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Conner J. Olsen for the degree of Honors Baccalaureate of Science in Environmental Engineering presented on May 29, 2014. Title: Testing a Theoretical Model that Predicts Extinction of Populations Forced by Random, Episodic Disturbances.

Abstract Approved: _____

Heather E. Lintz

We do not fully understand reasons behind extinction of populations and species. Consequently, our ability to anticipate extinction (which can be considered a permanent type of an ecological threshold) has remained elusive. In particular, it is not clear how the attributes of episodic disturbance regimes can elicit extinction. In this project, I test the application of a stochastic model that predicts population extinction based on attributes of the disturbance regime and population growth rates using phytoplankton in a test tube. I examined the response of phytoplankton (*Thalassiosira weissflogii* and *Synechocystis* sp.) to stochastic disturbances implemented by having MATLAB control a hydraulic pump that episodically removed portions of the population through time, in between episodes of population recovery. Model prediction of extinction was not observed in either species. In both cases, cells attached to the culture vessel provided refuge from disturbances, enabling the population to recover from a predicted extinction event. I conclude that model prediction might be improved by including a sub-population that is not subject to disturbance.

Key Words: ecological resilience, threshold, extinction, disturbance regime

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Testing a Theoretical Model that Predicts Extinction of Populations Forced by Random, Episodic Disturbances

by

Conner J. Olsen

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

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TESTING A THEORETICAL MODEL THAT PREDICTS THRESHOLDS IN POPULATIONS FORCED BY RANDOM, EPISODIC DISTURBANCES

INTRODUCTION

ENVIRONMENTAL MODELING

To predict system behavior in a complex world, we use simplifying models to understand phenomena and anticipate future responses to forcing. Modeling can be extremely useful for explaining and predicting some of the uncertainties we encounter. However, models are often susceptible to simplifying assumptions and approximations. Even if the simplest assumption turns out to be invalid, model results will fail at prediction. For this reason, it is necessary to test the validity of model assumptions and approximations.

A particular field of modeling that requires further testing is in the study of *ecological resilience* (Gunderson et al., 2009; Carpenter et al., 2011). C. S. Holling first introduced the idea of ecological resilience in 1973; it was defined as 'the amount of disturbance that an ecosystem can withstand without changing self-organized processes and structures' (Gunderson et al., 2000). This boundary where stressors start to inherently change the processes and structures of an ecosystem is known as an *ecological threshold*. More specifically, an ecological threshold is defined as the point at

which an ecological system exhibits an irreversible change (Groffman et al., 2006; CCSP, 2009). This shift from one stable state to another is typically associated with only a small change in either time or the driving condition (Scheffer et al., 2001; Beisner et al., 2003). Some examples of such ecological thresholds include eutrophication of waterways, dangerous shifts in wildfire regimes, desertification, and hypoxia of lakes.

Much research has gone into trying to uncover the causes of these threshold behaviors in an effort to make predictions about their occurrence. A classic example would be the work of Carpenter et al. (1999). This work presents the use of a deterministic, differential model to represent an ecological threshold caused by nutrient addition to lakes. This situation is what is known as a critical load threshold, because there is a specific concentration for which, when exceeded, the lake will almost always undergo eutrophication (Groffman et al., 2006). This approach and model is widely upheld in ecology and is appropriate for lake eutrophication. However, other model types and conceptual frameworks are warranted for different ecological systems and types of system responses. Perrings and Walker (1997) studied multiple locally-stable states in rangelands, experienced as a result of extreme events such as fire, flood, and drought. The unique component of this research was that it not only considered the effect of these extreme extrinsic factors, but it also took into account the influence of persistent stressors. In their case, the persistent stressor was grazing of the rangelands (Perrings and Walker, 1997).

POPULATION EXTINCTIONS

Population-level extinctions are another ecological phenomena that can exhibit threshold behavior (Beisner et al., 2003). The significant implication of this type of threshold is that it is permanent for the population, thus posing a major threat to the species (Groffman et al., 2006). Understanding what drives population extinctions is important to the sustainable future of species on Earth, including our species (Carpenter et al., 1999; Gunderson, 2000). For years, the dynamics of population-extinction thresholds have intrigued scientists, most notably due to the fact that they are yet to be fully understood (Lande, 1988; Dennis et al., 1991; Knowlton, 1992). A strong understanding of threshold behavior is imperative if we plan to maintain Earth's populations at a healthy state (Scheffer et al., 2001, Scheffer and Carpenter, 2003; Beisner et al., 2003).

One of the factors that can contribute to a population extinction threshold is the effect of episodic disturbances (Gunderson, 2006; Zinck, 2011). For the purposes of this paper, a "disturbance" is defined as any event that results in a decline in the expected population size. This is essentially the resultant effect of what Sibly and Hone (2002) call "environmental stressors;" which are defined as 'factors that reduce population growth rate when first applied.' A simple example of an environmental stressor would be inclement weather, for which extreme, episodic weather events will result in a disturbance to the population. Increasing frequency and intensity of extreme weather conditions and natural disasters is a particular area of concern in climate change scenarios (Scheffer et al., 2003; Keith et al., 2008). Disturbance regimes manifest as stochastic processes in nature, and for them to be modeled and better understood, they

need to be studied through experimentation, computer simulation, and/or quantification and pattern description in nature. In this paper, I will experimentally test the application of a model to help understand the nature of stochastic disturbance processes and whether they can contribute to a threshold in population extinction (Peckham and Waymire, 2014).

TESTING THE MODEL

Experimentation of a population-extinction threshold model requires the use of a live population existing within an environment that is of a relevant scale to its natural ecosystem (Scheffer and Carpenter, 2003). The major constraints for experimenting with a live population are that the species must be relatively well-understood in its growth characteristics, and it should reproduce at a rate that is commensurate for experimental time frames. Following these constraints, and for the purposes of imposing environmental disturbances in an effort to force a population to extinction, microbial populations are some of the only eligible test subjects. Bacteria and viruses are often studied due to the fact that they rapidly reproduce, but the population dynamics are complex, and genetic mutations are common. In this study, I use a phytoplankton culture to test whether a mathematical and stochastic model that predicts the existence of a population-extinction threshold applies in a controlled, *in vitro* environment.

The population dynamics of phytoplankton in nature are poorly understood. In aquatic systems, phytoplankton is subject to innumerable disturbances of varying degrees of intensities. Loss terms that can be quantified include dilution via mixing,

protist grazing, and the sinking of dead cells. Other disturbances such as mortality of individual cells, are completely unknown and must be empirically quantified. *In vitro*, many of these disturbances are insignificant and can be ignored for simple modeling scenarios.

The first species considered for experimentation purposes was *Thalassiosira weisflogii* (*Tw*) CCMP 1336, a marine diatom with a maximum growth rate of one division per day (r = 1.2 d^{-1}). This diatom exhibits logistic growth (Appendix A), and is considered to be highly resilient. In fact, this particular species is capable of fully populating any volume of desirable media from just a single cell. This means the population behavior of *Tw* lacks what is known in ecology as an *Allee effect*, an observed effect for which a population can only survive a disturbance event if the number of survivors exceeds a minimum population density (Allee, 1936). This proved to be useful for initial cell characterization and verification of experimental setup; however, the effect of this resiliency on experimentation was yet to be comprehended. The difficulties regarding lack of Allee effect will be reviewed in further detail in the discussion section of this paper.

After gaining a better understanding of the experimental constraints imposed by using a culture of *Tw*, alternative species of phytoplankton were considered. The requirements for this secondary culture were: 1) it should be comparable to *Tw* in growth characteristics, as to not require significant changes to the experimental setup; and 2) it should have an observed, if not known, Allee effect that will enable the experimental setup to induce an extinction event. The latter of these requirements has significant implications on the outcome of the experiment. Following these new

requirements, the second subject used for testing the model was *Synechocystis* sp. PCC 6803, a freshwater cyanobacteria species with similar growth rate (*r* is approximately 2 d^{-1}) and cell size to *Tw. Synechocystis* sp. also exhibits logistic growth (Appendix B). Although the Allee effect of these blue-green algae is not fully understood, it has been known to lose its viability following severe disturbance events, such as dilution to a small fraction of the original population. The significance of this is that the population will more accurately model an animal population because extinction is possible following extreme disturbance events, and should also be a possibility following the imposition of a disturbance regime with sufficient frequency and intensity of events.

"That we can make use of plankton to reveal the impact of climate is becoming increasingly evident as time-series lengthen and data-analysis techniques improve. There is strong evidence for systematic changes in plankton abundance and community structure over recent decades in many areas worldwide" (Hays et al., 2005). Residing in nearly every body of surface water, and providing nearly half of the total photosynthetic production for our planet, phytoplankton is an essential part of Earth's ecosystem (Edwards and Richardson, 2004; Hays et al., 2005). These factors have led to the realization that phytoplankton are an important indicator of the effects of global climate change. Hays et al. (2005) states, "these impacts of climate change might be compounded by the tendency for aquatic systems to undergo major and abrupt reorganization in plankton and fish communities." Although my experimental design is simplified in comparison to the natural ecosystem, the underlying themes of this study could have significant implications.

DISTURBANCE EVENTS

Testing a model such as this requires the imposition of precisely-quantified disturbance events. While the frequency of disturbance events is a variable that can be easily quantified and controlled for, the intensity is more complicated. Environmental disturbances are often far too complex to determine an intensity value prior to the event. Intensity values for environmental disturbances are typically calculated in the aftermath while observing the fraction of the population that survived the event. This method of quantification is useful for making sense of real-life disturbance events, but it does not lend itself well to the experimental testing of a population extinction threshold model. In an effort to impose consistently quantifiable disturbances to the populations of phytoplankton, these 'environmental disturbances' are simulated via fractional dilution of the population. Cells-and-media mixture is removed from the system and replaced with clean media, thus mimicking the scenario in which an event takes place and kills off a certain portion of the population, leaving only the survivors to repopulate the environment. While this may not be an ideal representation of an environmental disturbance, it is the simplest way to simulate such events with a known intensity value.

MATERIALS AND METHODS

THE MODEL (PECKHAM AND WAYMIRE, 2014)

Dr. Scott Peckham and Dr. Edward Waymire authored the model tested in the laboratory (Peckham and Waymire, 2014). Their model uses statistical probabilities of expected population values, along with the specific growth characteristics of a species, to predict the presence of an ecological threshold in which extinction of the population is certain. This is significant because there are few ecological models that allow a critical threshold to be computed based on environmental disturbances and population growth dynamics. Any population that obeys the assumptions of the model will follow the model prediction. The model is described as follows:

Population size is defined by the *logistic model*, an ordinary differential equation (ODE) that factors in specific growth rate, as well as a carrying capacity unique to the species and environment (Verhulst, 1838). Equation 1 gives the general solution to this ODE.

$$N(t) = \frac{r}{b+\left[\left(\frac{r}{N_0}\right)-b\right]e^{-rt}} \tag{1}$$

This equation shows that population size (N) at any time (t) is dependent upon the initial population size (N_0), growth rate (r), and a limiting constant (b) that is determined by the species and its environment. A larger value for b reflects higher levels of competition between cells, while a value of zero represents the case of

indefinite exponential growth. This constant is mathematically defined as the growth rate divided by the carrying capacity of the population.

When the population experiences a disturbance event, the right side of Equation 1 is multiplied by the survival rate, X_1. The resulting (surviving) population size (N_1) replaces N_0 as the new, initial population for subsequent growth. For this to work, t must also be redefined as zero following the disturbance event. This procedure is repeated for each of the n disturbance events within the disturbance regime. The model assumes a "disturbance regime" to be a series of population disturbance events that occur with stochastic frequency and intensity. The episodic nature of the regime is defined by two, unique probability distributions. The frequency of events is modeled as a Poisson process, with a frequency parameter (λ) representing the mean number of events per unit time. The probability (P) of n events occurring within the time interval [0, t] is given by Equation 2.

$$P(n) = \frac{e^{-\lambda t}(\lambda t)^n}{n!} \tag{2}$$

This probability function results in an exponential distribution with the modal value occurring at t = 0, meaning that clusters of events are a common trait of the Poisson process. Although this is quite effective for modeling the distribution of events over time, the intensity of each event requires a bit more mathematical manipulation. This is accomplished through the use of a *beta distribution*. A beta distribution is a range of values with fixed endpoints, in which the shape of the distribution is defined by the parameters '*a*' and '*b*' (Fente, 1999). Typical distributions include the standard bell

curve, triangular, or any variation of a "U" shape (Fente, 1999). Random values are drawn from the beta distribution to create a series of events that simulate the population being exposed to a sequence of disturbance events.

A different beta distribution is defined for each disturbance regime being tested. Each of these distributions has fixed endpoints at zero and one. The values within this interval represent a fraction of the existing population that will survive a given event. The flexibility of this distribution lies within the definition of a and b. The model being tested was also written as a Python script, which was used to determine the appropriate distribution parameters for the species being tested.

The experimental parameters for frequency and intensity defined the severity of the disturbance regime. The combination of these values resulted in the predicted threshold, which was represented by an indicator function (Equation 3).

$$I = r - \lambda(\eta - 1) \tag{3}$$

This function is essentially a balance of birth versus death rates of a population, with a threshold predicted to occur at $I = 0$. While r represents the mean birth rate of the population, the mean death rate was defined as a combination of the frequency and intensity of events or $\lambda(\eta - 1)$. As λ increased, so did the frequency of events. The intensity parameter, eta (η), was statistically defined as the negative of the expected value for the natural log of the survival rate. A series of experiments were defined as a result of these parameters and predictors, shown in Figure 1.

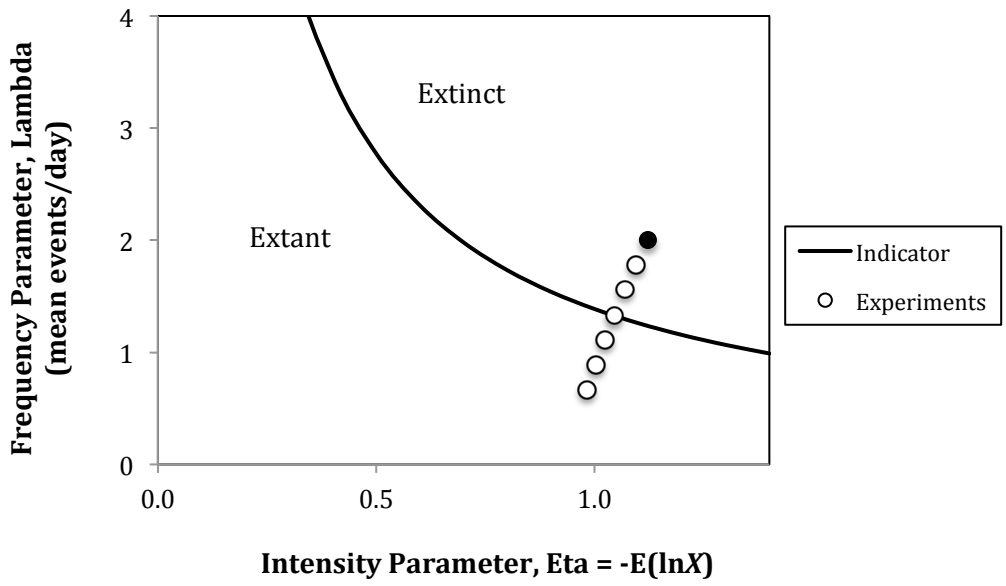

Figure 1: Experimental parameters for intensity (η) and frequency (λ) of disturbance events define the severity of each disturbance regime. Experiments step across the indicator curve representing the theoretical threshold. The solid point was the only one tested.

A series of seven experiments were designed with experimental constraints in mind (Appendix C). The seven experiments were intended to step across the threshold for a particular growth rate. A minimum of 20 disturbances per experiment was imposed to allow the population to reach a stable trajectory. Other practical constraints were imposed as well. A time constraint for each experiment of 30 days was imposed so that seven experiments could be completed. However, there were many unforeseen obstacles that only allowed for the completion of the most extreme disturbance regime (Figure 1). Some of the obstacles included contamination of the culture, midstream mathematical model overhaul, and anomalous variability in the performance of the pumping assembly.

CULTURING

Initial culturing was conducted using *Tw* CCMP 1336*.* Cells were grown in a 300-mL glass culture vessel at optimal conditions for growth (24-hr light cycle at 18°C) using f/2 culture medium (Guillard and Ryther, 1962). The environment was designed so that the limiting nutrient for growth was the silica content in the media. The media required for *Tw* was a seawater solution containing sodium silicate, sodium nitrate, iron, sodium selenate, and monosodium phosphate, plus trace metals and nutrients. A chelating agent ethylenediaminetetraacetic acid (EDTA) was also included to buffer metal ion concentrations.

The recipe required for the growth of *Synechocystis* sp*.* PCC 6803 was made using a similar method, but this cyanobacterium is a freshwater species, so the medium was made from natural lake water (Kalamath Lake, OR). The freshwater was supplemented with sodium nitrate, dipotassium phosphate, EDTA, along with trace metals (Rippka et al., 1979). The growth rate was limited by reducing the nitrate concentration in the freshwater media. The experimental setup was kept constant from previous *Tw* testing, although *Synechocystis* sp*.* prefers a slightly higher temperature (20-30°C). This resulted in a reduced growth rate of r = 1.5 d^{-1} (about two times that of *Tw*).

All media, culture vessels, and tubing were sterilized by autoclaving.

CELL COUNTS

While conducting these experiments, it was important to know the cell density within the test tube to a high level of accuracy. In order to accomplish this, a Coulter particle counter was used. This device used electrical impedance to measure the diameter of each particle that passed through a calibrated aperture, and then arranged them in a graphical output. The total number of cells in the sample was determined by summing all the particles with diameters lying between the known size-range for the species.

Samples were removed from the test tube using a 1-mL automatic pipette with a clean tip for each sample. Ten-milliliter samples were placed in a sampling cuvette (a flat-surfaced, transparent cup), which were then placed directly into the counter to get a value for cell density. As was often the case, the population density became too high, causing more than one cell (coincident cells) to pass through the aperture simultaneously, resulting in under-counted cells. For this situation, cells were diluted ten fold.

PUMPING

Dilution of the test tube required the use of a sterile syringe to remove a specific volume of culture. The same volume of clean media was then added to the test tube to keep volume constant throughout the process. In order to impose disturbance events to the highest level of accuracy, the dilutions were performed using an electronic, peristaltic pumping system. This consisted of a Cole-Parmer Masterflex adjustable-

speed pump fitted with small-diameter silicone tubing. Tubing was arranged to feed clean media from a 2-L bottle into the culture tube. Injection of media took place at the base of the test tube, while simultaneous extraction removed cells-and-media mixture from the top of the water column. This ensured a constant volume, while the thorough mixing from upwelling and air sparging created a dilution rate that resembled an exponential decay rate. The dilution rate for the pumping assembly was calibrated by collecting output over time. This flow rate was then converted to an exponential dilution rate using average cell counts from replicate trials of various pumping times.

CODING

Although the electronic pump automated the disturbances, the pump still had to be manually turned on and off at the proper times. This was not only inconvenient, but it became nearly impossible when imposing episodic disturbances at high frequency. In order to address this issue, a MATLAB code (Appendix C) was written to control the pumping through the use of a WebRelay remote relay control. This device made use of an Internet connection to send signals to an outlet fitted with a simple switch. The pump was plugged into this outlet, thus turning the pump on/off at the intervals defined by a MATLAB code.

Results

Disturbance events were defined in MATLAB as the length of time the pump was switched on. Accuracy of dilution fraction was therefore dependent on the consistency of the pumping assembly. Multiple tests of the dilution rate confirmed that the pumping rates were stable (Figure 2).

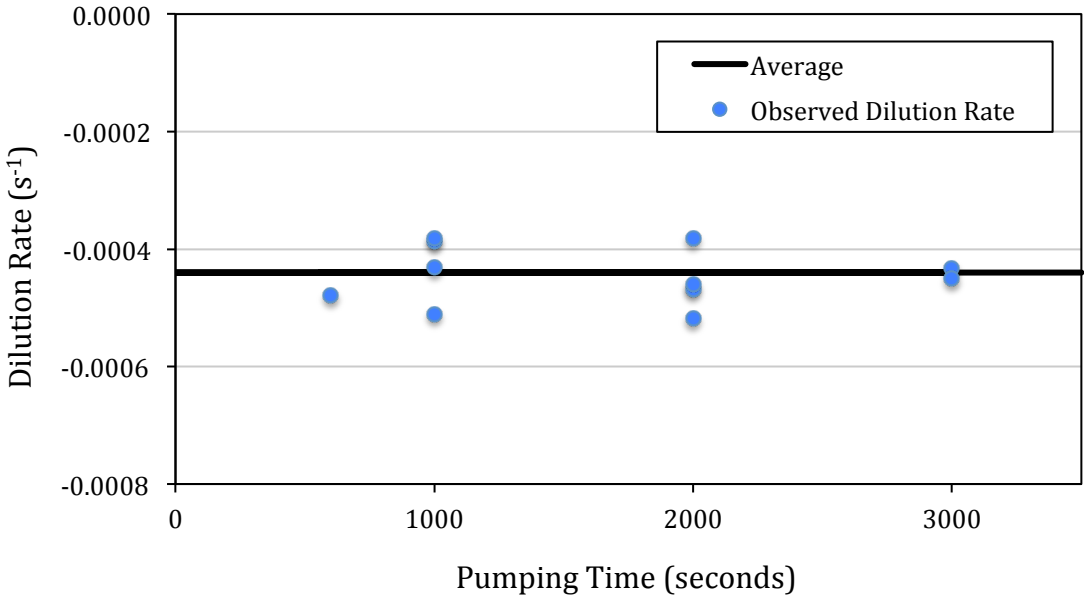

Figure 2: Calibration curve for the peristaltic pumping assembly using various pumping times. Dilution rate was constant with time.

The experimental setup proved to grow the cells at their maximum growth rates. The population density of the *Tw* consistently doubled daily, with the exception of the day(s) leading up to carrying capacity. The carrying capacity for *Tw* and *Synechocystis* sp. were observed to occur at approximately 10^5 and 10^6 cells/mL, respectively.

Tw survived every disturbance event and disturbance regime imposed upon it. The biomass over time mirrored the predicted growth curve for the first five days of the trial regime (1-6x underestimate), but then the test tube repopulated. Figure 3 shows a time-series plot of population throughout a severe disturbance regime in which the cells remained viable at the end of the experiment despite predicted extinction.

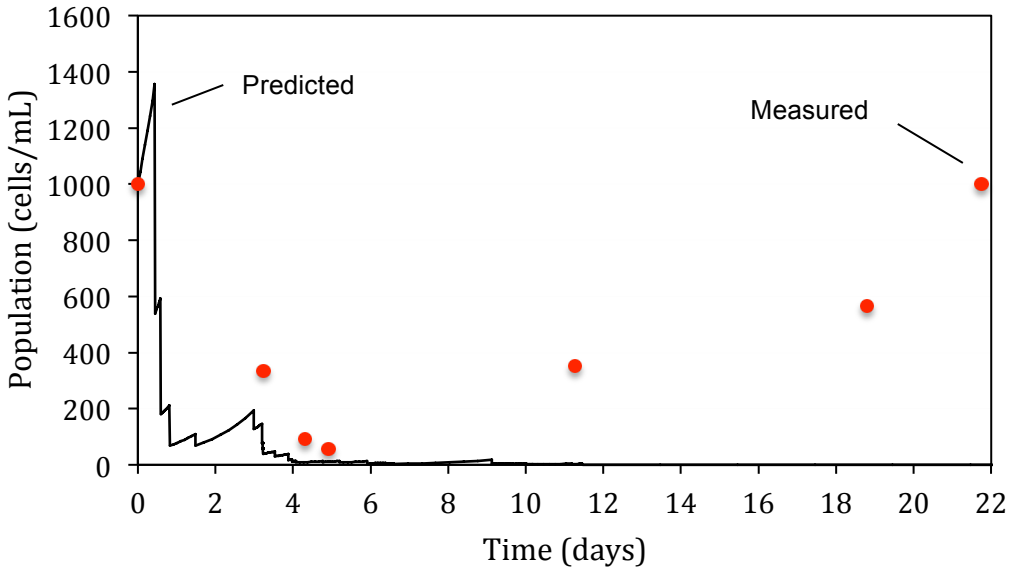

Figure 3: Plot of population versus time for a trial experiment using *Tw*. This severe disturbance regime was unable to kill the population.

Synechocystis sp. was chosen as the alternate species for experimentation because it is known to exhibit an Allee effect. The growth rate was about double that of the diatom, and the carrying capacity was only one order of magnitude greater. This resulted in pumping times that were longer, but still on the same order as the initial experimenting.

Experimental results for *Synechocystis* were similar to that of *Tw*. The population density followed the modeled population within 11% error for the first two days of pumping, but the culture remained viable beyond the expected extinction time. One significant deviation from the *Tw* trials is that *Synechocystis* accumulated masses of cells on the test tube and around the ends of the silicone tubing. This occurred within the first week of the disturbance regime, and persisted throughout. A plot of the most severe disturbance regime imposed in the *Synechocystis* sp. experiments is shown in Figure 4.

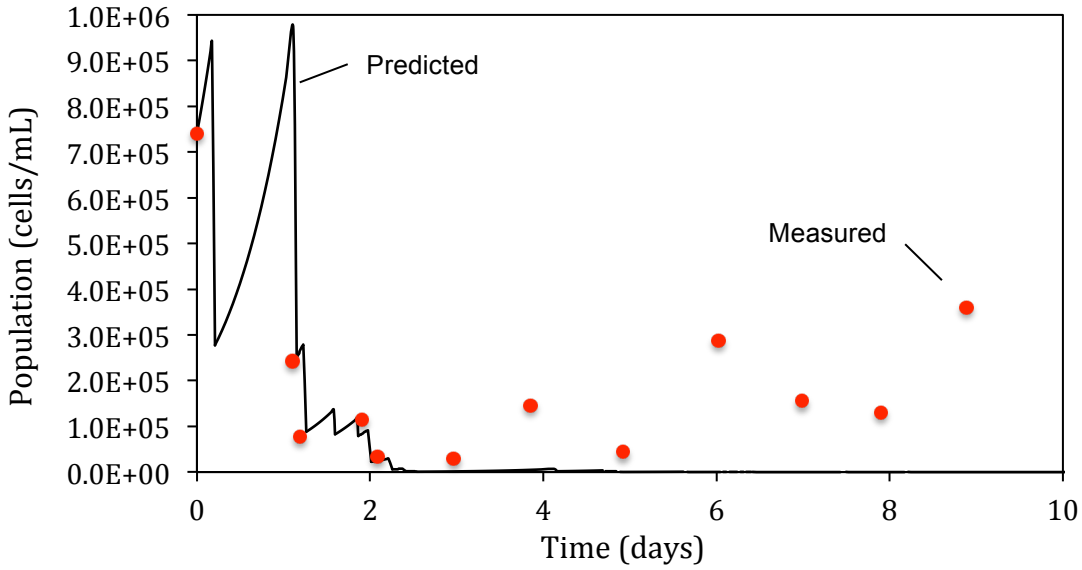

Figure 4: Plot of population versus time for the most severe disturbance regime using *Synechocystis* sp.. The population remained viable throughout the regime and following the end of the experiment.

DISCUSSION

CULTURING

Absent any disturbances, the cells grew as expected. The environment provided adequate nutrients and lighting to sustain exponential growth until reaching carrying capacity. This result was anticipated, but it was necessary for cell characterization purposes. Subsequent growth phases were diluted before reaching this maximum population density, which kept the cells in a quasi-continuous state of exponential growth. These steps allowed the culture to match the model assumption of logistic growth. Once this was accomplished, the pumping assembly could be applied to the culture tube for calibration and experimentation.

CALIBRATION

The experimental setup worked as it was designed to do. This allowed for disturbances to be imposed at the appropriate frequency and intensity. The *Tw* cells were useful in this stage of the project because the lack of Allee effect enabled for the imposition of disturbances at any intensity, without compromising the viability of the survivors. The population was able to recover, with minimal lag-phase, to be used in further calibration. By imposing disturbances at all intensities, the pumping assembly was validated over the entire range of pumping times.

Simultaneous injection and removal in the continuously-mixed test tube resulted in an exponential dilution rate. The pumping time required for a dilution fraction of 0.5 was roughly equivalent to the time it took to cycle the volume of the culture, while a survival rate of 0.25 took twice as long. The reason this value was not exactly represented by the time it took to cycle the working volume of the culture was because the cells were in exponential growth while dilutions took place. For this reason, and the fact that the pumping rate fluctuated slightly, the average exponential dilution rate was determined experimentally. The average dilution rate was consistent over long pumping times, which was in accordance with dilution event times (Figure 2).

EXPERIMENTATION

These experiments were unable to confirm the presence of an extinction threshold due to complexities associated with the population dynamics, and also with the pumping assembly. *Tw* was able to survive even the most severe disturbance regime imposed upon it. This was due to the fact that the *Tw* species lacks an Allee effect and can continue to reproduce from a single cell. The pumping assembly was unable to remove every single cell from the test tube, which allowed for the repopulation of the culture. Even if the pumping had been able to extract the last cell from the seawater media, there may have been cells stuck to the wall of the test tube and/or the silicone tubing. The problem of "sticky" cells led to the investigation of alternative species for experimentation.

One significant difference between *Synechocystis* and *Tw* was their behavior in a stressful environment. The cells became stressed when nearing carrying capacity because of the lack of nutrients in the media, and also during the imposed disturbance regime. When the *Synechocystis* culture became stressed, the cells would often conglomerate to form cell-masses. These masses of cells consisted of dead and senescent cells, as well as many viable cells. Cell-masses were observed after a few days of continued growth at the carrying capacity. Additionally, after a few days of imposing a disturbance regime, cell-masses began to form around the base of the silicone tubes and sometimes on the surface of the test tube. Figure 5 shows an extreme example of how the cells conglomerated in the test tube.

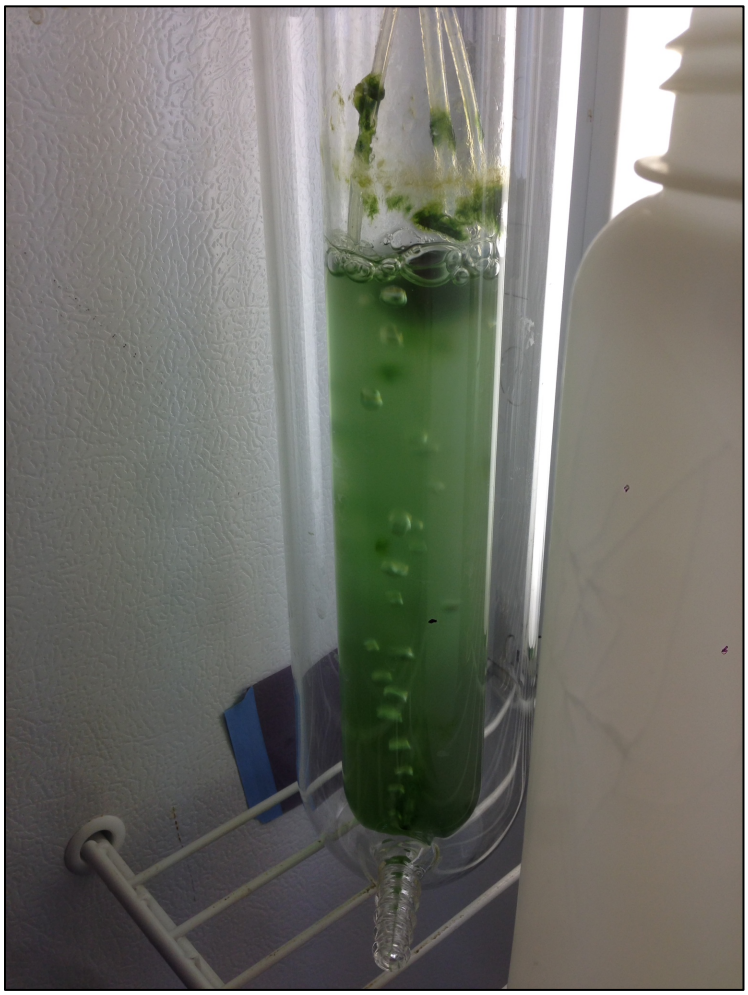

Figure 5: Picture of the *Synechocystis* culture after extended growth in a stressed environment. The dark masses of cells can be seen around the protruding test tubes and floating at the top of the tube.

The cell-masses served as a refuge for the species during disturbance events. This is likely a characteristic of the species that resulted from evolutionary adaptation to disturbance events in nature. This is a form of population resilience that was not predicted by the model. An attempt to physically remove cell masses failed and did not improve modeled population responses.

UNCERTAINTY

The ultimate trajectory of the population was said to be independent of the initial population size. Experiments were initiated at a moderate population density after cells were confirmed to be in a phase of exponential growth. The initial population for model prediction was defined as the measured population at the beginning of the disturbance regime. This meant that the accuracy of the predicted growth curve relied heavily upon the initial population measurement. The error associated with a cell count is inversely proportional to the number of cells counted. The result of the *Tw* trial experiment is a good example of how error can propagate as the experiment proceeds (Figure 3). The model prediction used an initial population of 1000 cells/mL, but subsequent measurements appear to be consistent with a larger initial cell density. The second measurement was about 74% greater than the prediction, while the second and third measurements exceeded 300% error.

The model makes predictions using mean values for disturbance frequency and intensity. A future study should impose at least three different disturbance regimes, thus providing a better representation of the mean outcome for the given indicator value (eq. 3). In this study, the failure of a single disturbance regime does not invalidate the model, nor does it absolutely disprove its application to natural systems. However, the fact that the most severe disturbance regime to be imposed in this study failed to cause extinction highlights the importance of refugia.

CONCLUSIONS AND IMPLICATIONS

The results of these experiments proved that assumptions and approximations are absolutely important to the ability of a model to be applied to a natural system. Because the model did not account for any form of refuge or population resilience, disturbance regimes that were predicted to force a population past its threshold for extinction were insufficient for this system. In the case of an ecological threshold, in which different stable states exist, the effects of species resilience are quite significant in determining the amount of disturbance required to force the population to an alternative state. Scheffer et al. (2001) concluded that "…all models of ecosystems with alternative stable states indicate that gradual change in environmental conditions, such as human-induced eutrophication and global warming, may have little *apparent* effect on the state of these systems, but still alter the 'stability domain' or resilience of the current state and hence the likelihood that a shift to an alternative state will occur…" So although the perceived effects of gradually-changing conditions are negligible, the cumulative effect could have extreme implications for the final state of the population. When the alternative state is extinction of the population, an understanding of the effects of these gradual changes in environmental conditions is highly sought-after for modeling and prediction purposes.

C.S. Holling first defined the term 'ecological resilience' over forty years ago, and it remains an abstract concept to this day. Ecological resilience and other factors of population dynamics continue to be complicated issues that cause uncertainty in models (Scheffer and Carpenter, 2003; Beisner et al., 2003). My results suggest that complexities related to ecological resilience and population dynamics (like sticky cells

and the forming of cell-masses) can impact populations' response to disturbance in unpredictable ways. This is just one of the many reasons why model assumptions must be valid, as well as comprehensive, if they are to be applied to real systems. In order to implement a comprehensive list of valid assumptions, experimentation, such as the methods used in this study, are necessary for pointing out the shortcomings of models.

It might have been possible to address the issue of sticking cells by treating the culture vessel by silinizing. However, these coatings only work to minimize sticking and cannot totally eliminate the problem. In the case of the *Tw* culture, a single cell stuck to the wall would cause the experiment to deviate from model assumptions. Sticking of cells is a process that naturally occurs in real systems. Perhaps accuracy is more important than tractability when developing a model for application to natural systems.

While the results of this study were inconclusive in determining the existence of a population extinction threshold, there are still conclusions that can be drawn. The fact that the cyanobacteria culture was stressed to the point of forming masses of conglomerated cells supports that a persistent disturbance regime can have a marked effect on the health of a population. Here, the cell-masses that seemed to be a sign of imminent extinction actually provided an unforeseen refuge that allowed more of the population to survive than expected. This deviation between theoretical and experimental survival rates resulted in an unpredicted failure to adhere to the model assumptions. In order to address this issue, the model could implement a sub-population that is decidedly exempt from the effects of disturbances, representing the portion of the population that exists in refuge. Although this phenomenon might be difficult to model, it could improve the model's ability to predict extinction in natural

systems. Because of this, I suggest more research should be done to study ecological resilience in the presence of episodic disturbance regimes.

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APPENDICES

APPENDIX A. *Thalassiosira weisflogii* Logistic Growth

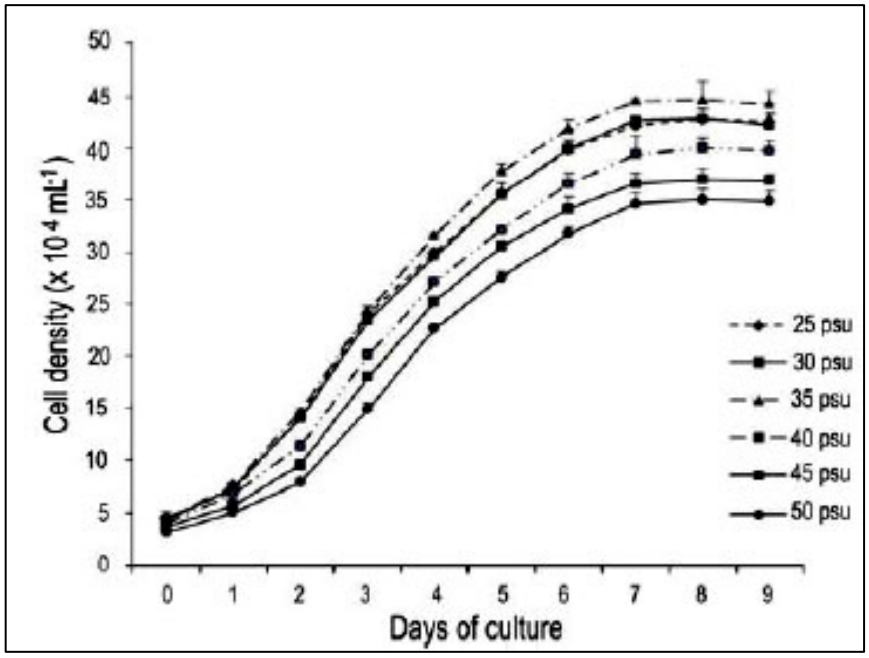

Figure 1: Growth (x10^4 cell mL^{-1}) of the estuarine diatom *Thalassiosira weissflogii* at different salinities. Horizontal bars indicate ± standard error (n = 3). From Garcia et al. (2012).

APPENDIX B. *Synechocystis* sp. Logistic Growth

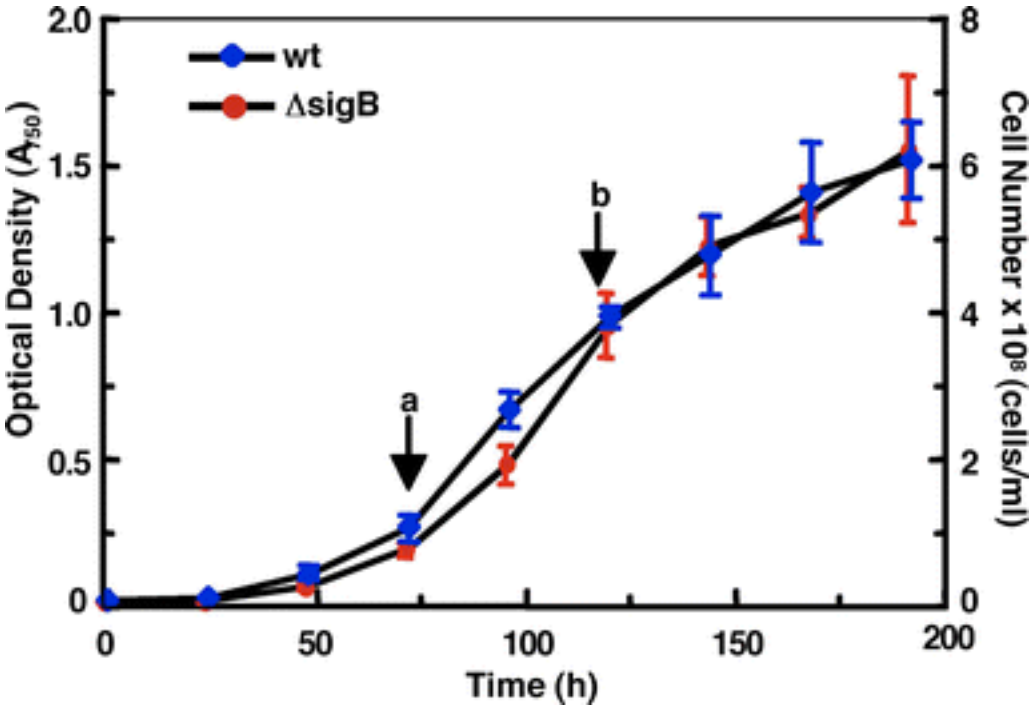

Figure 2: Growth curve for *Synechocystis* sp. PCC 6803 wild-type (*WT*, *diamond*) and *ΔsigB* strains (*circle*) over the first 200 h of growth. Cells enter early exponential growth at approximately 72 h (a) and continue logarithmic growth until approximately 120 h (b) where the cells appear to enter a linear growth phase for at least another 3 days. Note the *y*-axis is arithmetic and not logarithmetic. *Experimental error bars* are shown for $n = 3$. From Foster et al. (2006).

APPENDIX C. MATLAB Code

```
experiments = 7;  %Could make this 5 or just leave out 2 & 6, for example.
d = 86400;        %growth rate from days to seconds
num_hits = 20;    %initial constraint/guess
M_min = 10;       %Minimum experiment time
M_max = 30;       %duration of each experiment (days)
%r = 0.693;       %Tw daily growth rate
r = 1.5;        %Synechocystis growth rate
%No = 5e3;        %initial Tw population
No = 5e4;         %initial Synechocystis population
%K = 1e6;         %approximate carrying capacity Tw
K = 1e7;          %approximate carrying capacity Synechocystis
N = No;           %initialize experiment at population, No

%choose I_max less than r
%I_max = .6;  %Tw
I_max = 1.2;  %Synechocystis can use a larger I_max/min
I_min = -I_max;
lambda_min = num_hits/M_max;  %disturbance frequency regime lower bound
lambda_max = num_hits/M_min;  %experiment upper bound
m_min = (r-I_max)/lambda_min;  %disturbance intensity regime lower bound
%lambda_max = (r-I_min)/(m_min-1);  %disturbance freq. regime upper bound
%m_max = 0.647;  %Tw disturbance intensity regime upper bound
m_max = 1.293;  %Synechocystis
S = (lambda_max-lambda_min)/(m_max-m_min);
b = lambda_min-(S*m_min);
m_vec = m_min:((m_max-m_min)/6):m_max;
lam_vec = m_vec.*S+b;
m = m_vec;

%Get the random numbers for running the experiment
%%for each pair of (ETA!, lam) coordinates

%A = repmat(3, 7, 1);     %Alternate method for defining values
B = repmat(6, 7, 1);
for k = 1:7;
   %B(k,1) = invpsi(m_vec(k) + psi(A(k))) - A(k);
   x1 = 0.5 + (B(k)/(exp(m_vec(k))-1));

   for n = 1:100;
       xn1 = (x1-(m(k)-psi(x1+B(k))+psi(x1)))/((-psi(1,x1+B(k)))+psi(x1));
       X(n,1) = xn1;
       x1 = xn1;
   end

   XX(1:100,k) = X;
end

A = mode(XX);
BB = B';
Qa = A - 1;
Qb = (A + BB) - 2;

%Variance, Mode, Skewness of intensity values
```

```
Mode = Qa./Qb ;
Vary = [];
Mod = NaN(7,3);
moder = [];
[MeanB, Var] = betastat(A,BB);
eta = psi(A+BB) - psi(A);       %psi = digamma

%loop through a's & b's to get matrix of INTENSITY (M_hits_M) of hits

M_hits_M = [];
figure(1)
for k = 1:1:7;
    subplot(2,4,k)
    M_hits = betarnd(A(k),B(k),5000,1);  %Produces 5000 numbers from beta
    %M_hits = betarnd(5.4,4.8,5000,1);  %Produces 5000 numbers for Tw
    %M_hits = betarnd(5,6,5000,1);  %Produces 5000 numbers for Synecho
    hist(M_hits);
    St = std(M_hits);
    Stm(k,1) = St;
    xlabel(['A=' num2str(round(A(k)*100)/100)
        'B=' num2str(round(B(k)*100)/100)
        'Mode=' num2str(round(Mode(k)*100)/100)])
    M_hits_M = cat(2,M_hits_M,M_hits);
    vary = var(M_hits);
    Vm(k,1) = vary;
    skew = skewness(M_hits);
    moder = mode(M_hits);
    Mod(k,1) = vary;      %Variance
    Mod(k,2) = moder;     %Mode of intensity values
    Mod(k,3) = skew;      %Skewness
end

subplot(2,4,8)
scatter(MeanB, Var);
hold on
scatter(MeanB, Vm, '*', 'k')
R = {'varstat', 'varbrute'};
legend(R);
xlabel('Beta Mean')
ylabel('Beta Variance')

csvwrite('Intensity Hits.csv',M_hits_M);

%loop through lam's to get matrix of FREQUENCY (LAM_hits_M) of hits
LAM_hits_M = [];
figure(2)

for i = 1:7;
    L = lam_vec(i);
    subplot(2,4,i)
    LAM_hits = exprnd(1/L,5000,1);
    hist(LAM_hits)
    xlabel(['1/Lam=' num2str(1/L)])
    LAM_hits_M = cat(2,LAM_hits_M,LAM_hits);
end

flip_LAM = fliplr(LAM_hits_M);
```

```
csvwrite('Frequency Hits.csv',flip_LAM);
MaxIntensity = max(M_hits_M);

%End experiment (stop pumping) at 30 days
dur_vals = [];
Frequency = NaN(5000,7);
Intensity = NaN(5000,7);
dur_max = zeros(7,1);

for s = 1:7;

    for q = 1:length(LAM_hits_M);
        dur_vals = LAM_hits_M(1:q, s);
        Duration(q,s) = sum(dur_vals);  %Sum from 1 to q

        if Duration(q,s) <= 30;
        Frequency(q,s) = LAM_hits_M(q,s);  %Uses betarand
        Intensity(q,s) = M_hits_M(q,s);
        dur_max(s,1) = q;
        end
    end
end

Nt = [];
rr = r/d;
NNtt = NaN(3000000,7);
fin_length = NaN(100,1);

for i = 1:7;
    i;
    finlength = dur_max(i,1);
    Ntt = [];
    for k = 1:finlength;
        %Ntt = [];

        for j = 1:ceil(Frequency(k,i)*d);
            %Nb= rr/(bs+((rr/N)-bs)*exp(-rr*((j-1)-rr))); %Growth b/w hits
            %Nb = N*exp(rr*j); %Assuming exponential growth
            Nb = (K*N*exp(rr*j))/(K+N*(exp(rr*j)-1)); %Assuming logistic
            Nt = cat(1, Nt, Nb);
            N = Nb;

        end
        Ntt = cat(1,Ntt,Nt);
        N = Nt(length(Nt),1) * Intensity(k,i);

    end

    figure(3);
    subplot(7,1,i);
    plot( (1:length(Ntt)), Ntt )
    %NNtt(1:length(NNtt),i) = Ntt;
    clear Ntt
    xlabel('Time (seconds)')
    ylabel('Population, N(t)')
end
```

APPENDIX D. Data Tables

Synechocystis Experimental Parameters

Experiment Number	Indicator	Alpha	Beta	Lambda	Eta	Mode	Variance	Skewness
1	1.2	4.074	6.0	0.667	0.983	0.022	0.032	0.216
2	0.8	3.963	6.0	0.889	1.002	0.021	0.033	0.184
3	0.4	3.849	6.0	1.11	1.023	0.022	0.036	0.247
4	0	3.734	6.0	1.33	1.046	0.022	0.035	0.259
5	-0.4	3.617	6.0	1.56	1.069	0.022	0.022	0.338
6	-0.8	3.499	6.0	1.78	1.095	0.023	0.020	0.354
7	-1.2	3.378	6.0	2.00	1.122	0.022	0.025	0.314

Dilution Rate Calibration

N_0	N	t (s)	Dilution Rate (s^{-1})
83460	63020	600	-0.00047
8880000	6062000	1000	-0.00038
54860	32800	1000	-0.00051
7620000	4952000	1000	-0.00043
8716000	3410000	2000	-0.00047
5926000	2362000	2000	-0.00046
9554000	4454000	2000	-0.00038
8396000	2980000	2000	-0.00052
332600	94500	3000	-0.00042
1042400	269300	3000	-0.00045
		Average:	-0.00044

Tw Trial Experiment Prediction

t_events (days)	Fraction Remaining	Model Pop. (cells/mL)	Pumping Seconds	Growth Seconds	Cumulative Time (s)
0		1000	0	86400	86400
0.4391	0.397589184	539	635	37938	37938
0.574	0.30583662	181	816	11655	49594
0.8016	0.325589685	69	772	19665	69258
1.455	0.626626256	68	322	56454	125712
2.9617	0.657399276	127	289	130179	255891
3.1668	0.525967442	77	442	17721	273612
3.1764	0.748252186	58	200	829	274441
3.1848	0.668510909	39	277	726	275167
3.4866	0.624058188	30	325	26076	301242
3.8281	0.447247325	17	554	29506	330748
3.9203	0.662190967	12	284	7966	338714
4.0099	0.626530446	8	322	7741	346455
4.7142	0.767254785	10	182	60852	407307
5.1373	0.596693408	8	355	36556	443863
5.8445	0.382857191	5	661	61102	504965
6.3115	0.578813737	4	376	40349	545314
6.5216	0.64837803	3	298	18153	563466
9.0363	0.23339919	4	1002	217270	780736
9.45	0.750741345	4	197	35744	816480
9.9132	0.544068744	3	419	40020	856500
10.0607	0.601888776	2	349	12744	869244
11.0876	0.736256591	3	211	88724	957969
11.3496	0.555975468	2	404	22637	980605
12.1689	0	0	300	70788	1051393

Population Measurements for *Tw* Trial

Time (d)	0.00	3.24	4.32	4.92	11.27	18.79	21.74
Average Cell Count (mL^{-1})	1000	436	192	158	601	665	1100

Synechocystis Experiment #7 Prediction

t_events (days)	Fraction Remaining	Model Pop. (cells/mL)	Pumping Seconds	Growth Seconds	Cumulative Time (s)
0.00	0.29	740800	3058	14980	1.80E+04
0.21	0.26	277079	3370	78133	9.95E+04
1.15	0.32	251262	2886	6682	1.09E+05
1.26	0.60	88154	1264	27379	1.38E+05
1.59	0.67	82410	991	22113	1.61E+05
1.86	0.24	78971	3534	9541	1.74E+05
2.01	0.21	22382	3914	17151	1.95E+05
2.26	0.22	6153	3777	9898	2.09E+05
2.42	0.43	1591	2092	8455	2.19E+05
2.54	0.25	789	3445	136803	3.59E+05
4.16	0.33	1775	2767	45387	4.08E+05
4.72	0.20	1213	4056	10809	4.22E+05
4.89	0.20	285	3964	62866	4.89E+05
5.66	0.32	159	2863	32833	5.25E+05
6.08	0.31	86	2891	3379	5.31E+05
6.15	0.31	28	2914	7831	5.42E+05
6.27	0.36	10	2532	3484	5.48E+05
6.34	0.56	4	1432	4867	5.54E+05
6.42	0.11	2	5595	87549	6.47E+05
7.49	0.22	1	3780	58396	7.10E+05
8.21	0.28	1	3205	59215	7.72E+05
8.94	0.38	0	2413	28106	8.03E+05
9.29	0.45	0	2000	27193	8.32E+05
9.63	0.31	0	2890	86033	9.21E+05
10.66	0.33	0	2769	13228	9.37E+05

Population Measurements for *Synechocystis* Experiment #7

Time (d)	0.0	1.1	1.2	1.9	2.1	3.0	3.9
Average Cell Count (mL^{-1})	740800	243040	77240	115040	33400	29840	146320

4.9	6.0	7.0	7.9	8.9
43640	288000	156000	130600	360240