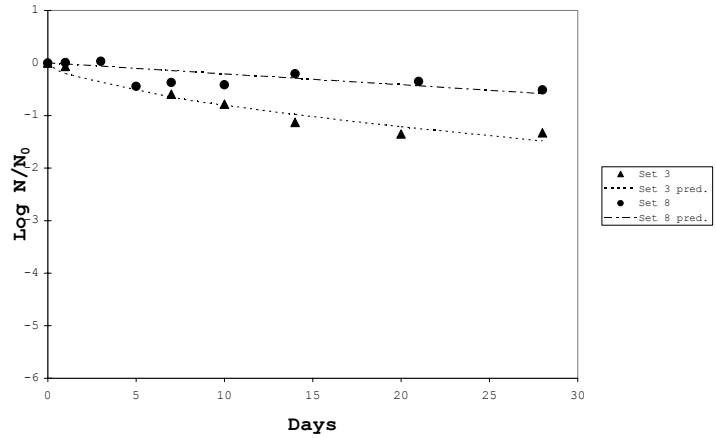
PRD-1, PBS, 22° C, Sets 4 & 6 (Bradenton water)



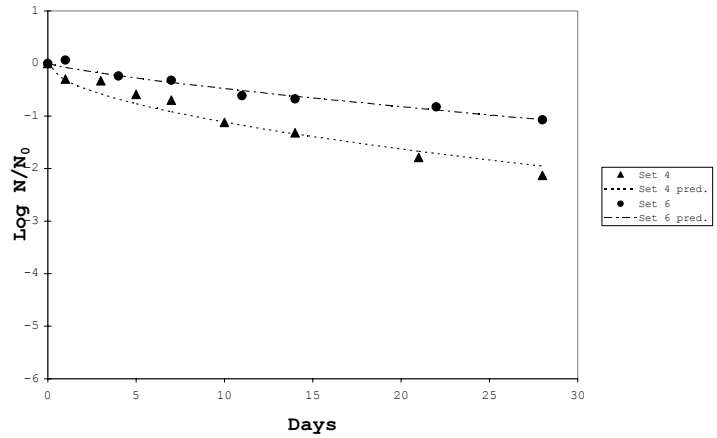
PRD-1, PBS, 22° C, Sets 5 & 7 (West Palm Bch. water)



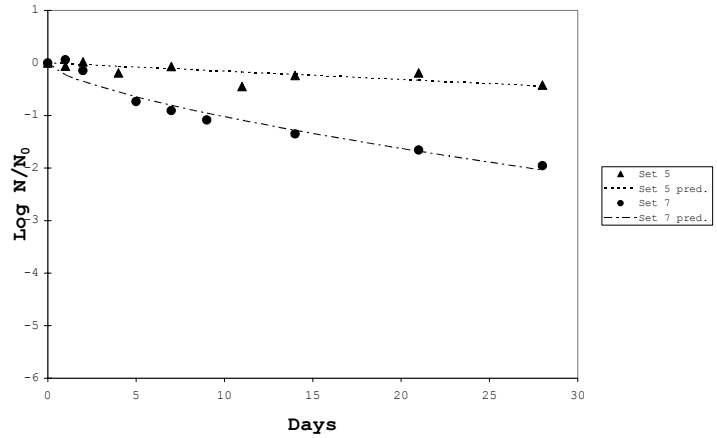
PRD-1, PBS, 30° C, Sets 1 & 2 (ASW trials)



PRD-1, PBS, 30° C, Sets 3 & 8 (ASW trials)



PRD-1, PBS, 30° C, Sets 4 & 6 (Bradenton water)

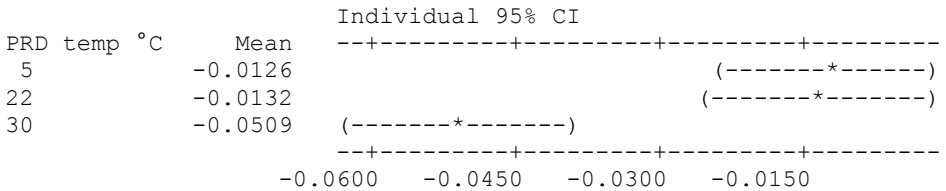


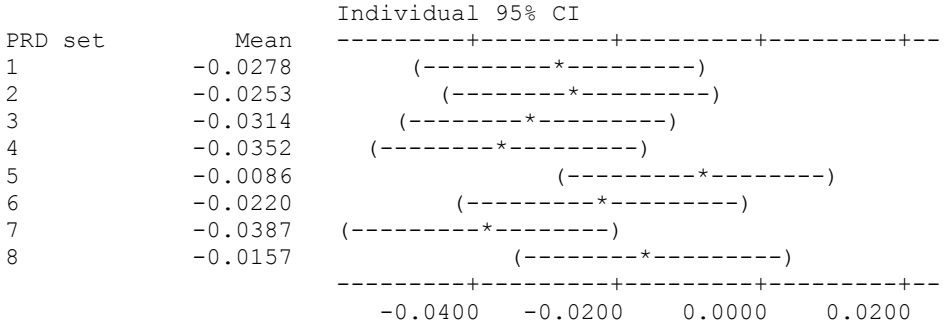
PRD-1, PBS, 30° C, Sets 5 & 7 (West Palm Bch. water)

PRD-1; 2-way ANOVA, PBS

Analysis of Variance for PRD rate

Source	DF	SS	MS	F	P
PRD temp	2	0.007713	0.003856	15.87	0.000
PRD set	7	0.002109	0.000301	1.24	0.345
Error	14	0.003402	0.000243		
Total	23	0.013223			





APPENDIX 5. Tables of predicted days for 2-log$_{10}$ (99%) declines in culturable counts, based on models fit to observed data

A. TDS-temperature trials

Fecal coliform

Trial	TDS	Temperature	Days to 2-log inactivation
1	200	5	101
2			19
3			76
mean ± std. dev.			**65 ± 42**
1	200	22	34
2			4
3			19
mean ± std. dev.			**19 ± 15**
1	200	30	46
2			3
3			9
mean ± std. dev.			**19 ± 23**
1	500	5	143
2			71
3			86
mean ± std. dev.			**100 ± 38**
1	500	22	26
2			18
3			27
mean ± std. dev.			**24 ± 5**
1	500	30	15
2			6
3			8
mean ± std. dev.			**10 ± 5**
1	1000	5	> 200
2			113
3			52
mean ± std. dev.			**122 ± 74**
1	1000	22	126
2			31
3			20
mean ± std. dev.			**59 ± 58**
1	1000	30	20
2			14
3			4
mean ± std. dev.			**13 ± 8**
1	3000	5	19
2			> 200
mean ± std. dev.			**110 ± 128**
1	3000	22	11
2			56
mean ± std. dev.			**34 ± 32**
1	3000	30	1
2			55
mean ± std. dev.			**28 ± 38**

Enterococci

Trial	TDS	Temperature	Days to 2-log inactivation
1	200	5	35
2			114
3			38
mean ± std. dev.			**62 ± 45**
1	200	22	3
2			5
3			4
mean ± std. dev.			**4 ± 1**
1	200	30	1
2			1
3			1
mean ± std. dev.			**1 ± 0**
1	500	5	110
2			57
3			40
mean ± std. dev.			**69 ± 37**
1	500	22	59
2			5
3			3
mean ± std. dev.			**22 ± 32**
1	500	30	7
2			2
3			1
mean ± std. dev.			**3 ± 3**
1	1000	5	25
2			61
3			29
mean ± std. dev.			**38 ± 20**
1	1000	22	4
2			8
3			4
mean ± std. dev.			**5 ± 2**
1	1000	30	1
2			3
3			1
mean ± std. dev.			**2 ± 1**
1	3000	5	113
2			76
mean ± std. dev.			**95 ± 26**
1	3000	22	21
2			49
mean ± std. dev.			**35 ± 20**
1	3000	30	4
2			25
mean ± std. dev.			**15 ± 15**

F+ RNA coliphage

Trial	TDS	Temperature	Days to 2-log inactivation
1	200	5	46
2			44
mean ± std. dev.			**45 ± 1**
1	200	22	32
2			42
mean ± std. dev.			**37 ± 7**
1	200	30	13
2			14
mean ± std. dev.			**14 ± 1**
1	500	5	82
2			39
mean ± std. dev.			**61 ± 30**
1	500	22	52
2			51
mean ± std. dev.			**52 ± 1**
1	500	30	21
2			27
mean ± std. dev.			**24 ± 4**
1	1000	5	64
2			50
mean ± std. dev.			**57 ± 10**
1	1000	22	41
2			32
mean ± std. dev.			**37 ± 6**
1	1000	30	11
2			13
mean ± std. dev.			**12 ± 1**
1	3000	5	155
2			140
mean ± std. dev.			**148 ± 11**
1	3000	22	47
2			42
mean ± std. dev.			**45 ± 4**
1	3000	30	6
2			5
mean ± std. dev.			**6 ± 1**

DNA coliphage

Trial	TDS	Temperature	Days to 2-log inactivation
1	200	5	141
2			65
mean ± std. dev.			**103 ± 54**
1	200	22	> 200
2			67
mean ± std. dev.			**134 ± 94**
1	200	30	63
2			12
mean ± std. dev.			**38 ± 36**
1	500	5	90
2			66
mean ± std. dev.			**78 ± 17**
1	500	22	89
2			76
mean ± std. dev.			**83 ± 9**
1	500	30	47
2			11
mean ± std. dev.			**29 ± 25**
1	1000	5	112
2			59
mean ± std. dev.			**86 ± 37**
1	1000	22	73
2			43
mean ± std. dev.			**58 ± 21**
1	1000	30	39
2			9
mean ± std. dev.			**24 ± 21**
1	3000	5	110
2			167
mean ± std. dev.			**139 ± 40**
1	3000	22	56
2			136
mean ± std. dev.			**96 ± 57**
1	3000	30	49
2			123
mean ± std. dev.			**86 ± 52**

PRD-1 bacteriophage (first order inactivation rate constants)

Trial	TDS	Temperature	Linear inact. rate (log_{10}/d)
1	200	5	-0.012
2			-0.023
mean ± std. dev.			**-.018 ± .008**
1	200	22	-0.020
2			-0.036
mean ± std. dev.			**-.028 ± .011**
1	200	30	-0.016
2			-0.083
mean ± std. dev.			**-.050 ± .047**
1	500	5	-0.022
2			-0.010
mean ± std. dev.			**-.016 ± .008**
1	500	22	-0.012
2			-0.012
mean ± std. dev.			**-.012 ± 0**
1	500	30	-0.023
2			-0.147
mean ± std. dev.			**-.085 ± .088**
1	1000	5	-0.022
2			-0.022
mean ± std. dev.			**-.022 ± 0**
1	1000	22	-0.014
2			-0.047
mean ± std. dev.			**-.031 ± .023**
1	1000	30	-0.011
2			-0.229
mean ± std. dev.			**-.120 ± .154**
1	3000	5	-0.010
2			-0.010
mean ± std. dev.			**-.010 ± 0**
1	3000	22	-0.014
2			-0.020
mean ± std. dev.			**-.017 ± .004**
1	3000	30	-0.034
2			-0.017
mean ± std. dev.			**-.026 ± .012**

Cryptosporidium parvum oocysts

Trial	TDS	Temperature	Days to 2-log inactivation
1	200	5	64
2			64
mean ± std. dev.			**64 ± 0**
1	200	22	26
2			33
mean ± std. dev.			**30 ± 5**
1	200	30	14
2			12
mean ± std. dev.			**13 ± 1**
1	500	5	64
2			63
mean ± std. dev.			**64 ± 1**
1	500	22	27
2			24
mean ± std. dev.			**26 ± 2**
1	500	30	16
2			16
mean ± std. dev.			**16 ± 0**
1	1000	5	60
2			117
mean ± std. dev.			**89 ± 40**
1	1000	22	25
2			26
mean ± std. dev.			**26 ± 1**
1	1000	30	20
2			18
mean ± std. dev.			**19 ± 1**

Giardia lamblia first order inactivation rates

TDS	Temperature	Linear inact. rate (log_{10}/d)
200	5	-0.0044
	22	-0.0047
	30	-0.094
500	5	-0.0029
	22	-0.0050
	30	-0.090
1000	5	-0.0043
	22	-0.0050
	30	-0.075

B. Fecal coliform inactivation in aquifer and reservoir water samples, as days predicted for 2-$\log_{10}$ decline

Fecal coliform in groundwater samples.

Trial	Water source	Temp.	Days to 2-log inactivation
1	Avon Park aq.	5	47
2	raw		99
mean ± std. dev.			**73 ± 37**
1	Avon Park aq.	22	22
2	raw		17
mean ± std. dev.			**20 ± 4**
1	Avon Park aq.	30	8
2	raw		12
mean ± std. dev.			**10 ± 3**
1	Avon Park aq.	5	63
2	pasteurized		63
mean ± std. dev.			**63 ± 0**
1	Avon Park aq.	22	110
2	pasteurized		159
mean ± std. dev.			**135 ± 35**
1	Avon Park aq.	30	51
2	pasteurized		51
mean ± std. dev.			**51 ± 0**
1	Lake Lytal aq.	5	14
2	raw		18
mean ± std. dev.			**16 ± 3**
1	Lake Lytal aq.	22	45
2	raw		35
mean ± std. dev.			**40 ± 7**
1	Lake Lytal aq.	30	11
2	raw		12
mean ± std. dev.			**12 ± 1**
1	Lake Lytal aq.	5	> 200
2	pasteurized		31
mean ± std. dev.			**116 ± 120**
1	Lake Lytal aq.	22	74
2	pasteurized		76
mean ± std. dev.			**75 ± 1**
1	Lake Lytal aq.	30	49
2	pasteurized		54
mean ± std. dev.			**52 ± 4**

Fecal coliform in surface water samples

Trial	Water source	Temp.	Days to 2-log inactivation
1	Bill Evers res.	5	24
2	raw		71
mean ± std. dev.			**48 ± 33**
1	Bill Evers res.	22	4
2	raw		6
mean ± std. dev.			**5 ± 1**
1	Bill Evers res.	30	2
2	raw		1
mean ± std. dev.			**2 ± 1**
1	Bill Evers res.	5	108
2	pasteurized		34
mean ± std. dev.			**71 ± 52**
1	Bill Evers res.	22	50
2	pasteurized		9
mean ± std. dev.			**30 ± 29**
1	Bill Evers res.	30	50
2	pasteurized		1
mean ± std. dev.			**26 ± 35**
1	Clear Lake res.	5	25
2	raw		26
mean ± std. dev.			**26 ± 1**
1	Clear Lake res.	22	6
2	raw		10
mean ± std. dev.			**8 ± 3**
1	Clear Lake res.	30	4
2	raw		5
mean ± std. dev.			**5 ± 1**
1	Clear Lake res.	5	119
2	pasteurized		93
mean ± std. dev.			**106 ± 18**
1	Clear Lake res.	22	55
2	pasteurized		60
mean ± std. dev.			**58 ± 4**
1	Clear Lake res.	30	38
2	pasteurized		49
mean ± std. dev.			**44 ± 8**

C. Enterococci inactivation in aquifer and reservoir water samples, as days predicted for 2-$\log_{10}$ decline

Enterococci in groundwater samples

Trial	Water source	Temp.	Days to 2-log inactivation
1	Avon Park aq.	5	60
2	raw		40
mean ± std. dev.			**50 ± 14**
1	Avon Park aq.	22	17
2	raw		5
mean ± std. dev.			**11 ± 8**
1	Avon Park aq.	30	7
2	raw		2
mean ± std. dev.			**5 ± 4**
1	Avon Park aq.	5	64
2	pasteurized		36
mean ± std. dev.			**50 ± 20**
1	Avon Park aq.	22	19
2	pasteurized		3
mean ± std. dev.			**11 ± 11**
1	Avon Park aq.	30	8
2	pasteurized		2
mean ± std. dev.			**5 ± 4**
1	Lake Lytal aq.	5	175
2	raw		> 200
mean ± std. dev.			**188 ± 18**
1	Lake Lytal aq.	22	32
2	raw		35
mean ± std. dev.			**34 ± 2**
1	Lake Lytal aq.	30	13
2	raw		16
mean ± std. dev.			**15 ± 2**
1	Lake Lytal aq.	5	158
2	pasteurized		115
mean ± std. dev.			**137 ± 30**
1	Lake Lytal aq.	22	41
2	pasteurized		56
mean ± std. dev.			**49 ± 11**
1	Lake Lytal aq.	30	10
2	pasteurized		18
mean ± std. dev.			**14 ± 6**

Enterococci in surface water samples

Trial	Water source	Temp.	Days to 2-log inactivation
1	Bill Evers res.	5	29
2	raw		37
mean ± std. dev.			**33 ± 6**
1	Bill Evers res.	22	4
2	raw		3
mean ± std. dev.			**4 ± 1**
1	Bill Evers res.	30	2
2	raw		1
mean ± std. dev.			**2 ± 1**
1	Bill Evers res.	5	177
2	pasteurized		73
mean ± std. dev.			**125 ± 74**
1	Bill Evers res.	22	33
2	pasteurized		2
mean ± std. dev.			**18 ± 22**
1	Bill Evers res.	30	4
2	pasteurized		1
mean ± std. dev.			**3 ± 2**
1	Clear Lake res.	5	28
2	raw		29
mean ± std. dev.			**29 ± 1**
1	Clear Lake res.	22	6
2	raw		7
mean ± std. dev.			**7 ± 1**
1	Clear Lake res.	30	4
2	raw		4
mean ± std. dev.			**4 ± 0**
1	Clear Lake res.	5	148
2	pasteurized		125
mean ± std. dev.			**137 ± 16**
1	Clear Lake res.	22	38
2	pasteurized		22
mean ± std. dev.			**30 ± 11**
1	Clear Lake res.	30	12
2	pasteurized		8
mean ± std. dev.			**10 ± 3**

D. F+ RNA coliphage inactivation in aquifer and reservoir water samples, as days predicted for 2-$\log_{10}$ decline

F+ RNA coliphage in groundwater

Trial	Water source	Temp.	Days to 2-log inactivation
1	Avon Park aq.	5	5
2	raw		3
mean ± std. dev.			**4 ± 1**
1	Avon Park aq.	22	1
2	raw		3
mean ± std. dev.			**2 ± 1**
1	Avon Park aq.	30	1
2	raw		1
mean ± std. dev.			**1 ± 0**
1	Avon Park aq.	5	28
2	pasteurized		46
mean ± std. dev.			**37 ± 13**
1	Avon Park aq.	22	3
2	pasteurized		5
mean ± std. dev.			**4 ± 1**
1	Avon Park aq.	30	1
2	pasteurized		1
mean ± std. dev.			**1 ± 0**
1	Lake Lytal aq.	5	33
2	raw		34
mean ± std. dev.			**34 ± 1**
1	Lake Lytal aq.	22	4
2	raw		4
mean ± std. dev.			**4 ± 0**
1	Lake Lytal aq.	30	1
2	raw		1
mean ± std. dev.			**1 ± 0**
1	Lake Lytal aq.	5	48
2	pasteurized		41
mean ± std. dev.			**45 ± 5**
1	Lake Lytal aq.	22	4
2	pasteurized		5
mean ± std. dev.			**5 ± 1**
1	Lake Lytal aq.	30	1
2	pasteurized		1
mean ± std. dev.			**1 ± 0**

F+ RNA coliphage in surface water

Trial	Water source	Temp.	Days to 2-log inactivation
1	Bill Evers res.	5	118
2	raw		82
mean ± std. dev.			**100 ± 25**
1	Bill Evers res.	22	7
2	raw		7
mean ± std. dev.			**7 ± 0**
1	Bill Evers res.	30	3
2	raw		2
mean ± std. dev.			**3 ± 1**
1	Bill Evers res.	5	60
2	pasteurized		51
mean ± std. dev.			**56 ± 6**
1	Bill Evers res.	22	11
2	pasteurized		12
mean ± std. dev.			**12 ± 1**
1	Bill Evers res.	30	3
2	pasteurized		3
mean ± std. dev.			**3 ± 0**
1	Clear Lake res.	5	21
2	raw		25
mean ± std. dev.			**23 ± 3**
1	Clear Lake res.	22	2
2	raw		2
mean ± std. dev.			**2 ± 0**
1	Clear Lake res.	30	1
2	raw		1
mean ± std. dev.			**1 ± 0**
1	Clear Lake res.	5	22
2	pasteurized		24
mean ± std. dev.			**23 ± 1**
1	Clear Lake res.	22	2
2	pasteurized		2
mean ± std. dev.			**2 ± 0**
1	Clear Lake res.	30	1
2	pasteurized		1
mean ± std. dev.			**1 ± 0**

E. DNA coliphage inactivation in aquifer and reservoir water samples, as days predicted for 2-$\log_{10}$ decline

DNA coliphage in groundwater

Trial	Water source	Temp.	Days to 2-log inactivation
1	Avon Park aq.	5	91
2	raw		190
mean ± std. dev.			**141 ± 70**
1	Avon Park aq.	22	45
2	raw		28
mean ± std. dev.			**37 ± 12**
1	Avon Park aq.	30	16
2	raw		10
mean ± std. dev.			**13 ± 4**
1	Avon Park aq.	5	112
2	pasteurized		71
mean ± std. dev.			**92 ± 29**
1	Avon Park aq.	22	90
2	pasteurized		44
mean ± std. dev.			**67 ± 33**
1	Avon Park aq.	30	16
2	pasteurized		39
mean ± std. dev.			**28 ± 16**
1	Lake Lytal aq.	5	62
2	raw		82
mean ± std. dev.			**72 ± 14**
1	Lake Lytal aq.	22	41
2	raw		24
mean ± std. dev.			**33 ± 12**
1	Lake Lytal aq.	30	14
2	raw		10
mean ± std. dev.			**12 ± 3**
1	Lake Lytal aq.	5	> 200
2	pasteurized		78
mean ± std. dev.			**139 ± 86**
1	Lake Lytal aq.	22	> 200
2	pasteurized		28
mean ± std. dev.			**114 ± 122**
1	Lake Lytal aq.	30	98
2	pasteurized		22
mean ± std. dev.			**60 ± 54**

DNA coliphage in surface water

Trial	Water source	Temp.	Days to 2-log inactivation
1	Bill Evers res.	5	30
2	raw		114
mean ± std. dev.			**72 ± 59**
1	Bill Evers res.	22	11
2	raw		19
mean ± std. dev.			**15 ± 6**
1	Bill Evers res.	30	11
2	raw		5
mean ± std. dev.			**8 ± 4**
1	Bill Evers res.	5	> 200
2	pasteurized		147
mean ± std. dev.			**174 ± 37**
1	Bill Evers res.	22	> 200
2	pasteurized		27
mean ± std. dev.			**114 ± 122**
1	Bill Evers res.	30	155
2	pasteurized		2
mean ± std. dev.			**79 ± 108**
1	Clear Lake res.	5	82
2	raw		156
mean ± std. dev.			**119 ± 52**
1	Clear Lake res.	22	19
2	raw		28
mean ± std. dev.			**24 ± 6**
1	Clear Lake res.	30	13
2	raw		6
mean ± std. dev.			**10 ± 5**
1	Clear Lake res.	5	> 200
2	pasteurized		149
mean ± std. dev.			**175 ± 36**
1	Clear Lake res.	22	> 200
2	pasteurized		170
mean ± std. dev.			**185 ± 21**
1	Clear Lake res.	30	163
2	pasteurized		102
mean ± std. dev.			**133 ± 43**

F. PRD-1 inactivation rates from natural water trials

PRD-1 inactivation in groundwater

Trial	Water source	Temp.	Linear inact. rate (log_{10}/d)
1	Avon Park aq.	5	-0.001
2	raw		-0.017
mean ± std. dev.			**-.009 ± .011**
1	Avon Park aq.	22	-0.011
2	raw		-0.022
mean ± std. dev.			**-.017 ± .008**
1	Avon Park aq.	30	-0.010
2	raw		-0.019
mean ± std. dev.			**-.015 ± .006**
1	Avon Park aq.	5	0.004
2	pasteurized		-0.026
mean ± std. dev.			**-.011 ± .021**
1	Avon Park aq.	22	0.006
2	pasteurized		-0.016
mean ± std. dev.			**-.005 ± .016**
1	Avon Park aq.	30	-0.003
2	pasteurized		-0.039
mean ± std. dev.			**-.021 ± .025**
1	Lake Lytal aq.	5	-0.005
2	raw		-0.036
mean ± std. dev.			**-.021 ± .022**
1	Lake Lytal aq.	22	-0.015
2	raw		-0.040
mean ± std. dev.			**-.028 ± .018**
1	Lake Lytal aq.	30	-0.038
2	raw		-0.052
mean ± std. dev.			**-.045 ± .010**
1	Lake Lytal aq.	5	0.004
2	pasteurized		-0.018
mean ± std. dev.			**-.007 ± .016**
1	Lake Lytal aq.	22	-0.020
2	pasteurized		-0.043
mean ± std. dev.			**-.032 ± .016**
1	Lake Lytal aq.	30	-0.007
2	pasteurized		-0.032
mean ± std. dev.			**-.020 ± .018**

PRD-1 inactivation in surface water

Trial	Water source	Temp. °C	Linear inact. rate (log_{10}/d)
1	Bill Evers res.	5	-0.072
2	raw		-0.023
mean ± std. dev.			**-.048 ± .035**
1	Bill Evers res.	22	-0.139
2	raw		-0.068
mean ± std. dev.			**-.104 ± .050**
1	Bill Evers res.	30	-0.124
2	raw		-0.220
mean ± std. dev.			**-.172 ± .068**
1	Bill Evers res.	5	-0.005
2	pasteurized		0.0004
mean ± std. dev.			**-.002 ± .004**
1	Bill Evers res.	22	-0.009
2	pasteurized		-0.052
mean ± std. dev.			**-.031 ± .030**
1	Bill Evers res.	30	-0.004
2	pasteurized		-0.377
mean ± std. dev.			**-.191 ± .264**
1	Clear Lake res.	5	-0.025
2	raw		-0.043
mean ± std. dev.			**-.034 ± .013**
1	Clear Lake res.	22	-0.088
2	raw		-0.079
mean ± std. dev.			**-.084 ± .006**
1	Clear Lake res.	30	-0.089
2	raw		-0.14
mean ± std. dev.			**-.115 ± .036**
1	Clear Lake res.	5	-0.005
2	pasteurized		-0.015
mean ± std. dev.			**-.010 ± .007**
1	Clear Lake res.	22	-0.004
2	pasteurized		-0.022
mean ± std. dev.			**-.013 ± .013**
1	Clear Lake res.	30	-0.004
2	pasteurized		-0.024
mean ± std. dev.			**-.014 ± .014**

G. *Cryptosporidium parvum* first-order inactivation rates from infectivity data in natural water trials, both surface and groundwater

Site	Water Type	Temperature	Linear inact. rate (log_{10}/d)
BER	surface	5	-0.0017
CLR			-0.0037
mean ± std. dev.			**-0.0027 ± 0.001**
BER	surface	22	-0.045
CLR			-0.066
mean ± std. dev.			**-0.055 ± 0.015**
BER	surface	30	-0.20
CLR			-0.18
mean ± std. dev.			**-0.19 ± 0.01**
AP	ground	5	0.0088
LLP			0.00090
mean ± std. dev.			**0.005 ± 0.006**
AP	ground	22	-0.0010
LLP			-0.042
mean ± std. dev.			**-0.022 ± 0.029**
AP	ground	30	-0.11
LLP			-0.12
mean ± std. dev.			**-0.11 ± 0.004**

H. *Giardia lamblia* first-order inactivation rates from viability staining in natural water trials, both surface and groundwater

Site	Water Type	Temperature °C	Linear inact. rate (log_{10}/d)
BER	surface	5	-0.0034
CLR			-0.0034
mean ± std. dev.			**-0.0034 ± 0**
AP	ground	5	-0.0042
LLP			-0.0050
mean ± std. dev.			**-0.0046 ± .0006**
BER	surface	22	-0.0050
CLR			-0.0042
mean ± std. dev.			**-0.0046 ± 0.0006**
AP	ground	22	-0.0395
LLP			-0.0304
mean ± std. dev.			**-0.035 ± 0.0064**
BER	surface	30	-0.081
CLR			-0.076
mean ± std. dev.			**-0.078 ± 0.0034**
AP	ground	30	-0.098
LLP			-0.11
mean ± std. dev.			**-0.10 ± 0.0086**

BER = Bill Evers reservoir
CLR = Clear Lake reservoir
AP = Avon Park well
LLP = Lake Lytal Park well

APPENDIX 6. Results of statistical comparisons on days for predicted 2-log_{10} (99%) decline

A. Fecal coliform Temperature-TDS trials

Fecal coliform, 2-log ANOVA, all TDS concentrations, unbalanced design

```
Factor     Type Levels Values
Temp-bac   fixed      3  5 22 30
TDS-bact   fixed      4  200  500 1000 3000

Analysis of Variance for FC days, using Adjusted SS for Tests

Source               DF     Seq SS     Adj SS     Adj MS       F      P
Temp-bac              2      40786      39868      19934    9.33  0.001
TDS-bact              3       4588       4588       1529    0.72  0.554
Temp-bac*TDS-bact     6       3913       3913        652    0.31  0.927
Error                21      44887      44887       2137
Total                32      94174

Unusual Observations for FC days

Obs   FC days       Fit   StDev Fit  Residual   St Resid
 19   200.000   121.667      26.693    78.333      2.08R
 28    19.000   109.500      32.692   -90.500     -2.77R
 29   200.000   109.500      32.692    90.500      2.77R

R denotes an observation with a large standardized residual.
```

Regression analysis of fecal coliform inactivation periods at 4 TDS concentrations

The regression equation is
FC days = 106 - 3.35 Temp-bact + 0.00688 TDS-bact

Predictor	Coef	StDev	T	P
Constant	106.19	16.86	6.30	0.000
Temp-bac	-3.3500	0.6980	-4.80	0.000
TDS-bact	0.006885	0.007388	0.93	0.359

S = 41.80 R-Sq = 44.3% R-Sq(adj) = 40.6%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	2	41760	20880	11.95	0.000
Residual Error	30	52414	1747		
Total	32	94174			

Source	DF	Seq SS
Temp-bac	1	40243
TDS-bact	1	1517

Unusual Observations

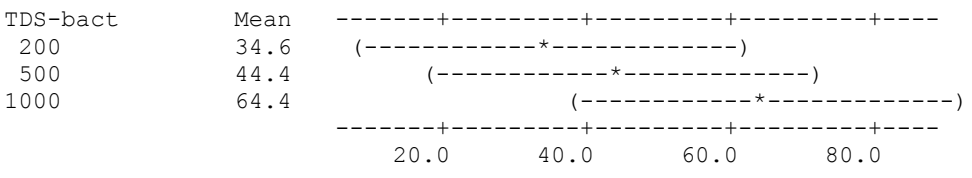
Obs	Temp-bac	FC days	Fit	StDev Fit	Residual	St Resid
19	5.0	200.00	96.32	12.18	103.68	2.59R
22	22.0	126.00	39.37	7.57	86.63	2.11R
28	5.0	19.00	110.09	19.10	-91.09	-2.45R
29	5.0	200.00	110.09	19.10	89.91	2.42R

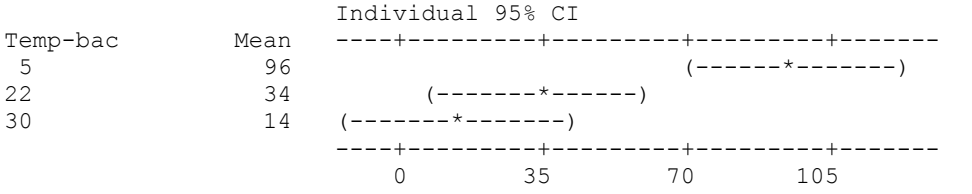
R denotes an observation with a large standardized residual

Fecal coliform, 2-way ANOVA, TDS of 200, 500, & 1000 mg/L only

Analysis of Variance for FC days

Source	DF	SS	MS	F	P
TDS-bact	2	4173	2087	1.44	0.262
Temp-bac	2	32712	16356	11.31	0.001
Interaction	4	3688	922	0.64	0.642
Error	18	26036	1446		
Total	26	66610			





B. Enterococci Temperature-TDS trials

Enterococci, 2-log ANOVA, all TDS concentrations, unbalanced design

```
Factor     Type Levels Values
Temp-bac   fixed      3  5 22 30
TDS-bact   fixed      4  200  500 1000 3000

Analysis of Variance for Ent Days, using Adjusted SS for Tests

Source              DF     Seq SS     Adj SS     Adj MS       F      P
Temp-bac             2    21873.2    22335.8    11167.9   21.71  0.000
TDS-bact             3     4280.7     4280.7     1426.9    2.77  0.067
Temp-bac*TDS-bact    6     1505.2     1505.2      250.9    0.49  0.810
Error               21    10805.0    10805.0      514.5
Total               32    38464.1

Unusual Observations for Ent Days

Obs  Ent Days       Fit   StDev Fit  Residual   St Resid
  2   114.000    62.333      13.096    51.667      2.79R
 10   110.000    69.000      13.096    41.000      2.21R

R denotes an observation with a large standardized residual.
```

Regression analysis results for enterococci at all TDS concentrations.

```
The regression equation is
Ent Days = 65.7 - 2.44 Temp-bact + 0.00825 TDS-bact

Predictor        Coef       StDev          T        P
Constant       65.662       8.987       7.31    0.000
Temp-bac      -2.4428      0.3720      -6.57    0.000
TDS-bact     0.008252    0.003937       2.10    0.045

S = 22.27       R-Sq = 61.3%     R-Sq(adj) = 58.7%

Analysis of Variance

Source            DF          SS          MS         F        P
Regression         2       23579       11789     23.76    0.000
Residual Error    30       14885         496
Total             32       38464

Source       DF      Seq SS
Temp-bac      1       21399
TDS-bact      1        2180

Unusual Observations
Obs   Temp-bac   Ent Days         Fit   StDev Fit    Residual    St Resid
  2        5.0     114.00       55.10        7.23       58.90        2.80R
 10        5.0     110.00       57.57        6.80       52.43        2.47R

R denotes an observation with a large standardized residual
```

Enterococci, 2-way ANOVA, TDS of 200, 500, & 1000 mg/L only

```
Analysis of Variance for Ent days
Source        DF        SS        MS        F        P
TDS-bact       2      1222       611     1.16    0.337
Temp-bac       2     15497      7748    14.67    0.000
Interaction    4       975       244     0.46    0.763
Error         18      9508       528
Total         26     27201

                      Individual 95% CI
TDS mg/L        Mean  -+---------+---------+---------+---------+
 200            22.4        (-------------*------------)
 500            31.6                  (------------*-------------)
1000            15.1  (-------------*------------)
                      -+---------+---------+---------+---------+
                     0.0      12.0      24.0      36.0      48.0

                      Individual 95% CI
Temp °C         Mean  ------+---------+---------+---------+-----
 5              56.6                          (------*-----)
22              10.6       (-----*------)
30               2.0  (------*-----)
                      ------+---------+---------+---------+-----
                          0.0      25.0      50.0      75.0
```

C. F+ RNA coliphage Temperature-TDS trials

F+ RNA coliphage, 2-way ANOVA, all TDS concentrations

```
Analysis of Variance RNA days
Source        DF        SS        MS        F        P
temp-pha       2     16313      8156    77.59    0.000
TDS-phag       3      4220      1407    13.38    0.000
Interaction    6      9766      1628    15.48    0.000
Error         12      1262       105
Total         23     31560

                      Individual 95% CI
temp °C         Mean  --------+---------+---------+---------+---
 5              77.5                                    (---*---)
22              42.4                   (---*---)
30              13.8    (---*---)
                      --------+---------+---------+---------+---
                           20.0      40.0      60.0      80.0

                      Individual 95% CI
TDS mg/L        Mean  -----+---------+---------+---------+------
 200            31.8  (-----*-----)
 500            45.3            (-----*-----)
1000            35.2     (-----*------)
3000            65.8                          (-----*-----)
                      -----+---------+---------+---------+------
                        30.0      45.0      60.0      75.0
```

Regression analysis results for F+ RNA coliphage at all TDS concentrations

The regression equation is
RNA days = 78.7 + 0.0110 TDS - 2.47 temp

Predictor	Coef	StDev	T	P
Constant	78.65	11.49	6.84	0.000
TDS-phag	0.010981	0.004496	2.44	0.024
temp-pha	-2.4743	0.4709	-5.25	0.000

S = 24.05 R-Sq = 61.5% R-Sq(adj) = 57.9%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	2	19416.3	9708.1	16.79	0.000
Residual Error	21	12143.7	578.3		
Total	23	31560.0			

Source	DF	Seq SS
TDS-phag	1	3449.6
temp-pha	1	15966.7

Unusual Observations

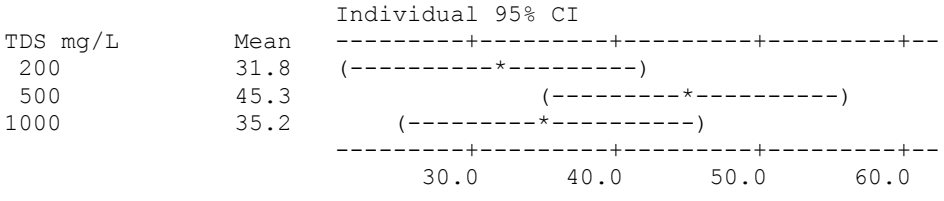
Obs	TDS-phag	RNA days	Fit	StDev Fit	Residual	St Resid
19	3000	155.00	99.22	11.61	55.78	2.65R

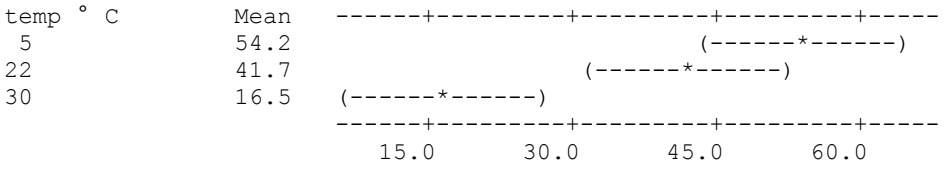
R denotes an observation with a large standardized residual

F+ RNA coliphage, 2-way ANOVA, TDS of 200, 500, & 1000 mg/L only

Analysis of Variance for RNA days

Source	DF	SS	MS	F	P
TDS phg	2	593	297	2.35	0.151
temp phg	2	4417	2208	17.50	0.001
Interaction	4	132	33	0.26	0.895
Error	9	1136	126		
Total	17	6278			





D. DNA coliphage Temperature-TDS trials

DNA coliphage, 2-way ANOVA, all TDS concentrations

```
Analysis of Variance for DNA days
Source        DF        SS        MS        F        P
temp-pha       2     15859      7930     3.45    0.065
TDS-phag       3     10963      3654     1.59    0.243
Interaction    6      6509      1085     0.47    0.816
Error         12     27558      2297
Total         23     60889

                      Individual 95% CI
temp °C         Mean  --------+---------+---------+---------+---
 5               101                  (----------*---------)
22               96                 (---------*----------)
30               44  (----------*---------)
                     --------+---------+---------+---------+---
                             35        70       105       140

                      Individual 95% CI
TDS mg/L        Mean  -------+---------+---------+---------+----
 200              95              (-----------*-----------)
 500              63     (-----------*-----------)
1000              56    (-----------*-----------)
3000             107                 (------------*-----------)
                     -------+---------+---------+---------+----
                           35        70       105       140
```

Regression analysis for DNA coliphage at all TDS concentrations.

```
The regression equation is
DNA days = 106 + 0.0104 TDS-phage - 1.98 temp-phage

Predictor        Coef       StDev          T        P
Constant       105.75       22.75       4.65    0.000
TDS-phag     0.010372    0.008901       1.17    0.257
temp-pha      -1.9793      0.9322      -2.12    0.046

S = 47.61       R-Sq = 21.8%     R-Sq(adj) = 14.4%

Analysis of Variance

Source            DF          SS          MS         F        P
Regression         2       13295        6647      2.93    0.075
Residual Error    21       47595        2266
Total             23       60889

Source       DF      Seq SS
TDS-phag      1        3077
temp-pha      1       10217

Unusual Observations
Obs   TDS-phag   DNA days         Fit   StDev Fit    Residual    St Resid
  3        200     225.00       64.28       13.33      160.72        3.52R

R denotes an observation with a large standardized residual
```

DNA coliphage, 2-way ANOVA, TDS of 200, 500, & 1000 mg/L only

```
Analysis of Variance for DNA days
Source        DF        SS        MS        F        P
TDS phg        2      5345      2673     1.20    0.344
temp phg       2     15509      7755     3.49    0.075
Interaction    4      3750       938     0.42    0.789
Error          9     19996      2222
Total         17     44601

                      Individual 95% CI
TDS mg/L          Mean   -------+---------+---------+---------+----
 200               95               (-----------*-----------)
 500               63      (-----------*-----------)
1000               56    (-----------*-----------)
                        -------+---------+---------+---------+----
                              35        70       105       140

                      Individual 95% CI
temp °C         Mean   ----+---------+---------+---------+-------
 5                89                  (----------*----------)
22                95                    (----------*----------)
30                30    (----------*---------)
                        ----+---------+---------+---------+-------
                            0        40        80       120
```

E. PRD-1 bacteriophage Temperature-TDS trials

PRD-1, 2-way ANOVA, all TDS concentrations

```
Analysis of Variance for PRD rate
Source        DF        SS        MS        F        P
temp-pha       2   0.01393   0.00696     2.41    0.132
TDS-phag       3   0.00496   0.00165     0.57    0.644
Interaction    6   0.00591   0.00098     0.34    0.902
Error         12   0.03466   0.00289
Total         23   0.05945

                      Individual 95% CI
temp °C        log/d   --+---------+---------+---------+---------
 5            -0.016                  (-----------*-----------)
22            -0.022                (-----------*-----------)
30            -0.070   (-----------*-----------)
                       --+---------+---------+---------+---------
                     -0.105    -0.070    -0.035     0.000

                      Individual 95% CI
TDS-phag        Mean   -+---------+---------+---------+---------+
 200          -0.032           (-------------*-------------)
 500          -0.038          (-------------*-------------)
1000          -0.057    (-------------*------------)
3000          -0.017              (-------------*-------------)
                       -+---------+---------+---------+---------+
                   -0.105    -0.070    -0.035     0.000     0.035
```

Regression analysis for PRD-1 at all TDS concentrations

The regression equation is
PRD rate = - 0.0092 - 0.00186 temp-phage +0.000007 TDS-phage

Predictor	Coef	StDev	T	P
Constant	-0.00923	0.02307	-0.40	0.693
temp-pha	-0.0018600	0.0009453	-1.97	0.062
TDS-phag	0.00000722	0.00000903	0.80	0.433

S = 0.04827 R-Sq = 17.7% R-Sq(adj) = 9.8%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	2	0.010515	0.005257	2.26	0.130
Residual Error	21	0.048937	0.002330		
Total	23	0.059452			

Source	DF	Seq SS
temp-pha	1	0.009023
TDS-phag	1	0.001491

Unusual Observations

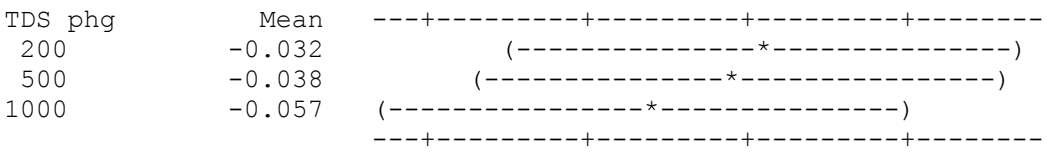
Obs	temp-pha	PRD rate	Fit	StDev Fit	Residual	St Resid
18	30.0	-0.22900	-0.05781	0.01441	-0.17119	-3.72R

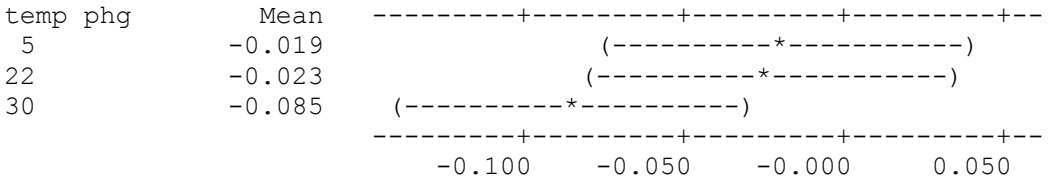
R denotes an observation with a large standardized residual

PRD-1, 2-way ANOVA, TDS of 200, 500, & 1000 mg/L only

Analysis of Variance for PRD rate

Source	DF	SS	MS	F	P
TDS phg	2	0.00219	0.00110	0.29	0.758
temp phg	2	0.01637	0.00819	2.14	0.174
Interaction	4	0.00322	0.00080	0.21	0.926
Error	9	0.03450	0.00383		
Total	17	0.05629			



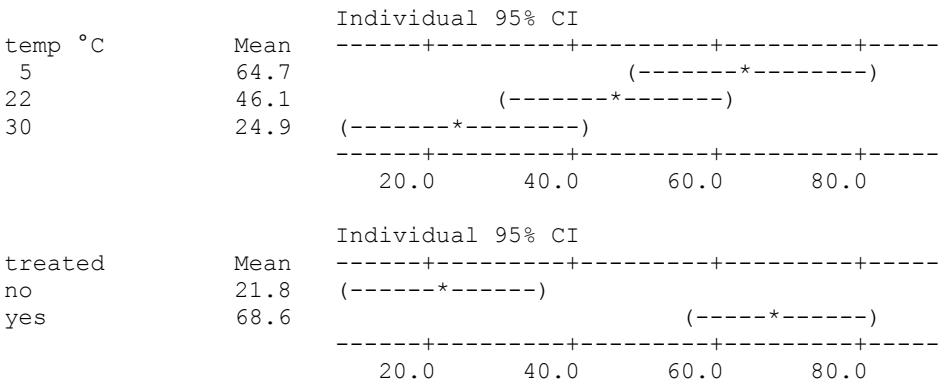


F. Fecal coliform natural water trials

Fecal coliform; 2-way ANOVA, all natural water

Analysis of Variance for 2-log days

Source	DF	SS	MS	F	P
temp	2	12700	6350	5.87	0.006
treated	1	26274	26274	24.29	0.000
Interaction	2	815	408	0.38	0.688
Error	42	45430	1082		
Total	47	85218			



Fecal coliform; 3-way ANOVA, all natural water

Factor	Type	Levels	Values
wate type	fixed	2	ground surface
treated	fixed	2	no yes
temp	fixed	3	5 22 30

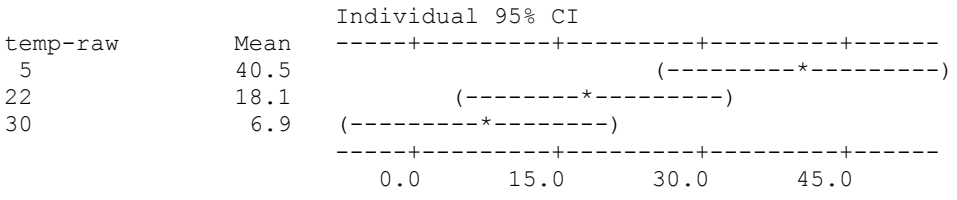
Analysis of Variance for 2-log days

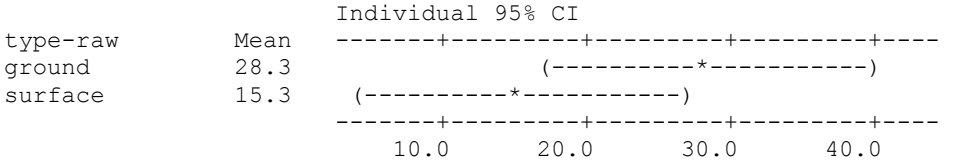
Source	DF	SS	MS	F	P
type	1	4622	4622	4.62	0.038
treated	1	26274	26274	26.25	0.000
temp	2	12700	6350	6.34	0.004
type*treated	1	527	527	0.53	0.473
type*temp	2	3195	1598	1.60	0.217
treated*temp	2	815	408	0.41	0.669
type*treated*temp	2	1051	525	0.52	0.596
Error	36	36036	1001		
Total	47	85218			

Fecal coliform; 2-way ANOVA, raw surface and ground

Analysis of Variance for days-raw

Source	DF	SS	MS	F	P
temp-raw	2	4688	2344	6.28	0.009
type-raw	1	1014	1014	2.72	0.117
Interaction	2	315	158	0.42	0.662
Error	18	6721	373		
Total	23	12737			

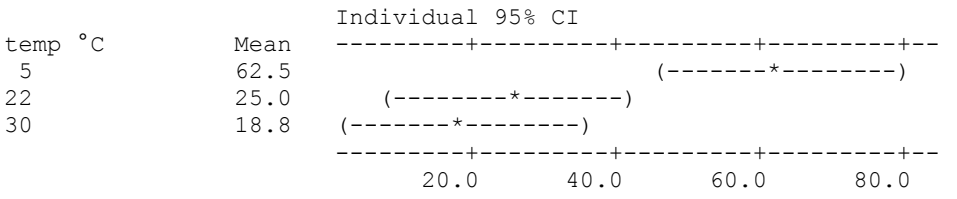


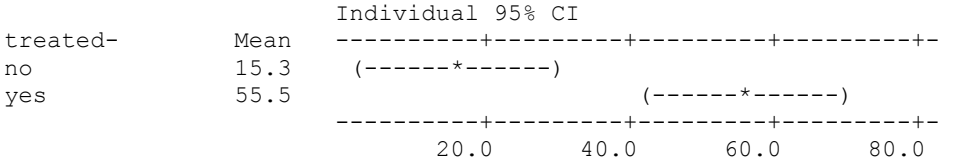


Fecal coliform; 2-way ANOVA, surface water

Analysis of Variance for days-sw

Source	DF	SS	MS	F	P
temp-sw	2	8958	4479	8.82	0.002
treated-	1	9680	9680	19.06	0.000
Interaction	2	450	225	0.44	0.649
Error	18	9141	508		
Total	23	28230			





Fecal coliform; 1-way ANOVA, raw surface water

```
Analysis of Variance for days sw
Source     DF        SS        MS        F        P
temp sw     2      2713      1356     7.54    0.012
Error       9      1618       180
Total      11      4331
                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Temp °C     N      Mean     StDev  -------+---------+---------+---------
 5          4     36.50     23.01                    (------*-------)
22          4      6.50      2.52     (------*-------)
30          4      3.00      1.83   (-------*------)
                                    -------+---------+---------+---------
Pooled StDev =    13.41                  0        20        40
```

Fecal coliform; 2-way ANOVA, groundwater

```
Analysis of Variance for days gw
Source        DF        SS        MS        F        P
temp gw        2      9588      4794     1.68    0.215
treated        1     22022     22022     7.70    0.012
Interaction    2      1287       643     0.22    0.801
Error         18     51491      2861
Total         23     84388

                      Individual 95% CI
temp °C         Mean  ---+---------+---------+---------+--------
 5                78               (----------*----------)
22                67          (----------*----------)
30                31    (----------*----------)
                      ---+---------+---------+---------+--------
                         0        35        70       105

                      Individual 95% CI
treated         Mean  --+---------+---------+---------+---------
no                28    (---------*----------)
yes               89                        (----------*---------)
                      --+---------+---------+---------+---------
                        0        30        60        90
```

Fecal coliform; 1-way ANOVA, raw groundwater

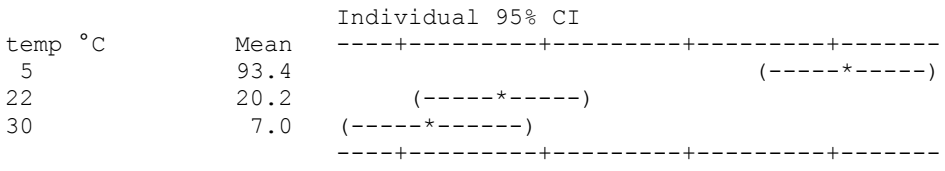
```
Analysis of Variance for days gw
Source     DF        SS        MS        F        P
temp gw     2      2290      1145     2.02    0.189
Error       9      5102       567
Total      11      7393
                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Temp °C     N      Mean     StDev  -------+---------+---------+---------
 5          4     44.50     39.20                (----------*----------)
22          4     29.75     12.69          (----------*----------)
30          4     10.75      1.89   (---------*----------)
                                       -------+---------+---------+--------
-
Pooled StDev =    23.81                    0        25        50
```

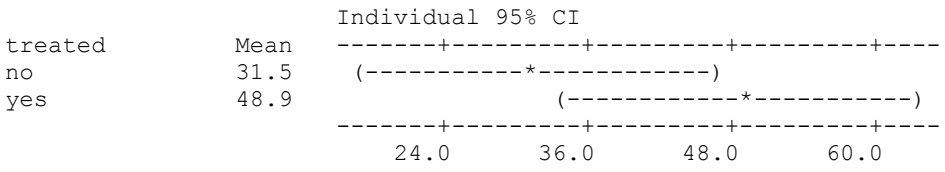
G. Enterococci natural water trials

Enterococci; 2-way ANOVA, all natural water

Analysis of Variance for 2-log days

Source	DF	SS	MS	F	P
temp	2	69285	34643	25.76	0.000
treated	1	3623	3623	2.69	0.108
Interaction	2	2629	1314	0.98	0.385
Error	42	56483	1345		
Total	47	132019			





Enterococci; 3-way ANOVA, all natural water

Factor	Type	Levels	Values
temp	fixed	3	5 22 30
treated	fixed	2	no yes
water ty	fixed	2	ground surface

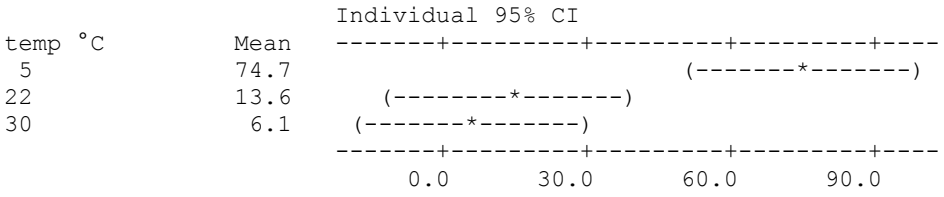
Analysis of Variance for 2-log days

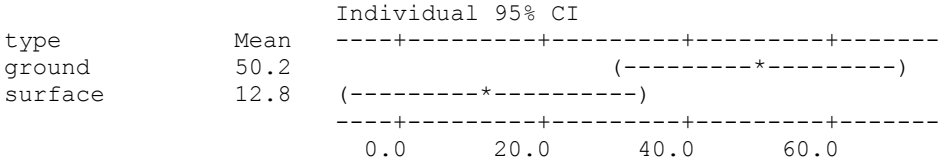
Source	DF	SS	MS	F	P
temp	2	69285	34643	33.34	0.000
treated	1	3623	3623	3.49	0.070
water ty	1	2338	2338	2.25	0.142
temp*treated	2	2629	1314	1.27	0.294
temp*water ty	2	853	426	0.41	0.666
treated*water ty	1	6557	6557	6.31	0.017
temp*treated*water ty	2	9332	4666	4.49	0.018
Error	36	37403	1039		
Total	47	132019			

Enterococci; 2-way ANOVA, raw surface and groundwater

Analysis of Variance for days-r

Source	DF	SS	MS	F	P
temp-r	2	22672	11336	10.11	0.001
type-r	1	8363	8363	7.46	0.014
Interaction	2	7812	3906	3.48	0.053
Error	18	20188	1122		
Total	23	59034			

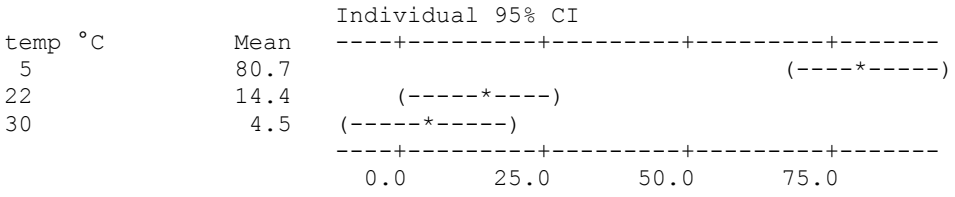


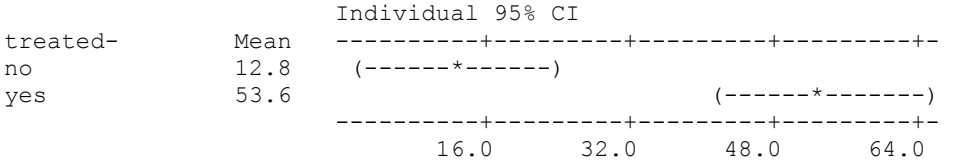


Enterococci; 2-way ANOVA, surface water

Analysis of Variance for days-sw

Source	DF	SS	MS	F	P
temp-sw	2	27513	13756	36.91	0.000
treated-	1	9963	9963	26.74	0.000
Interaction	2	10764	5382	14.44	0.000
Error	18	6708	373		
Total	23	54948			





Enterococci; 1-way ANOVA, raw surface water

```
Analysis of Variance for days sw
Source     DF        SS        MS        F        P
temp sw     2   1936.17    968.08   125.36    0.000
Error       9     69.50      7.72
Total      11   2005.67

                                 Individual 95% CIs For Mean
                                 Based on Pooled StDev
Temp °C     N      Mean     StDev  -+---------+---------+---------+-----
 5          4    30.750     4.193                                (--*--)
22          4     5.000     1.826     (--*--)
30          4     2.750     1.500   (--*--)
                                    -+---------+---------+---------+-----
Pooled StDev =    2.779              0         10        20        30
```

Enterococci; 2-way ANOVA, groundwater

```
Analysis of Variance for days gw
Source         DF        SS        MS        F        P
treated         1       216       216     0.13    0.726
temp gw         2     42625     21313    12.50    0.000
Interaction     2      1197       598     0.35    0.709
Error          18     30695      1705
Total          23     74733

                       Individual 95% CI
treated         Mean   --------+---------+---------+---------+---
no              50.2         (---------------*----------------)
yes             44.2    (---------------*----------------)
                       --------+---------+---------+---------+---
                            30.0      45.0      60.0      75.0

                       Individual 95% CI
temp °C         Mean   ------+---------+---------+---------+-----
 5               106                            (-------*------)
22                26         (-------*------)
30                10    (------*-------)
                       ------+---------+---------+---------+-----
                             0        40        80       120
```

Enterococci; 1-way ANOVA, raw groundwater

```
Analysis of Variance for days gw
Source     DF        SS        MS        F        P
temp gw     2     28547     14274     6.39    0.019
Error       9     20119      2235
Total      11     48666

                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Temp °C     N      Mean     StDev  --------+---------+---------+-------
-
 5          4    118.75     80.45                     (--------*-------
-)
22          4     22.25     13.94     (--------*--------)
30          4      9.50      6.24   (--------*-------)
                                  --------+---------+---------+-------
-
Pooled StDev =    47.28                   0        60       120
```

H. F+ RNA coliphage natural water trials

F+ RNA coliphage; 2-way ANOVA, all natural water

```
Analysis of Variance for 2-log days
Source        DF        SS        MS        F        P
temp           2     14709      7354    24.49    0.000
treated        1         4         4     0.01    0.908
Interaction    2         8         4     0.01    0.986
Error         42     12610       300
Total         47     27331

                      Individual 95% CI
temp            Mean  -----+---------+---------+---------+------
 5              40.1                           (-----*-----)
22               4.6     (-----*-----)
30               1.4   (-----*-----)
                      -----+---------+---------+---------+------
                         0.0      15.0      30.0      45.0

                      Individual 95% CI
treated         Mean  -+---------+---------+---------+---------+
no              15.1    (-----------------*-----------------)
yes             15.7     (-----------------*-----------------)
                      -+---------+---------+---------+---------+
                     8.0      12.0      16.0      20.0      24.0
```

F+ RNA coliphage; 3-way ANOVA, all natural water

Factor	Type	Levels	Values
temp	fixed	3	5 22 30
water ty	fixed	2	ground surface
treated	fixed	2	no yes

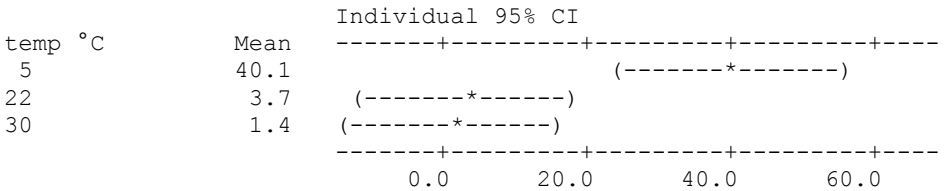
Analysis of Variance for 2-log da

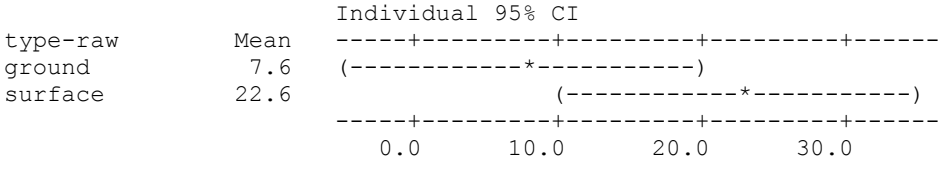
Source	DF	SS	MS	F	P
temp	2	14708.6	7354.3	29.65	0.000
water ty	1	736.3	736.3	2.97	0.093
treated	1	4.1	4.1	0.02	0.899
temp*water ty	2	984.3	492.1	1.98	0.152
temp*treated	2	8.3	4.1	0.02	0.983
water ty*treated	1	616.3	616.3	2.48	0.124
temp*water ty*treated	2	1342.8	671.4	2.71	0.080
Error	36	8930.5	248.1		
Total	47	27331.2			

F+ RNA coliphage; 2-way ANOVA, raw surface and groundwater

Analysis of Variance for days-raw

Source	DF	SS	MS	F	P
temp-raw	2	7548	3774	9.07	0.002
type-raw	1	1350	1350	3.24	0.088
Interaction	2	2311	1155	2.78	0.089
Error	18	7491	416		
Total	23	18700			

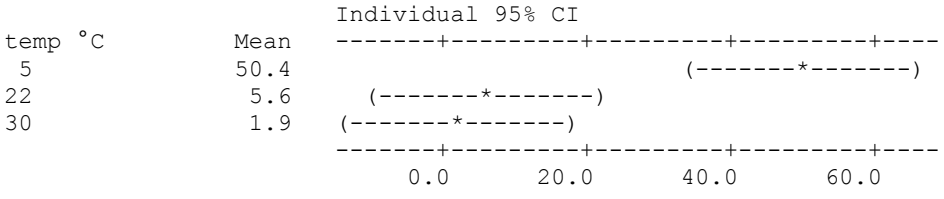


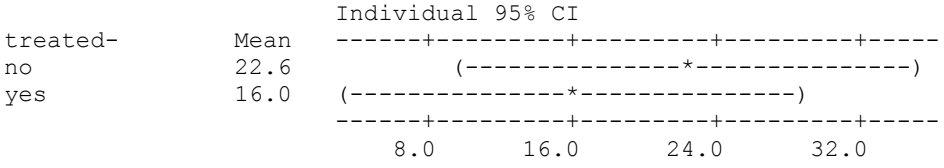


F+ RNA coliphage; 2-way ANOVA, surface water

Analysis of Variance for days-sw

Source	DF	SS	MS	F	P
temp-sw	2	11650	5825	13.43	0.000
treated-	1	260	260	0.60	0.449
Interaction	2	740	370	0.85	0.442
Error	18	7806	434		
Total	23	20457			

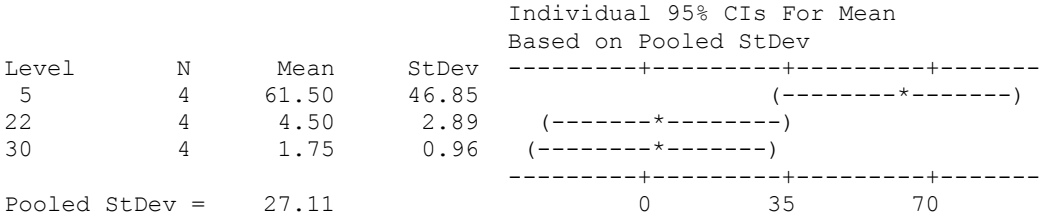




F+ RNA coliphage; 1-way ANOVA, raw surface water

Analysis of Variance for days sw

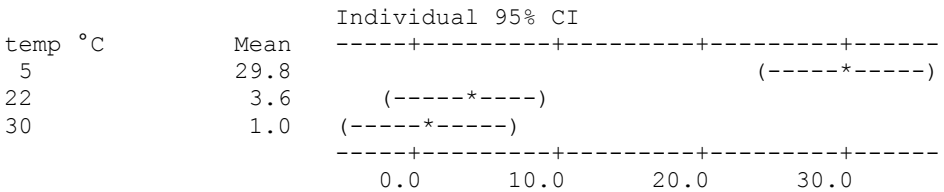
Source	DF	SS	MS	F	P
temp sw	2	9102	4551	6.19	0.020
Error	9	6613	735		
Total	11	15715			

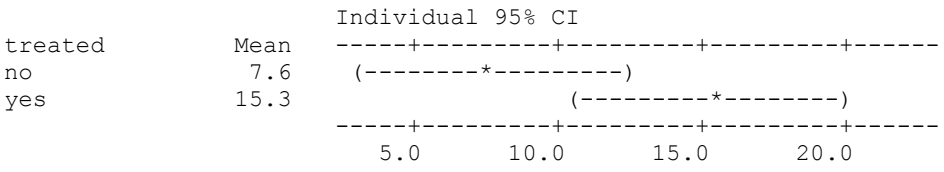


F+ RNA coliphage; 2-way ANOVA, groundwater

Analysis of Variance for days gw

Source	DF	SS	MS	F	P
temp gw	2	4042.6	2021.3	32.36	0.000
treated	1	360.4	360.4	5.77	0.027
Interaction	2	610.7	305.4	4.89	0.020
Error	18	1124.3	62.5		
Total	23	6138.0			

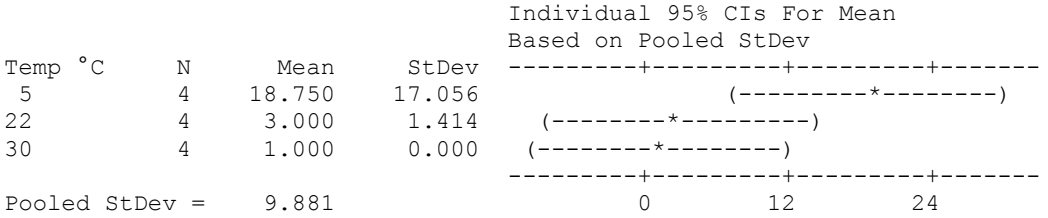




F+ RNA coliphage; 1-way ANOVA, raw groundwater

Analysis of Variance for days gw

Source	DF	SS	MS	F	P
temp gw	2	756.2	378.1	3.87	0.061
Error	9	878.8	97.6		
Total	11	1634.9			



I. DNA coliphage natural water trials

DNA coliphage; 2-way ANOVA, all natural water

```
Analysis of Variance for 2-log days
Source        DF        SS        MS        F        P
temp           2     52285     26143     9.68    0.000
treated        1     53734     53734    19.89    0.000
Interaction    2      4902      2451     0.91    0.411
Error         42    113466      2702
Total         47    224388

                      Individual 95% CI
temp °C         Mean  ------+---------+---------+---------+-----
 5               123                          (------*-------)
22               73           (-------*------)
30               43    (------*-------)
                      ------+---------+---------+---------+-----
                           35        70       105       140

                      Individual 95% CI
treated         Mean  --+---------+---------+---------+---------
no               46   (------*-------)
yes             113                         (------*------)
                      --+---------+---------+---------+---------
                       30        60        90       120
```

DNA coliphage; 3-way ANOVA, all natural water

```
Factor     Type Levels Values
water ty  fixed      2  ground surface
treated   fixed      2    no   yes
temp      fixed      3     5    22    30


Analysis of Variance for 2-log days

Source                   DF         SS         MS       F      P
water ty                  1       7450       7450    2.94  0.095
treated                   1      53734      53734   21.19  0.000
temp                      2      52285      26143   10.31  0.000
water ty*treated          1      14560      14560    5.74  0.022
water ty*temp             2        110         55    0.02  0.979
treated*temp              2       4902       2451    0.97  0.390
water ty*treated*temp     2         36         18    0.01  0.993
Error                    36      91310       2536
Total                    47     224388
```

DNA coliphage; 2-way ANOVA, raw surface and groundwater

```
Analysis of Variance for days-raw
Source        DF        SS        MS        F        P
type-raw       1       590       590     0.57    0.462
temp-raw       2     37027     18513    17.74    0.000
Interaction    2       134        67     0.06    0.938
Error         18     18789      1044
Total         23     56541

                      Individual 95% CI
type-raw        Mean  ---+---------+---------+---------+--------
ground          51.1            (-----------------*---------------)
surface         41.2     (---------------*-----------------)
                      ---+---------+---------+---------+--------
                      24.0      36.0      48.0      60.0

                      Individual 95% CI
temp-raw        Mean  ----+---------+---------+---------+-------
 5               101                              (------*------)
22                27         (------*------)
30                11    (------*------)
                      ----+---------+---------+---------+-------
                          0        35        70       105
```

DNA coliphage; 2-way ANOVA, surface water

```
Analysis of Variance for days-sw
Source        DF        SS        MS        F        P
temp-sw        2     24831     12416     4.62    0.024
treated-       1     62118     62118    23.11    0.000
Interaction    2      2727      1364     0.51    0.610
Error         18     48378      2688
Total         23    138055

                      Individual 95% CI
temp °C         Mean  ------+---------+---------+---------+-----
 5               135                       (---------*--------)
22                84           (---------*---------)
30                57     (--------*---------)
                      ------+---------+---------+---------+-----
                           40        80       120       160

                      Individual 95% CI
treated-        Mean  --------+---------+---------+---------+---
no                41   (-------*-------)
yes              143                               (-------*-------)
                      --------+---------+---------+---------+---
                             40        80       120       160
```

DNA coliphage; 1-way ANOVA, raw surface water

```
Analysis of Variance for days surface water, raw
Source     DF        SS        MS        F        P
temp sw     2     17933      8967     9.31    0.006
Error       9      8664       963
Total      11     26598

                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Temp °C     N      Mean     StDev  ------+---------+---------+---------+
 5          4     95.50     53.15                    (------*------)
22          4     19.25      6.95     (------*------)
30          4      8.75      3.86   (------*------)
                                  ------+---------+---------+---------+
Pooled StDev =    31.03               0        50       100       150
```

DNA coliphage; 2-way ANOVA, groundwater

```
Analysis of Variance for days gw
Source        DF        SS        MS        F        P
temp gw        2     27564     13782     5.78    0.012
treated        1      6176      6176     2.59    0.125
Interaction    2      2211      1106     0.46    0.636
Error         18     42931      2385
Total         23     78883

                      Individual 95% CI
temp °C         Mean  ---+---------+---------+---------+--------
 5               111                       (--------*--------)
22                62             (--------*--------)
30                28    (--------*--------)
                      ---+---------+---------+---------+--------
                         0        40        80       120

                      Individual 95% CI
treated         Mean  --+---------+---------+---------+---------
no                51    (----------*-----------)
yes               83               (-----------*-----------)
                      --+---------+---------+---------+---------
                       25        50        75       100
```

DNA coliphage; 1-way ANOVA, raw groundwater

```
Analysis of Variance for days gw
Source     DF        SS        MS        F        P
temp gw     2     19228      9614     8.55    0.008
Error       9     10125      1125
Total      11     29353

                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Temp °C     N      Mean     StDev  ------+---------+---------+---------+
 5          4    106.25     57.13                      (------*-------)
22          4     34.50     10.08       (-------*------)
30          4     12.50      3.00   (-------*------)
                                  ------+---------+---------+---------+
Pooled StDev =    33.54               0        50       100       150
```

J. PRD-1 natural water trials, inactivation rate statistics

PRD-1; 2-way ANOVA, all natural water

```
Analysis of Variance for rate
Source        DF        SS        MS        F        P
temp           2   0.02579   0.01289     3.15    0.053
treated        1   0.00922   0.00922     2.25    0.141
Interaction    2   0.00065   0.00033     0.08    0.923
Error         42   0.17217   0.00410
Total         47   0.20783

                      Individual 95% CI
temp °C         Mean  ------+---------+---------+---------+-----
 5            -0.018                     (----------*----------)
22            -0.039               (----------*----------)
30            -0.074    (---------*----------)
                      ------+---------+---------+---------+-----
                       -0.090    -0.060    -0.030     0.000

                      Individual 95% CI
treated         Mean  --+---------+---------+---------+---------
no           -0.0573  (------------*-------------)
yes          -0.0296                (------------*------------)
                      --+---------+---------+---------+---------
                  -0.0800   -0.0600   -0.0400   -0.0200
```

PRD-1; 3-way ANOVA, all natural water

```
Factor     Type Levels Values
temp       fixed      3     5    22    30
water ty   fixed      2  ground surface
treated    fixed      2    no   yes

Analysis of Variance for rate

Source                   DF         SS         MS       F      P
temp                      2   0.025786   0.012893    3.83  0.031
water ty                  1   0.028743   0.028743    8.53  0.006
treated                   1   0.009224   0.009224    2.74  0.107
temp*water ty             2   0.015638   0.007819    2.32  0.113
temp*treated              2   0.000655   0.000327    0.10  0.908
water ty*treated          1   0.005491   0.005491    1.63  0.210
temp*water ty*treated     2   0.000957   0.000479    0.14  0.868
Error                    36   0.121337   0.003370
Total                    47   0.207831
```

PRD-1; 2-way ANOVA, raw surface and groundwater

```
Analysis of Variance for rate-r
Source        DF        SS        MS         F        P
temp-r         2  0.013808  0.006904      7.76    0.004
type-r         1  0.029681  0.029681     33.34    0.000
Interaction    2  0.007660  0.003830      4.30    0.030
Error         18  0.016024  0.000890
Total         23  0.067173

                      Individual 95% CI
temp °C         Mean  ----+---------+---------+---------+-------
 5            -0.028                          (--------*--------)
22            -0.058              (--------*--------)
30            -0.087    (-------*--------)
                      ----+---------+---------+---------+-------
                    -0.100    -0.075    -0.050    -0.025

                      Individual 95% CI
type           Mean   -------+---------+---------+---------+----
ground       -0.0222                          (-----*-----)
surface      -0.0925   (-----*-----)
                      -------+---------+---------+---------+----
                       -0.0900   -0.0600   -0.0300    0.0000
```

PRD-1; 2-way ANOVA, surface water

```
Analysis of Variance for rate
Source        DF        SS        MS         F        P
temp-sw        2   0.04072   0.02036      3.16    0.067
treated-       1   0.01447   0.01447      2.24    0.152
Interaction    2   0.00158   0.00079      0.12    0.886
Error         18   0.11615   0.00645
Total         23   0.17293

                      Individual 95% CI
temp °C         Mean  -+---------+---------+---------+---------+
 5            -0.023                   (---------*---------)
22            -0.058              (---------*---------)
30            -0.123    (---------*--------)
                      -+---------+---------+---------+---------+
                  -0.180    -0.120    -0.060     0.000     0.060

                      Individual 95% CI
treated         Mean  -+---------+---------+---------+---------+
no            -0.093    (-------------*-------------)
yes           -0.043                  (-------------*-------------)
                      -+---------+---------+---------+---------+
                  -0.140    -0.105    -0.070    -0.035     0.000
```

PRD-1; 1-way ANOVA, raw surface water

```
Analysis of Variance for rate sw
Source     DF        SS        MS        F        P
temp sw     2   0.02102   0.01051     6.89    0.015
Error       9   0.01372   0.00152
Total      11   0.03474

                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Temp °C     N      Mean     StDev  --+---------+---------+---------+----
 5          4  -0.04075   0.02269                    (------*-------)
22          4  -0.09350   0.03142           (------*-------)
30          4  -0.14325   0.05542   (------*------)
                                  --+---------+---------+---------+----
Pooled StDev =  0.03904           -0.180    -0.120    -0.060     0.000
```

PRD-1; 2-way ANOVA, groundwater

```
Analysis of Variance for rate gw
Source        DF        SS        MS        F        P
temp gw        2  0.000704  0.000352     1.22    0.318
treated        1  0.000241  0.000241     0.84    0.373
Interaction    2  0.000034  0.000017     0.06    0.943
Error         18  0.005183  0.000288
Total         23  0.006162

                     Individual 95% CI
temp gw         Mean --------+---------+---------+---------+---
 5           -0.0119                (-----------*-----------)
22           -0.0201       (------------*-----------)
30           -0.0250   (------------*-----------)
                     --------+---------+---------+---------+---
                       -0.0300   -0.0200   -0.0100    0.0000

                     Individual 95% CI
treated         Mean -------+---------+---------+---------+----
no           -0.0222    (-------------*--------------)
yes          -0.0158              (-------------*--------------)
                     -------+---------+---------+---------+----
                      -0.0280   -0.0210   -0.0140   -0.0070
```

PRD-1; 1-way ANOVA, raw groundwater

```
Analysis of Variance for rate gw
Source     DF        SS        MS        F        P
temp gw     2  0.000450  0.000225     0.88    0.448
Error       9  0.002304  0.000256
Total      11  0.002754

                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev  --+---------+---------+---------+----
 5          4  -0.01475   0.01571             (-----------*-----------)
22          4  -0.02200   0.01283       (-----------*-----------)
30          4  -0.02975   0.01887  (-----------*-----------)
                                  --+---------+---------+---------+----
Pooled StDev =  0.01600           -0.045    -0.030    -0.015     0.000
```

K. *Cryptosporidium parvum* TDS-temperature trials statistics

Cryptosporidium parvum, 2-way ANOVA, TDS of 200, 500, & 1000 mg/L

```
Analysis of Variance for days
Source        DF        SS        MS        F        P
temp           2     10587      5293    28.73    0.000
TDS            2       331       165     0.90    0.441
Interaction    4       544       136     0.74    0.589
Error          9      1658       184
Total         17     13120

                      Individual 95% CI
temp °C      Mean days ---------+---------+---------+---------+--
 5              72.0                                (-----*-----)
22              26.8         (-----*------)
30              16.0    (-----*-----)
                      ---------+---------+---------+---------+--
                            20.0      40.0      60.0      80.0

                      Individual 95% CI
TDS mg/L     Mean days --------+---------+---------+---------+---
 200            35.5    (------------*-----------)
 500            35.0   (------------*------------)
1000            44.3             (-----------*------------)
                      --------+---------+---------+---------+---
                           30.0      40.0      50.0      60.0
```

L. *Cryptosporidium parvum* natural water trials, inactivation rate statistics

Cryptosporidium parvum; 2-way ANOVA, raw surface and ground

```
Analysis of Variance for rate
Source        DF        SS        MS        F        P
temp           2  0.050863  0.025432   113.52    0.000
type           1  0.004528  0.004528    20.21    0.004
Interaction    2  0.002310  0.001155     5.16    0.050
Error          6  0.001344  0.000224
Total         11  0.059045

                      Individual 95% CI
temp °C     rate log/d -----+---------+---------+---------+------
 5            0.0011                                    (--*---)
22           -0.0385                              (--*---)
30           -0.1525    (---*--)
                      -----+---------+---------+---------+------
                     -0.1500   -0.1000   -0.0500   -0.0000

                      Individual 95% CI
type            Mean  ---------+---------+---------+---------+--
ground       -0.0439                       (------*-------)
surface      -0.0827  (-------*------)
                      ---------+---------+---------+---------+--
                         -0.0800   -0.0600   -0.0400   -0.0200
```

M. *Giardia lamblia* natural water trials, inactivation rate statistics

Giardia lamblia; 2-way ANOVA, raw surface and ground

```
Analysis of Variance for rate
Source        DF        SS        MS        F        P
type           1 0.0010849 0.0010849    51.44    0.000
temp           2 0.0172935 0.0086467   409.98    0.000
Interaction    2 0.0004879 0.0002440    11.57    0.009
Error          6 0.0001265 0.0000211
Total         11 0.0189928

                      Individual 95% CI
type   Mean rate log/d -----+---------+---------+---------+------
ground       -0.0479   (------*-----)
surface      -0.0288                             (------*-----)
                      -----+---------+---------+---------+------
                     -0.0490   -0.0420   -0.0350   -0.0280

                      Individual 95% CI
temp °C    Mean rate   ---------+---------+---------+---------+--
 5           -0.0040                                     (-*--)
22           -0.0198                                (-*-)
30           -0.0913   (-*--)
                      ---------+---------+---------+---------+--
                         -0.0750   -0.0500   -0.0250   -0.0000
```