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EMPIRICAL MODELING OF POPULATION RECOVERY USING MARINE ROTIFERS

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Abstract

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Three quarters of the worlds fisheries are classified as overexploited or depleted. Management programs have mainly focused on reducing the fishing pressure on these stocks. However, some stocks fail to rebound even after fishing effort is reduced and hatchery programs may be used to facilitate population recovery. Despite substantial investment in hatchery supplementation, failed programs outnumber successful ones. It therefore seems vital to explore the abiotic and biotic factors that hinder their success. This thesis addresses the performance of several active recovery policies through the use of multispecies microcosms. Specifically, I ask 1) whether one or several supplementation efforts are needed before a sustainable stock population is established and 2) what factors influence the success or failure of recovery in these microcosms. My results show that the community within an ecosystem may strongly influence a recovery programs likelihood of success and that multiple small additions may offer a better chance of success than one or several large additions. My results support previously made arguments that community ecology is an important framework for fisheries management. Moreover, commercial fishing alters community structure and this may happen in a way that inhibits population recovery. I suggest reconceiving population recovery as facilitated invasion may provide useful guidance for designing future recovery programs.

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Introduction

Fisheries expanded rapidly following WWII (Pauly *et al.* 2005) leading to a dramatic increase in the number of over-exploited stocks (FAO 2016, Pikitch *et al.* 2004), *i.e.* stocks that have biomass below the biomass at maximum sustainable yield. This trend continued for the remainder of the 20th century; In 1974, 10% of the worlds marine fisheries were categorized as over-exploited. By 2013, this category had grown to 31.4% (FAO 2016).

To control overfishing and increase harvestable biomass, a variety of measures have been adopted including harvest restrictions, protection of essential fish habitat, and hatchery supplementation (Levin *et al.* 2001). Although harvest restrictions and habitat restoration (Levin *et al.* 2001) often result in increased biomass, some over-fished populations fail to recover (Caddy and Agnew 2004). In these cases, hatchery supplementation may be a viable recovery option. Although there are negative effects of hatcheries including disease, nutrient loading, and genetic bottlenecks, here we focus on their use in population recovery.

Hatcheries require a significant amount of funds to run and maintain; in the US alone, hatchery programs cost over 60 million each year (USFW 2013). Thus one

important question is to identify an optimal restoration policy to guide these efforts. Here I specifically focus on the rate at which hatchery fish should be produced and the period of time over which this should be carried out. Several previous authors have addressed this from a theoretical point of view (Stickney 2011, Lorenzen 2005, Blankenship and Leber 1995). Most recently, Lampert and Hastings (2014) developed a model integrating both biological and economic considerations. This model aims to restore a population to its biological threshold, a point where the population continues to increase even after supplementation ends, but restricts efforts by an economic threshold. The economic threshold is defined as the point at which the lost revenue from the stock being depleted is equal to the natural recovery rate times the cost of active recovery (Lampert and Hastings 2014). Similar economic theories have been applied to the removal of invasive species (Epanchin-Niell and Hastings 2010) and when setting maximum sustainable yields (Sharov and Liebhold 1998). Although these models have provided useful insights into the economics of recovery, they assume single-species dynamics with linear controls and costs. As a consequence, in the absence of economic or biological constraints the optimal recovery policy is to inundate the system with all of the hatchery stock needed to instantly reach the biological restoration threshold (Clark 1990).

Despite their considerable investment cost, such active recovery plans have mixed success (Lorenzen 2005). Though it seems straightforward that putting fish into the system ought to increase the number of fish that come out, this is not always the case. Chinook salmon in the Columbia River Basin (Levin *et al* 2001), red sea bream (Kitada and Kishino 2006), and Japanese flounder off the coast of Japan (Kitada and

Kishino 2006) show no signs of steady increase even after sustained periods of active recovery. However, in the Chesapeake Bay, hatchery use has had a positive impact on the oyster population, which is currently on its way to rebuilt status (Kemp *et al.* 2005). Positive effects on abundances have also been seen in seagrass (van Katwijk *et al.* 2009) and lobsters (Agnalt 2008). Araki and Schmid (2010) analyzed 266 empirical studies dealing with the ecological and genetic impacts of hatchery rearing on stock enhancement. Out of 70 cases that compared wild and hatchery stocks, only a handful showed a positive impact on the abundance of a targeted population, though this was not the primary focus of their review.

One possible explanation for the variation in success of these programs is the ecosystem context in which they were carried out. Fishing can lead to changes in community composition (Pauly *et al.* 2002, Roberts 1997, Hilborn *et al* 2003), increase the success rate of invaders (Jackson *et al.* 2001), and habitat destruction (Pikitch *et al.* 2004, Turner *et al.* 1999). Thus it seems plausible that recovery failures derive from harvest-related changes in the ecosystem. In light of this and the considerable expense incurred by restoration failures we must ask whether the insights derived from single-species recovery models provide relevant guidance.

In order to experimentally test recovery policies, we need a model system that will respond on a tractable time scale. Studies in micro-organisms have been the source of numerous foundational ideas in ecology and population dynamics (Jessup *et al* 2005). Examples include, the effects of rapid evolution on predator-prey relationships to better understand population oscillations in nature (Fussman *et al* 2000, Yoshida *et*

al 2003), the effects of habitat quality and disturbances on community composition and diversity (Altermatt and Holyoak 2012), coexistence of competitors (Luckinbill 1973), and phenotypic diversity and niche specificity (Rainey and Travisano 1998), among many others.

This thesis therefore addresses the performance of several active recovery policies in a multispecies ecosystem through the use of multispecies microcosms. In light of the all-at-once prediction from existing theory, I determine 1) whether one or several additions are needed before a sustainable stock population is established and 2) what factors influence the success or failure of recovery in these microcosms.

Methods

The marine rotifer *Brachionus plicatilis* (hereafter referred to simply as rotifers) is a well-studied zooplankter that has long been used to study ecology and population dynamics. They exhibit a cosmopolitan distribution and are typically found in salt lakes and coastal lagoons (Gomez *et al* 2002). This species is euryhaline and survives in salinity raging from 3-50ppt (Lawrence *et al.* 2012). Reproductive temperatures for this species range from10C-35C (Lawrence *et al.* 2012). Depending on temperature they mature in 0.5 to 1.5 days and will live 3-30 days, reaching a lorica (outer shell) length of between 250-350m (Henry 2016). This species is widely used in aquaculture as food for newly hatched fish. Rotifers used in this research were ordered from Reed Mariculture (Reed Mariculture Inc., Campbell, CA).

I conducted three experiments looking at different ecological aspects of population recovery. Each experiment involved rearing multiple rotifer colonies. Daily care involved water changes and feeding at rates based on preliminary experiments (Appendix A). Water for the microcosms was created by combining deionized water and Instant Ocean artificial sea salt (Aquarium Systems, Inc.) for a salinity of 30 ppt. To control temperature, groups of colonies were held in sea tables heated with immersion

heaters. Temperatures ranged from 24C, 26C and 29C and were held constant (+/- 1oC) in all replicates. Water in each colony was aerated to provide air and water movement. Daily 25% water changes were performed on each microcosm to remove waste build up. Colonies were moved to clean microcosms once a week to also deal with waste build up and to reduce the accumulation of biofilm in the microcosms. Preliminary experiments showed that without the microcosm change colonies were more likely to crash (Appendix B). Water for refilling came from an outside reservoir of artificial seawater (*i.e.* Instant Ocean and deionized water at 30 ppt salinity) that was provided with both air and heat.

All colonies were fed RG Complete (Reed Mariculture Inc., Campbell, CA), a concentrated microalgae-based feed, at 2.2ml food/L water. Based on preliminary trials, this feeding rate was sufficient to maintain colonies at 100 rotifers/ml (Appendix A). Therefore, during recovery a population size of 100 rotifers/ml was targeted for all experiments.

Population size was tracked through daily counts via microscopy on three 1 ml samples that were set aside for each microcosm from the water removed for daily water changes. The total number of rotifers in each ml was counted and the three counts per colony were averaged to give the estimated population size.

Each experiment contained an acclimation period, harvest period, passive recovery period and active recovery period. The length of each period varied from experiment to experiment (See Figures 1 and 2 for full experimental time series for Experiment 1 and Experiment 2) as did the size of the microcosms used and the maximum harvest

level reached. These variations were based off of observations made as I moved from one experiment to the next. The maximum harvest level reached was based off of the reduction in population numbers through the harvest period for each experiment. The length of each period was changed from Experiment 1 to 2 and Experimet 2 to 3, based on what occurred in the microcosms during each period. For example, none of the colonies recovered during the twenty-one day passive recovery period in Experiment 1, so this interval was reduced to ten days for experiment 2. Microcosm sizes were changed based on what was available to me at the time.

Each experimental setup (microcosms, sea tables, etc) was established one week prior to inoculation with rotifers to ensure stable environmental conditions. In general, the acclimation period started when each microcosm was initially seeded with rotifers. During this period the rotifers received standard daily care and were allowed to adjust to their new environment and establish population dynamics. The harvest periods were aimed to mimic an unsustainable fishery. We simulated an over-fished fishery by incrementally increasing the total volume removed from each colony until a crash in the overall population was seen. We started at the standard daily water change (25%, 4.5L) and increased in increments of 5% (0.9L) over a set numbers of days. This pattern was followed until the maximum removal rate was achieved (between 35% and 45%). Following the overfishing period, the colonies were allowed a passive recovery period. During this time they were returned to the conditions experienced during the acclimation period which simulates a period during which harvest controls have been enacted and overfishing has been eliminated in the hope that populations will return to

pre-harvest levels without active intervention.

The goal of the active recovery period was to use a supplementation program to try to bring the colonies back up to a sustainable level, roughly 100 rotifers per ml. In Experiment 1, I used active recovery periods of 1, 3, 5, and 10 days. Subsequent experiments focused on 1 and 10 day recovery periods. Since the total number of colonies is limited due to space and time constraints, the 3 and 5 day treatments were eliminated so that I could increase the number of replicates in the 1 and 10 day treatments. The 1 day treatment was retained because the all at once strategy has some theoretical justification and (as seen in the results from Experiment 1) the 10 day treatment produced qualitatively different results.

Based on population counts, the number of rotifers required to bring each colony to the target density of 100 rotifers per ml was determined. This number was then added to each colony, divided evenly across the days of the recovery treatment. That is, for each colony, I counted the rotifers per ml, subtracted from 100, and divided by the treatment duration (1,3,5 or 10 days) to determine the number of rotifers that needed to be added each day. These additional rotifers were added after the daily water change. Thus the total number of rotifers added was colony specific, but the density immediately following the recovery period should be 100 per ml for all colonies.

Experiment 1

In this experiment, our goal was to compare the population responses to active recovery periods of 1 ,3, 5, and 10 days to assess the best strategy for active recovery. To do this we used buckets to establish eighteen 18L microcosms that were seeded with both rotifers and brine shrimp (*Artemia salina*). The addition of brine shrimp, whose target population density was determined in a previous experiment (Appendix C), was to ensure some level of ecological complexity in our system. Estimates of brine shrimp pre-adult stages vary from 9-19 (Abatzopoulus *et al.* 2002). Throughout these stages brine shrimp compete with rotifers for food. Adult brine shrimp are also filter feeders and do not necessarily consume rotifers. However, based on my preliminary experiments, adult brine shrimp clearly destroy rotifers, either inadvertently or by consumption (Appendix D). In either case, I expected the brine shrimp to affect the rotifer dynamics.

Replicates were split evenly between three temperature treatments: 24C, 26C, and 29C. Microcosms were partially covered to regulate heat loss and light exposure, but remained open to potential colonization by bacteria, ciliates, and other micro-organisms. Although this meant the community composition in my microcosms changed through time, this was a realistic representation of natural systems which typically contain substantially more than two species.

The acclimation period for this experiment lasted for a twenty-seven days. The daily water removals were done using a 1L beaker. Each microcosm was assigned its own beaker to prevent crossover between replicates. During the harvest period, removals

increased by 5% every eight days and reached a total daily removal of 45% (25% daily water change + 20% harvest). The following passive recovery period for this experiment lasted for three weeks and standard care was followed during this time. None of the populations recovered during this interval.

The treatments used during the active recovery period were 1, 3, 5 and 10 days. Four buckets were assigned to the 1 day treatment and two buckets were assigned to the 3, 5, and 10 day treatment, all with approximately the same population size at the end of the passive recovery period. The rotifers used for the supplementation program were kept in a separate 18L microcosm that provided rotifers for all active recovery treatments. After each treatment reached its final day of active recovery, it was returned to the standard care used in both the acclimation and passive recovery periods.

Experiment 2

Experiment 1 set up an open system that allowed for invasion and colonization by other microorganisms. This proved to be highly successful and my microcosms were colonized by a variety of other visible species including ciliates and nematodes in addition to being presumptively colonized by bacteria. This led to the determination that brine shrimp were no longer needed to ensure complexity. More importantly, it led me to wonder if having open verses closed systems affected the results of my active recovery strategy. I addressed this question by directly comparing closed verses open

systems.

Six, 1L microcosms were established for each of the open and closed treatments and held at 26C for the duration of the experiment. Closed microcosms were established in 1L glass flasks with foam stoppers to limit colonization by other microorganisms. Airlines for the flasks were fitted with a filter to further limit invasion. All Flasks were maintained under a singular hood with its own light source. A vacuum attached to the hood created an updraft with the hope of reducing aerosols falling into the flasks during brief periods in which the corks were removed.The fact that only one hood was available means that all closed colonies were not independent. This was unavoidable due to space and financial constraints. Microcosms for the open treatment were established in 1L glass beakers maintained outside of the hood. Airlines for this set up were also fitted with filters so the only source of invasion was from the air surrounding the beakers. As with Experiment 1, the microcosms were set up for one week prior to inoculation with rotifers.

The acclimation period for this experiment lasted seventeen days. Standard care was provided to each colony. Water was removed from the flasks using a small tube that was inserted into the flasks with the foam corks in place. All flasks had their own siphoning tube. Water was removed from each of the open microcosms using a 100ml beaker. Each open microcosm had its own 100ml beaker.

As in Experiment 1, harvesting involved removing additional water from each microcosm and increased in 5% increments. In this experiment, each increment was maintained for three days and only 35% total daily removal was reached before the

colonies exhibited a crash. The colonies then entered a ten day passive recovery period following the harvest period. During the active recovery period only two treatments were focused on: 1 and 10 days. Two flasks and two beakers were assigned to each treatment. The project was run for ten more days after the last day of active recovery.

Experiment 3

Experiment 2 showed that, although open systems were significantly more variable than closed systems during the acclimation period, this had little impact on the outcome of recovery. In light of this, I next hypothesized that apparent success of the 10 day recovery was either due to differences in initial conditions (*i.e.* the community into which I was putting the rotifers following their collapse) or due to other processes leading to stochastic dynamics. To test this hypothesis, I repeated the 1 and 10 day recovery trials with identical communities obtained by mixing and subdividing communities in several microcosms. If initial conditions were responsible for the divergence among colonies, I expected that these identical communities would remain similar for an extended period of time. If stochastic dynamics were the main cause, I expected that colonies in microcosms with identical communities should diverge at the same rate as colonies in different communities. Of course, these hypotheses are not mutually exclusive it is certainly possible to have both community effects and stochasticity. The question is really how much more slowly identical communities diverge than independent ones.

I set up ten microcosms matching the closed system of Experiment 2, but eliminated the hood. Thus the microcosms for Experiment 3 were still open to invasion, but the foam corks reduced the rate of colonization. The acclimation period was 35 days. The harvesting period reached 35% prior to the crash. Duplicate communities were created by splitting ten of the colonies in half. To avoid diluting these twenty communities, the flasks were maintained at 500ml for thirty days and re-seeded with rotifers. All of these crashed immediately, leading me to conclude that the half-full flasks were not suitable environments for rotifers (this hypothesis is tested explicitly in Appendix F). Rather than re-starting the experiment from scratch, I created 3 sets of four duplicate 1L communities by pooling eight 500ml communities, mixing well, and dividing them evenly across four clean 1L flasks. Two flasks from each set of duplicates were assigned to each recovery treatment (1 and 10 days).

Statistical Methods

For each experiment, I tested for effects of the recovery treatment (1, 3, 5 and 10 day) in the post-recovery population sizes and population growth rates. To do so, I used one-way ANOVA to compare population sizes across recovery treatments, using colony means as the response variable. To control for the different recovery durations, I compared the treatments on the same number of days after the recovery period was complete. Daily population growth rates were calculated for each colony as rt = ln(Nt+1)-ln(Nt) where Nt is the average rotifer density (rotifers/ml) on day t. I re-

peated this one-way ANOVA for each day post recovery. To compensate for the fact that I am using many tests on the same experiment, I used a Bonferonni correction, dividing the nominal significance level of 0.05 by the number of days being tested.

However, the recovery treatments had relatively little impact on the average population sizes. Rather the primary effect of the different recovery periods was on the differences between colonies within a recovery treatment. To explore this, I also conducted a series of one-way ANOVAs within treatments to determine the time required for the replicate colonies to become significantly different from one another. To do so, I used the three daily counts for each colony as the response variable and colony identity as the treatment. Although my focus is on the number of days post-recovery required for the colonies to diverge, I repeated this analysis for all of the colonies over the per-harvest acclimation period. Because the initial conditions for all of the colonies are, in principle, identical during this period, this estimate of the divergence time serves as a useful baseline for evaluating the divergence times post-recovery.

Results

In all three experiments, the duration of the active recovery period had a substantial impact on the subsequent population sizes and growth rates. Qualitatively, in Experiment 1 treatments 1, 3 and 5 day produced highly variable results and the results from the 10 day treatment were the least variable (Figure 3). Similar results were found in Experiments 2 (Figures 4) and 3 (Figures 5 and 6), where the 10 day replicate population produced population trajectories that trended together and the 1 day replicates populations produced population trajectories that diverged almost immediately after seeding.

To quantify these descriptive results, I tested for differences in the population size and growth between treatment levels for each experiment. For each day post recovery, I used a one-way ANOVA to test for significant differences between recovery treatments. I then plotted the F-ratios for the days post recovery (Figures 8-13). For each test the numerator degrees of freedom is the number of treatments minus one and the denominator degrees of freedom is the number of observed responses minus the number of treatments. For the Bonferonni correction I used the number of days post active recovery that contained all treatments (Since all the colonies in a given experiment

were terminated the same calendar day, the 10 day recovery treatment had a shorter post-recovery period than the all the other treatments).

I found no effects of recovery treatment on either the average population size or on population growth for either Experiment 1 or 2. Based on the population trajectories (Figures 3-6), I suspect this was caused by the large variance among replicate colonies within each recovery treatment. I then looked within each recovery treatment to determine whether there were differences in the divergence time (*i.e.* when these replicate populations become significantly different from one another). These analyses are described below, separately for each experiment.

Experiment 1

My goal was to assess the consistency of success in four different strategies for active recovery of overharvested rotifer populations. My treatments consisted of 1, 3, 5 and 10 day active recovery periods. To establish a benchmark divergence time for this experiment, I used a one-way ANOVA to test for significant differences among colonies during the pre-harvest acclimation period. Replicate counts are the response in this analysis. During this interval, the microcosms have just been seeded with rotifers and brine shrimp from the same sources and are held under the same experimental conditions. The F-ratios indicate that colonies become significantly different from each other within one to four days (Figures 14 and 15). This is clearly visible in the population trajectories over this time period (Figure 14).

Shifting now to the post-recovery analysis, I found that the 10 day treatment produced the least variableresults (Figure 2); F-ratios indicate that colonies within the 1, 3, and 5 day treatments significantly diverged from one another shortly within zero to two days post-recovery while the colonies within the 10 day treatment did not diverge until four days (Figure 2).

Experiment 2

I hypothesized that some of the variability in Experiment 1 resulted from leaving the colonies open to the air and allowing invasion by other microorganisms. I hypothesized that the 1 and 10 day results would be more similar in closed microcosms without this added ecological complexity. The objective of Experiment 2 was therefore to determine whether keeping our population in closed flasks or open beakers led to different results for the 1 and 10 day active recovery treatments.

To establish a baseline for the divergence time, I used one-way ANOVA on the closed and open microcosms during the pre-harvest acclimation period following the same statistical protocol as in Experiment 1 with the Bonferroni correction based on fourteen days of acclimation. I found that the colonies diverged within one to two days (Figure 16). But the variance in initial counts among replicates was 220.2 in the open treatment and 103.1 in the closed treatment. This immediate divergence among replicates seemed large, so I attempted to correct for differences in initial conditions by removing the mean on day one (Figure 17) and re-analyzing. After doing so, replicates

within the open treatment still diverge rapidly (significantly different by day two) while the replicates in the closed treatment take three days to diverge.

Following active recovery, the results from the 1 day treatment again showed a larger variation in dynamics in both closed and open microcosms, than in the 10 day treatment (Figures 4). Again, I found that they diverged within 1-3 days. The 1d and 10d treatments showed no significant difference between open and closed microcosms (Figure 4).

Experiment 3

Based on the results of the previous two experiments, I hypothesized that apparent success of the 10 day recovery was either due to differences in community or stochasticity. To test this hypothesis, I repeated the 1 and 10 day recovery trials with identical communities obtained by mixing and subdividing communities in several colonies.

Three sets of identical experimental communities were created by pooling, mixing, and dividing crashed colonies into 1L microcosms and repeating the 1 and 10 day recovery treatments. Again, I used one way ANOVA to look at the F-ratios for each day of the recovery period in this experiment, with Bonferroni correction based on twelve days.

As with Experiments 1 and 2, I found that the communities within treatments diverged rapidly (one to two days) post recovery (Figure 7). However, within each

community we see that, for both the 1 and 10 day treatment, duplicates trend closely together for an extended period of time (Figures 5 and 6). The 1 day treatment shows significant differences between duplicates after two to seven days (Figure 5), while the duplicates in the 10 day treatment either took five to eleven days to diverge or never did (Figure 6).

Discussion

In this study I explored several active recovery strategies in an attempt to see which would provide the least variable results. Theory suggested that without biological or economic constraints we should put everything back all at once. I incorporated this theory into my research as the 1 day active recovery treatment and in Experiment 1 I compared it to 3, 5 and 10 day active recovery treatments. I found no significant differences in the average population size or growth rate resulting from these treatments in any of my experiments. However, after the initial rotifer seeding, all colonies adopted individualistic population trajectories and the primary effect of the recovery strategy was on the variance in response among replicates. I found that active recovery over 1, 3, or 5 days provided highly variable results and replicates in these treatments showed significant differences within one to two days. The 10 day treatment proved less variable; replicates in this treatment took ten days (after the final day of active recovery) to show significant differences from one another. The population trajectories for the 10 day treatment trended closely together until the end of the experiment.

The results from Experiment 1 led me to ask what caused the early divergence in the buckets during the pre-harvest period. Since the rotifers all came from the

same source and were placed in to clean microcosms, this rapid divergence was rather surprising. However, the microcosms had been set up one week in advance of inoculation with rotifers and were consequently open to colonization by other microorganisms during this time. I therefore hypothesized the early divergence resulted from the open-ness of the system.

Open and closed systems were used for Experiment 2 and I limited my active recovery treatments to just 1 and 10 days. During the pre-harvest acclimation period, I found that the variance among replicates in closed microcosms on day one was roughly half the variance among the open microcosms. Although this might be due to chance, I made every effort to inoculate each microcosm with the same number of rotifers. The lower variance in the closed microcosms at the first count is, however, consistent with the fact that these microcosms were stoppered for the prior week while the open microcosms were more easily invaded. Moreover, the open microcosms became significantly different within one to two days while the closed microcosms took two to five days to diverge. However, the magnitude of divergence among all microcosms was much less than in Experiment 1.

Returning to Experiment 2, the 1 day results again provided highly variable recovery results and the 10 day treatment provide less variableresults. Interestingly, open and closed microcosms diverged to the same degree in both the 1 and 10 day treatments, with similar trajectories. These post-recovery results suggest that either the closed microcosms had already been invaded by the start of the recovery period or the disparity between the 1 and 10 day recovery plans was not due to open-ness of the

microcosms to airborne invaders.

In Experiment 3, I hypothesized that the source of discrepancy between 1 and 10 day recovery trajectories was either due to stochasticity or differences in communities within the microcosms at the outset of the recovery period. Linear stochastic systems are expected to drift apart at a rate proportional to the square root of time. If the divergence between colonies was driven primarily by stochasticity, then identical communities should have diverged at the same rate as the replicate colonies in previous experiments. This did not occur. Rather, identical communities diverged much more slowly than the replicate colonies. This is consistent with the hypothesis that the communities in each microcosm were different. Since all colonies were started and maintained under the same conditions until the active recovery period, changes likely stem from invasion.

Although I think the community explanation is the most reasonable, I dont have any independent data (*e.g.* 16S sequences, etc) to support this, so it is worthwhile considering alternatives. First, we should consider the possibility that these results emerge from single-species dynamics. It is certainly possible that repeatedly adding rotifers to the microcosms pushes their dynamics into a chaotic regime where sensitive dependence on initial conditions could explain rapid divergence. However, if this were the case, we should see the greatest divergence in treatments with repeated additions (*i.e.* the 10 day treatment). The 1 day treatment, in contrast, is more analogous to re-setting each colony to the same initial population density.

Another alternative explanation for the results is that different responses of

each colony to recovery stem from rapid evolution. While it is likely that rotifers in each microcosm are evolving independently, the supplemental rotifers, added during the active recovery period, are from the same stock for all colonies. Since the colonies are at exceedingly low densities ($< 10/ml$) at the start of active recovery, genetic differences among them will be erased by the addition of new rotifers. Thus genetic differences between colonies should be negligible following active recovery.

It is interesting to note that the post-recovery trajectories in Experiment 3 were much more similar than in the previous experiments. I suspect this has to do with the fact that I pooled eight 500ml colonies to create four identical 1L communities. If the microcosms are invaded at random from a relatively small pool of potential invaders, this pooling will tend to make the community composition of independent replicates more similar to each other at the start of recovery than in Experiments 1 and 2.

In light of all of these results, I suspect that divergence in trajectories is driven by differences in community composition. But, the question of why 10 days of active recovery works while the 1 day does not, remains open. I suspect this has to do with competitive exclusion but further experiments are needed to establish what micro-organisms actually invaded and their ecological roles.

Taking a step back, however, my study suggests that flooding a system all at once does not guarantee successful reestablishment of the population. This is not to say it never works; however, failed recoveries currently out number successes (Araki and Schmid 2010) and with the extreme costs involved in running and maintaining hatcheries it is a rather expensive gamble (Shea and Possingham 2000). The act of simply putting

stocks back from where they came from has some common-sense appeal, but in many cases, I suspect that during the course of over-harvesting the ecosystem has changed.

The removal or elimination of a population can lead to increased success rate of invaders (Pauly *et al.* 2002, Roberts 1997, Hilborn *et al.* 2003.), habitat destruction (Pikitch *et al.* 2004, Turner *et al.* 1999), or increased predator presence (Fraser 2008, Lorenzen 2014). The resulting community and habitat might no longer have a place for the recovering population. From this view point we see an important and largely overlooked connection between invasion theory and population recovery. For both areas of research establishment of a new population is often dependent on the community make up and a species ability to increase from low density (Shea and Chesson 2002).

Experiments with microbial and plant communities have shown that the more complex a community and the fewer limiting resources an ecosystem has, the less susceptible it is to invasion (Shea and Chesson 2002, Jessup et al 2004, McGrady-Steed *et al.* 1997). An ecosystem is harder to invade if there are more obstacles an invader has to overcome. Informally, each member of a community plays a role and if the role of an invader is already taken then it first needs to out-compete that community member before it can establish itself fully in that system. Shea and Chesson (2002) pointed out that the likelihood of invasion is dependent on what opportunities the invaded community provides and this can better be understood by incorporating community ecology theory. In the context of recovery, this would imply that all else being equal - as the complexity of a community increases, the chances of a recovery programs success decreases. Understanding the type of interactions hatchery stocks will encounters after

seeding is important.

An example that highlights the use of environmental and community data is the ongoing effort, since 2005, to restore the oyster population in the Chesapeake Bay. The success of this project was dependent on moving away from a traditional single species approach and adopting a more ecosystem-based one. Over-harvesting oysters caused detrimental changes to the bay including destruction of oyster habitat (Jaskson *et al.* 2001, Schulte and Burke 2014) and runoff from agriculture had led to increased water pollution in the bay (Kemp *et al.* 2005). Acknowledgment of these changes as a potential obstacle for recovery efforts prompted managers to develop a plan to first deal with the habitat degradation in order to create a more suitable environment for hatchery seed. This stock is now on its way to rebuilt status through the use of rebuilt reefs, reduced take, and hatchery-raised seed (Schulte and Burke 2014). Similarly, work to recover Zostera noltii beds along the Basque coast has shown that knowledge of the sediment is important to successful transplants (Valle *et al.* 2015).

Accounting for all the interactions in a large scale open system is a non-trivial task. But my experiment does offer some hope of success in cases when we dont know how the system works. Specifically, I found that low magnitude augmentation over an extended period can lead to less variable recovery dynamics. The relevance of this result likely depends on whether recovery failure is due to species interactions or abiotic factors. If recovery is prevented by species interactions such as competition or predation, then I suspect my results are applicable and suggest that repeated perturbations can provide a means of successfully recovering a depleted population. On the other hand, if

recovery failure stems from habitat destruction, pollution, or disease, my guess is that we would need to fix the habitat first, as in the Chesapeake.

Figures

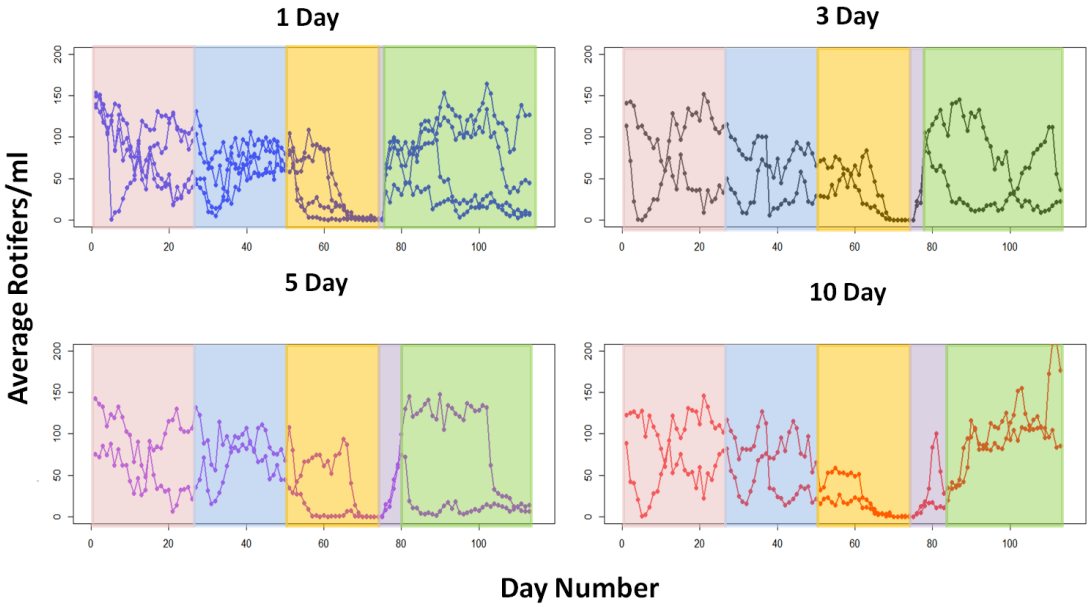

Figure 1: Full time series of population densities vs. time in Experiment 1 for 1 day, 3 day, 5 day and 10 day treatments. Each line represents the daily average density of rotifers (number/ml) for a different replicate within each recovery treatment. Colors indicate different experimental time periods (left to right): pink- acclimation period, blue- over harvest period, orange- passive recovery period, purple- active recovery period, green- post-recovery period.

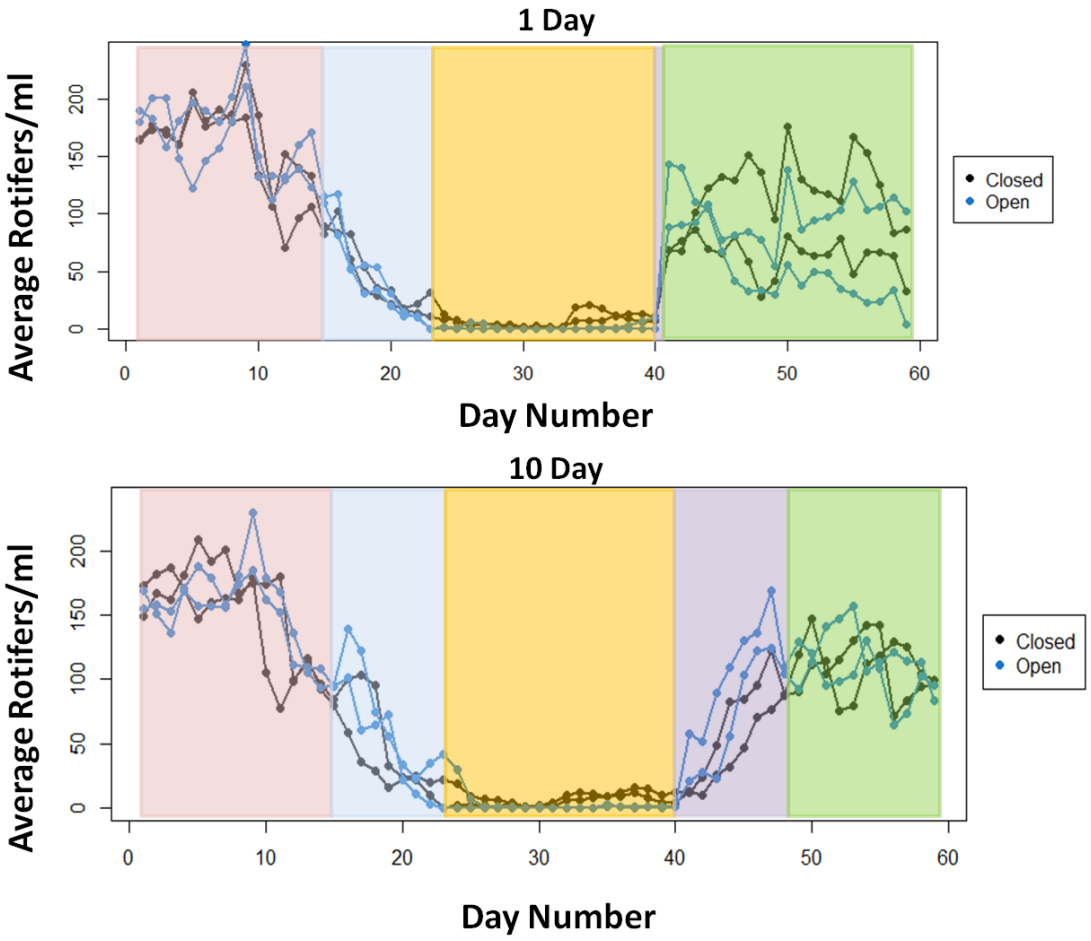

Figure 2: Full time series of population densities vs. time in Experiment 2 for 1 day and 10 day treatments. Each line represents the daily average density of rotifers (number/ml) for a different replicate within each recovery treatment. Blue markers represent open microcosms and black markers represent closed microcosms. Colors indicate different experimental time periods (left to right): pink- acclimation period, blue- over harvest period, orange- passive recovery period, purple- active recovery period, green- post-recovery period.

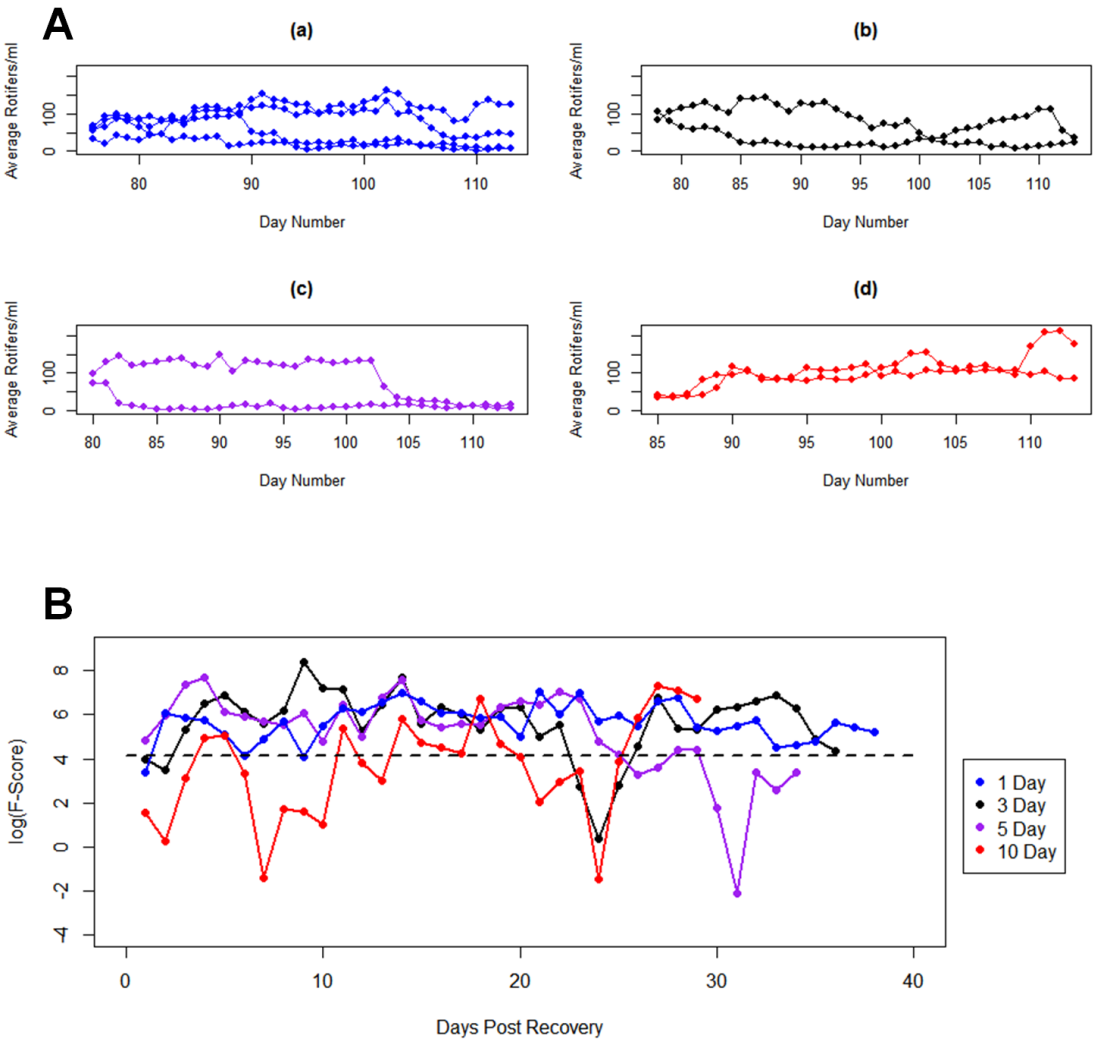

Figure 3: Post-recovery population densities vs. time in Experiment 1 for A) 1 day (a), 3 day (b), 5 day (c) and 10 day (d) treatments. Each line represents the daily average density of rotifers (number/ml) for a different replicate within each recovery treatment. B) Experiment 1 ANOVA results for comparison among colonies with counts as the response. F-ratios are plotted for each day during the post-recovery period for 1 (blue), 3 (black), 5(purple) and 10 (red) day treatment. The dashed line represents the significance threshold for a nominal p=0.05 test with Bonferroni correction for 29 tests (number of days that contain all treatments).

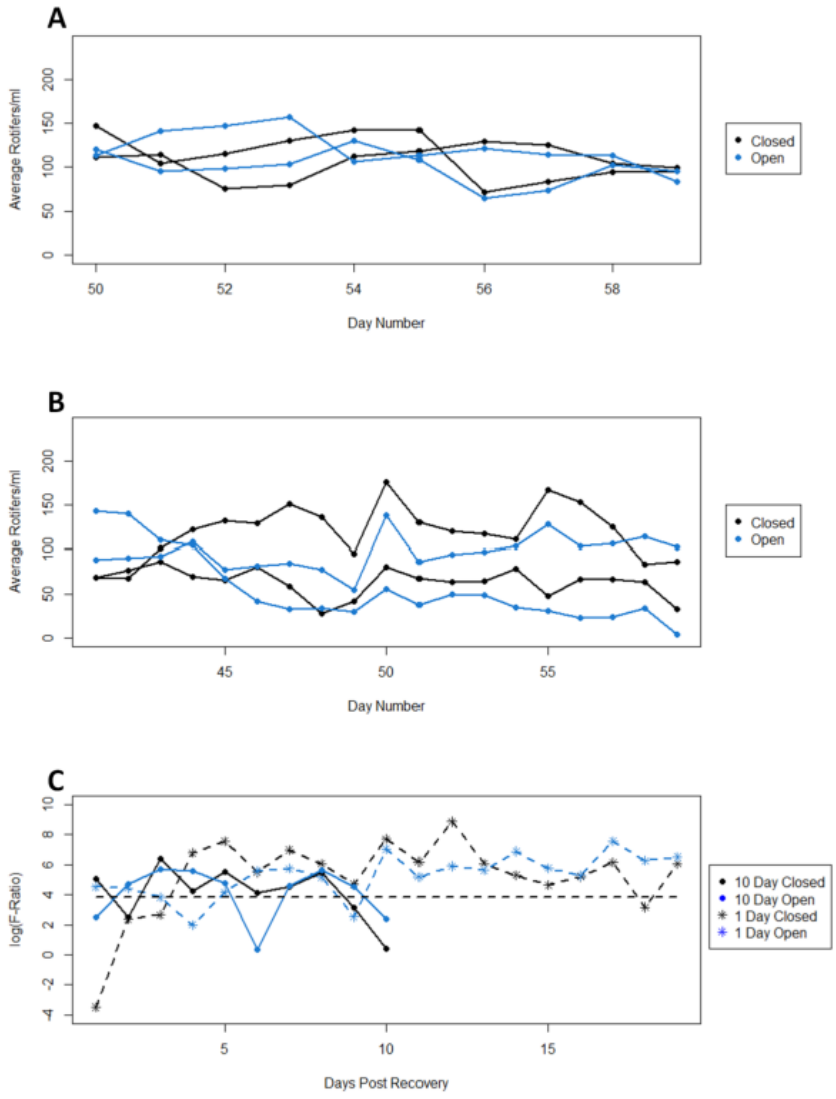

Figure 4: Post-recovery trajectories in Experiment 2 for the A) 1 day treatment and B) 10 day treatment. Each line represents the average population density of rotifers (number/ml) vs. time. Blue markers represent open microcosms and black markers represent closed microcosms. C) Experiment 2 ANOVA results for comparison among colonies with counts as the response. F-ratios are plotted for each day during the post-recovery period for 1 (stars), and 10 (circles) day treatment. Blue markers represent open microcosms and black markers represent closed microcosms. The dashed line represents the significance threshold for a nominal p=0.05 test with Bonferroni correction for 10 tests (number of days that contain all treatments).

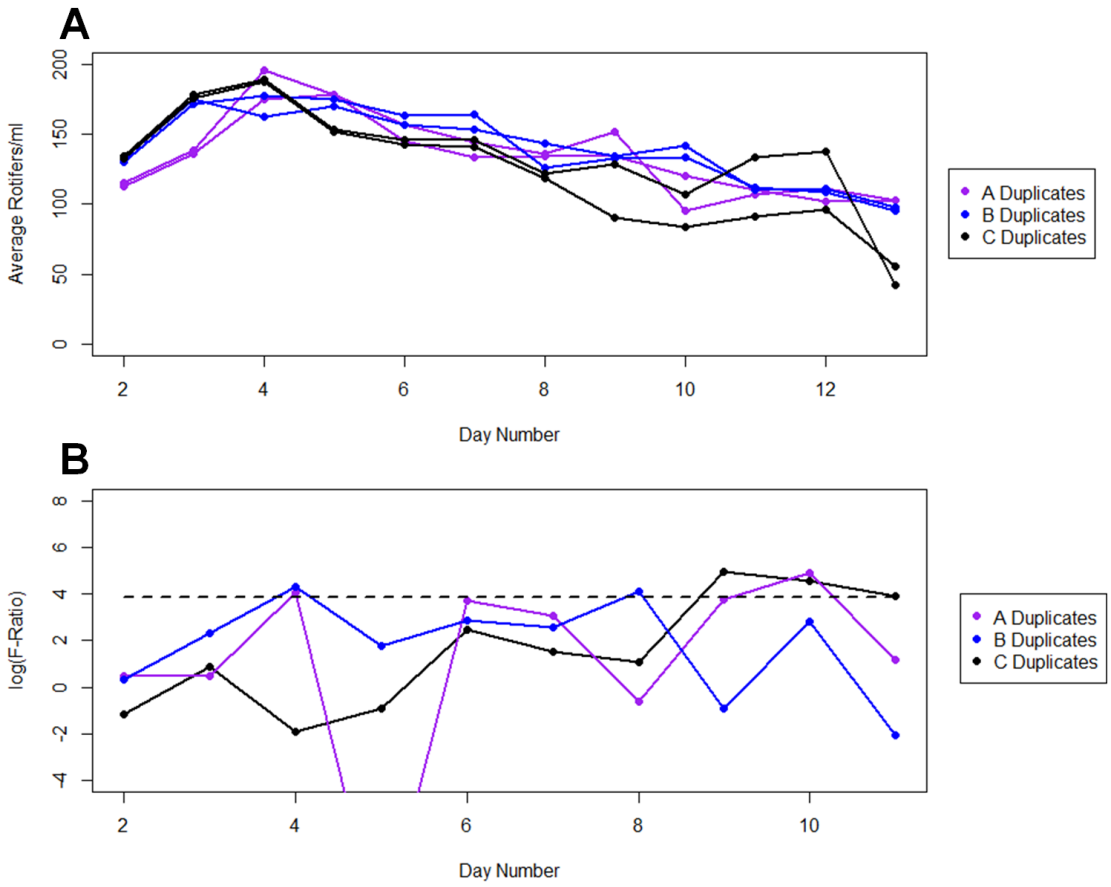

Figure 5: Post-recovery trajectories in Experiment 3 for the 1 day treatment. Each line represents the average population density of rotifers (number/ml) vs. time. Purple markers represent colony A split, blue marker represent B colony splits and black represent C colony splits. B) Experiment 3 ANOVA results for comparison among colonies with counts as the response. F-ratios are plotted for each day during the post-recovery period for the 1 day treatment. The dashed line represents the significance threshold for a nominal p=0.05 test with Bonferroni correction for 21 tests.

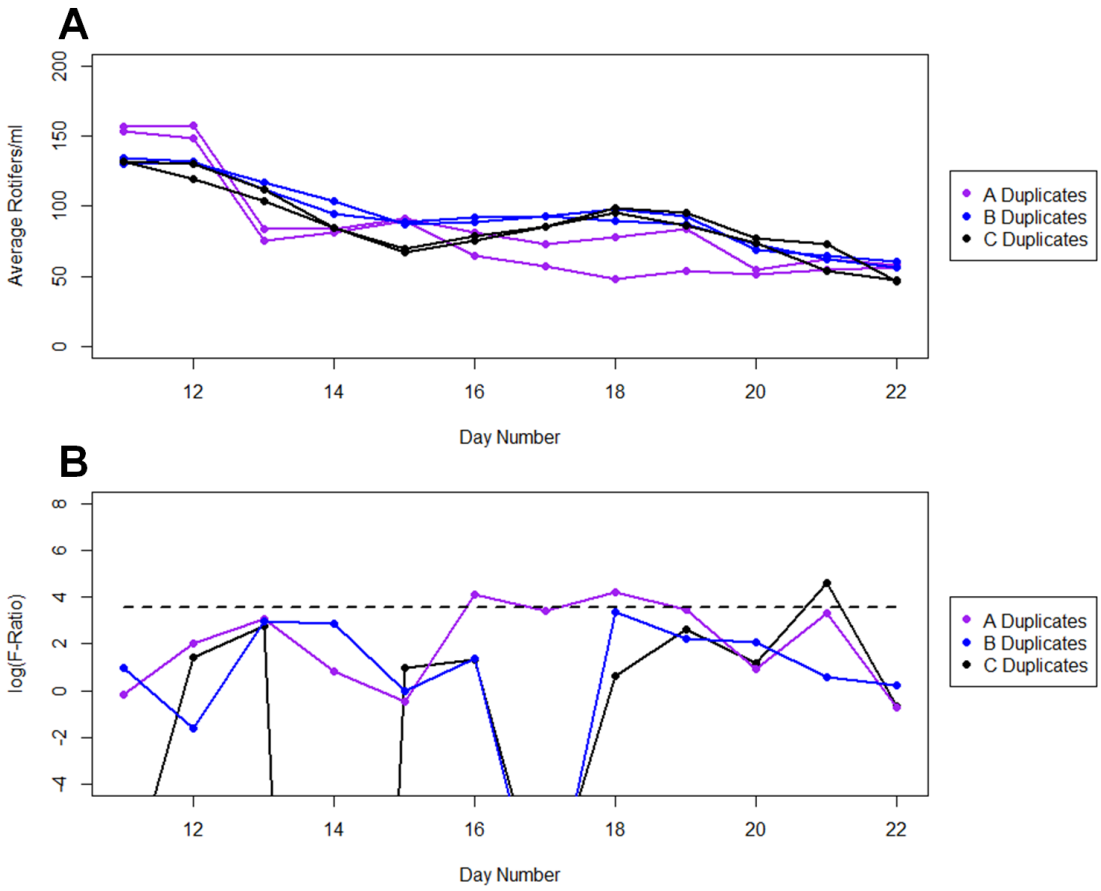

Figure 6: Post-recovery trajectories in Experiment 3 for the 10 day treatment. Each line represents the average population density of rotifers (number/ml) vs. time. Purple markers represent colony A split, blue marker represent B colony splits and black represent C colony splits. B) Experiment 3 ANOVA results for comparison among colonies with counts as the response. F-ratios are plotted for each day during the post-recovery period for the 10 day treatment. The dashed line represents the significance threshold for a nominal p=0.05 test with Bonferroni correction for 12 tests.

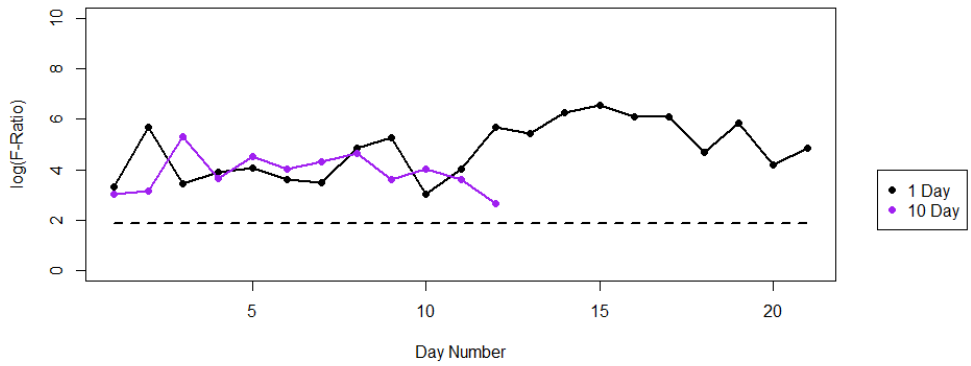

Figure 7: Experiment 3 ANOVA results for comparison among colonies with average population density of rotifers (number/ml) as the response. F-ratios are plotted for each day during the pre-harvest period for. Black markers represent the 1 day treatment and purple markers represent the 10 day treatment. The dashed line represents the significance threshold for a nominal p=0.05 test with Bonferroni correction for 12 tests (number of days that contain all treatments).

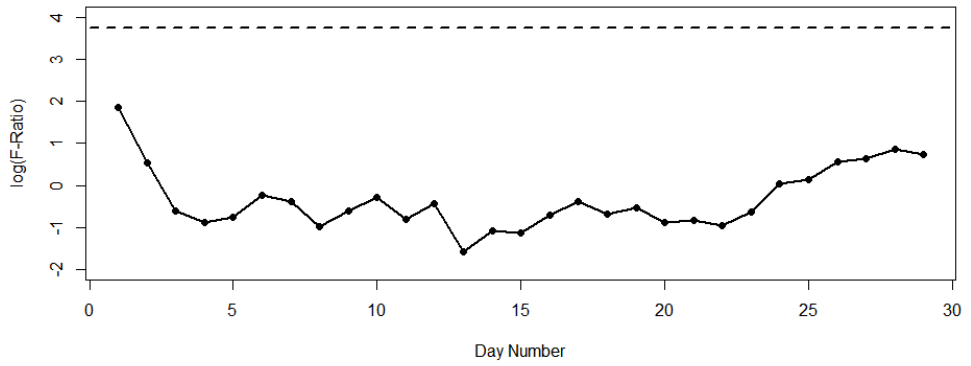

Figure 8: ANOVA results testing for treatment effects on the average rotifer density in Experiment 1. The F-ratios are plotted for each day during the post-recovery period. The dashed line represents the significance threshold for a nominal p=0.05 test with Bonferroni correction for 29 tests. As all points remain under the line of significance, there is no effect of recovery treatment on average population size.

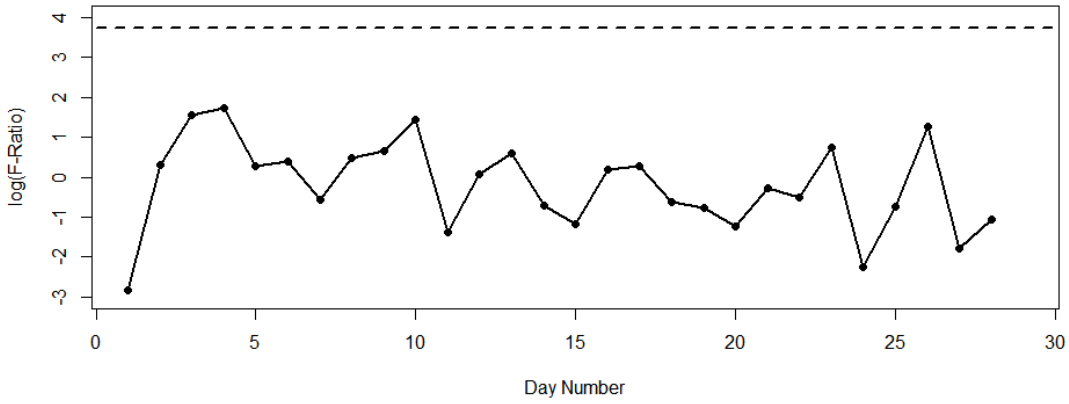

Figure 9: ANOVA results testing for treatment effects on the population growth in Experiment 1. The F-ratios are plotted for each day during the post-recovery period. The dashed line represents the significance threshold for a nominal p=0.05 test with Bonferroni correction for 29 tests. As all points remain under the line of significance, there is no effect of recovery treatment on population growth.

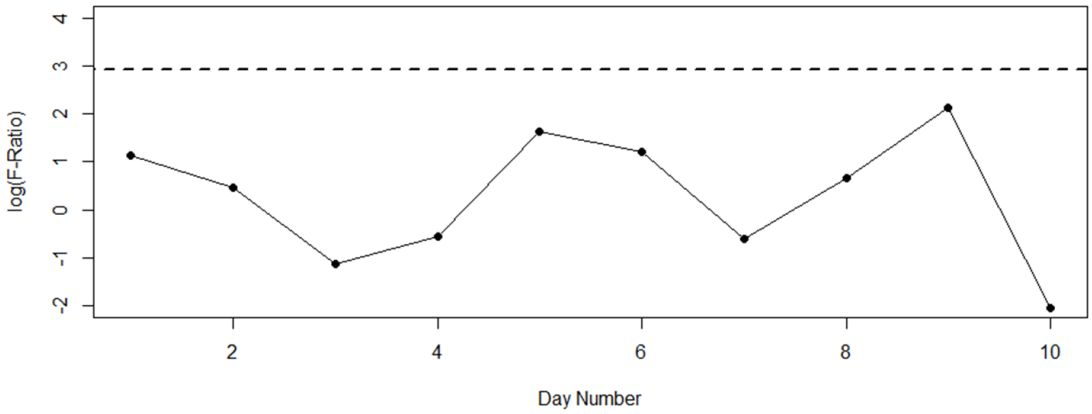

Figure 10: ANOVA results testing for treatment effects on the average rotifer density in Experiment 2. The F-ratios are plotted for each day during the post-recovery period. The dashed line represents the significance threshold for a nominal p=0.05 test with Bonferroni correction for 10 tests. As all points remain under the line of significance, there is no effect of recovery treatment on average population size.

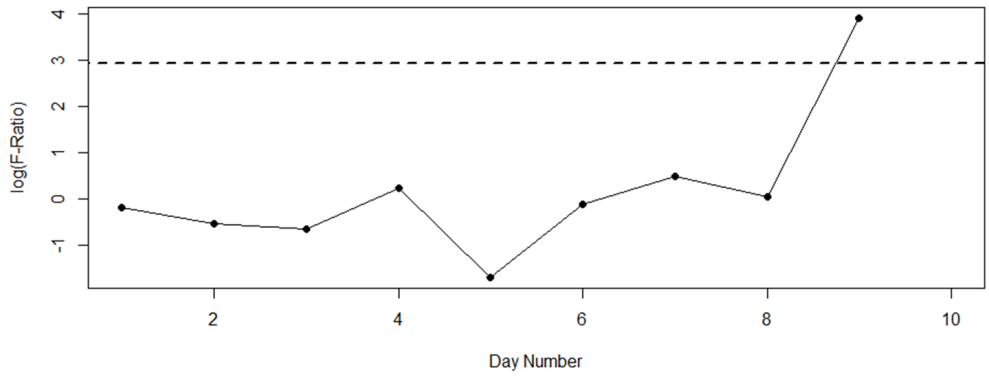

Figure 11: ANOVA results testing for treatment effects on the population growth in Experiment 2. The F-ratios are plotted for each day during the post-recovery period. The dashed line represents the significance threshold for a nominal p=0.05 test with Bonferroni correction for 10 tests. As all points remain under the line of significance until the last day, there is no effect of recovery treatment on population growth.

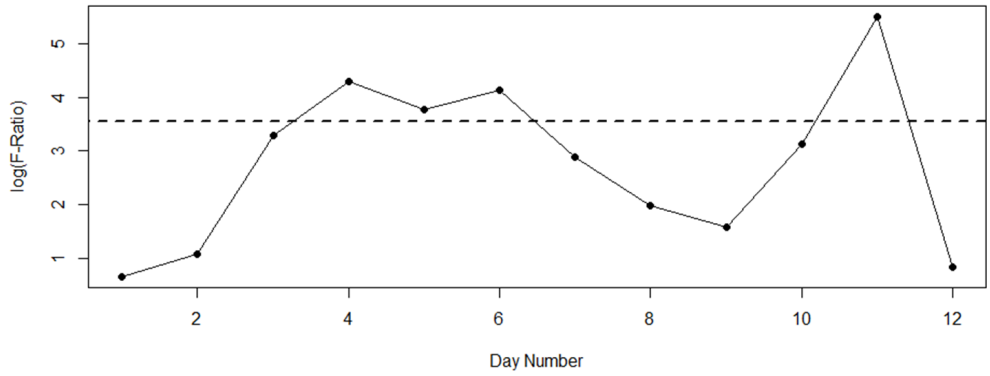

Figure 12: ANOVA results testing for treatment effects on the average rotifer density in Experiment 3. The F-ratios are plotted for each day during the post-recovery period. The dashed line represents the significance threshold for a nominal p=0.05 test with Bonferroni correction for 12 tests. In contrast with Experiments 1 and 2, there is a significant in average density between the 1d and 10d treatments.

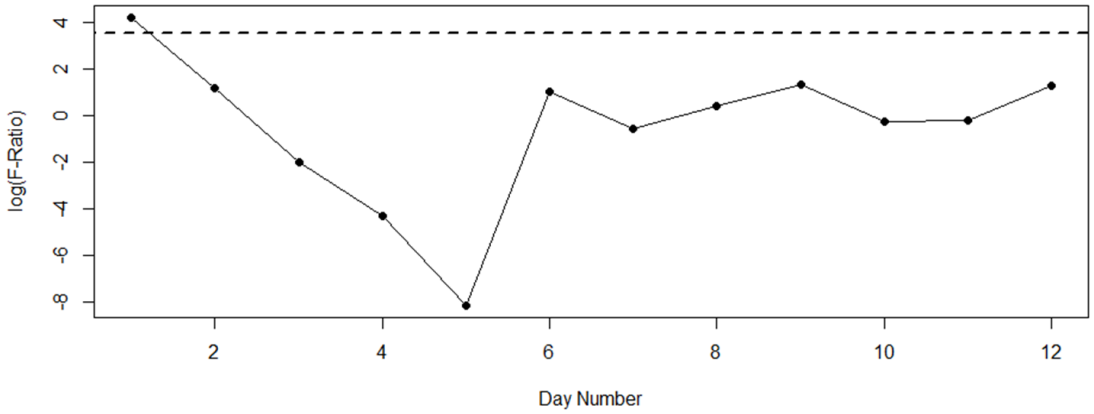

Figure 13: ANOVA results testing for treatment effects on the population growth in Experiment 3. The F-ratios are plotted for each day during the post-recovery period. The dashed line represents the significance threshold for a nominal p=0.05 test with Bonferroni correction for 10 tests. Apart from the first day, all points remain under the line of significance.

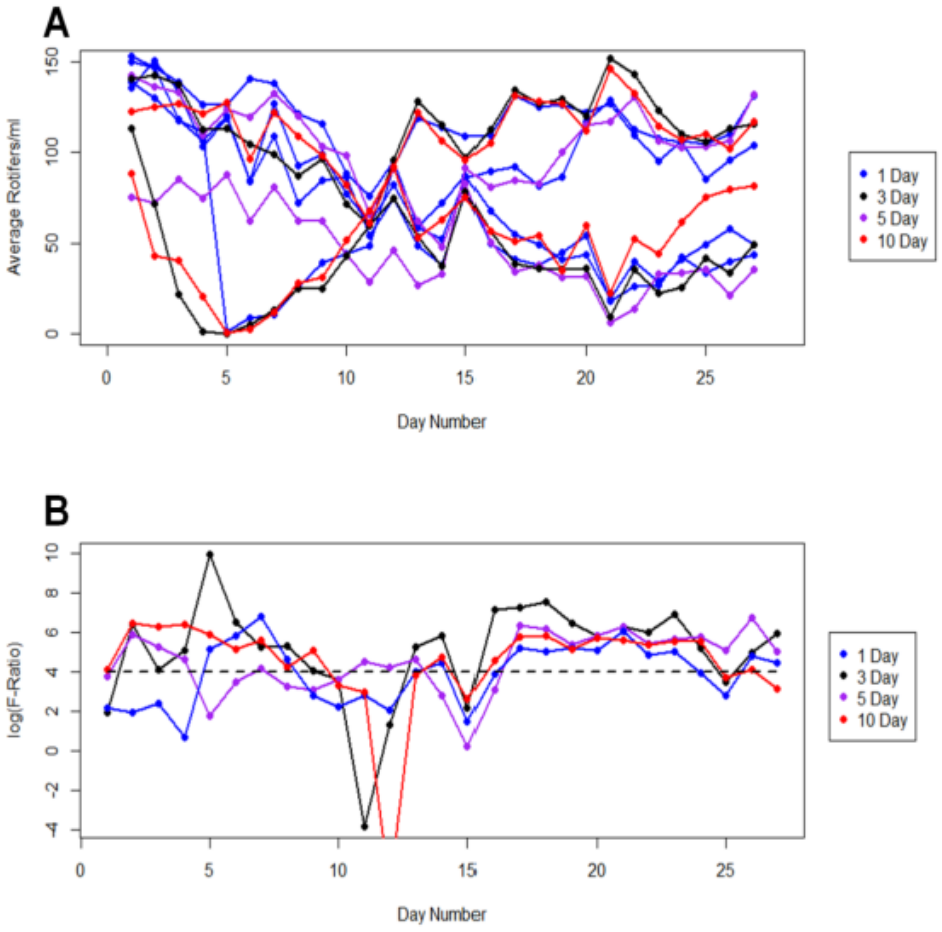

Figure 14: Pre-harvest population densities vs. time in Experiment 1 for A) 1 day (blue), 3 day (black), 5 day (purple) and 10 day (red) treatments. Each line represents the daily average density of rotifers (number/ml) for a different replicate within each recovery treatment. B) Experiment 1 ANOVA results for comparison among colonies with counts as the response. F-ratios are plotted for each day during the pre-harvest period for 1 (blue), 3 (black), 5(purple) and 10 (red) day treatment. The dashed line represents the significance threshold for a nominal p=0.05 test with Bonferroni correction for 27 tests.

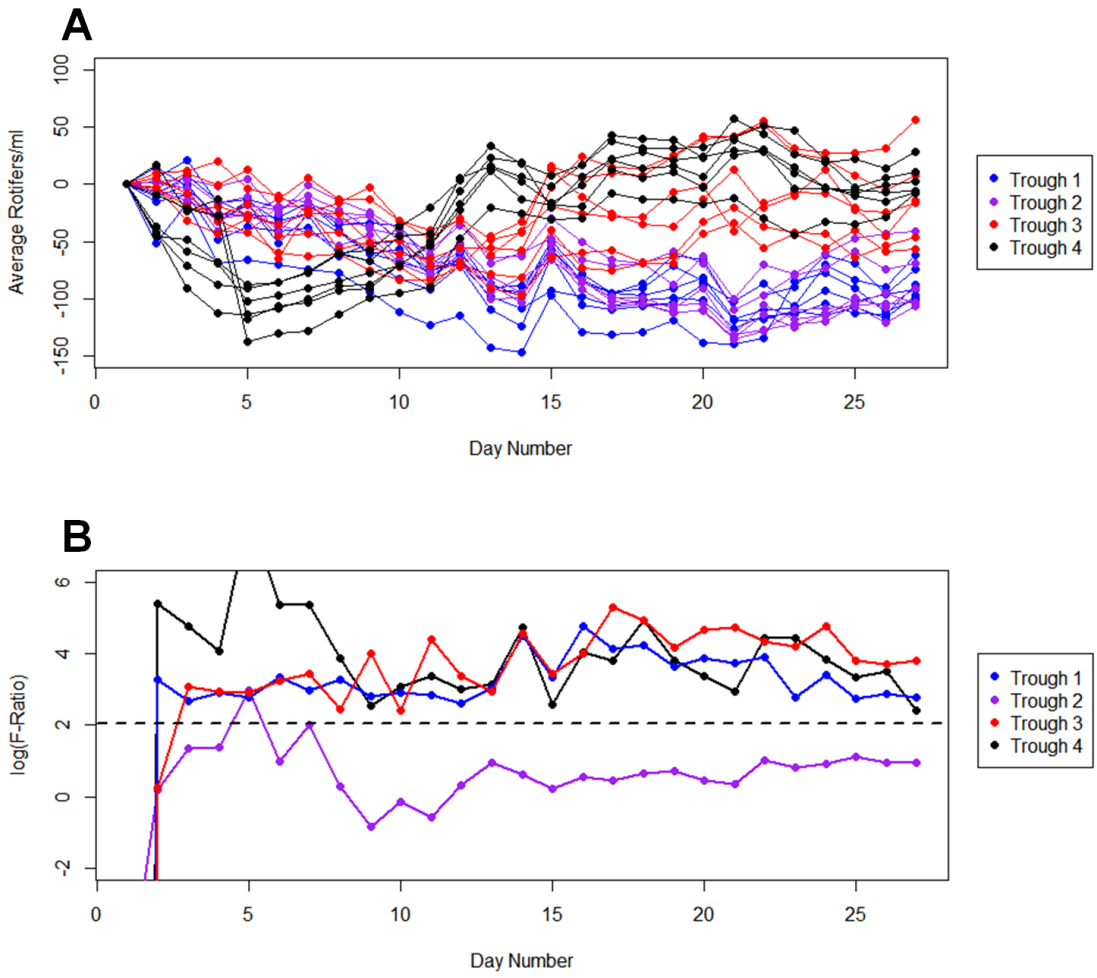

Figure 15: Pre-harvest population densities vs. time in Experiment 1 for A) trough 1 (blue), trough 2 (purple), trough 3 (red) and trough (black). Each line represents the daily average density of rotifers (number/ml) for a different replicate within each recovery treatment. Averages have been adjusted for initial mean density. B) Experiment 1 ANOVA results for comparison among colonies with adjusted average population density of rotifers (number/ml) as the response. F-ratios are plotted for each day during the pre-harvest period for trough 1 (blue), trough 2 (purple), trough 3 (red) and trough (black). The dashed line represents the significance threshold for a nominal p=0.05 test with Bonferroni correction for 27 tests.

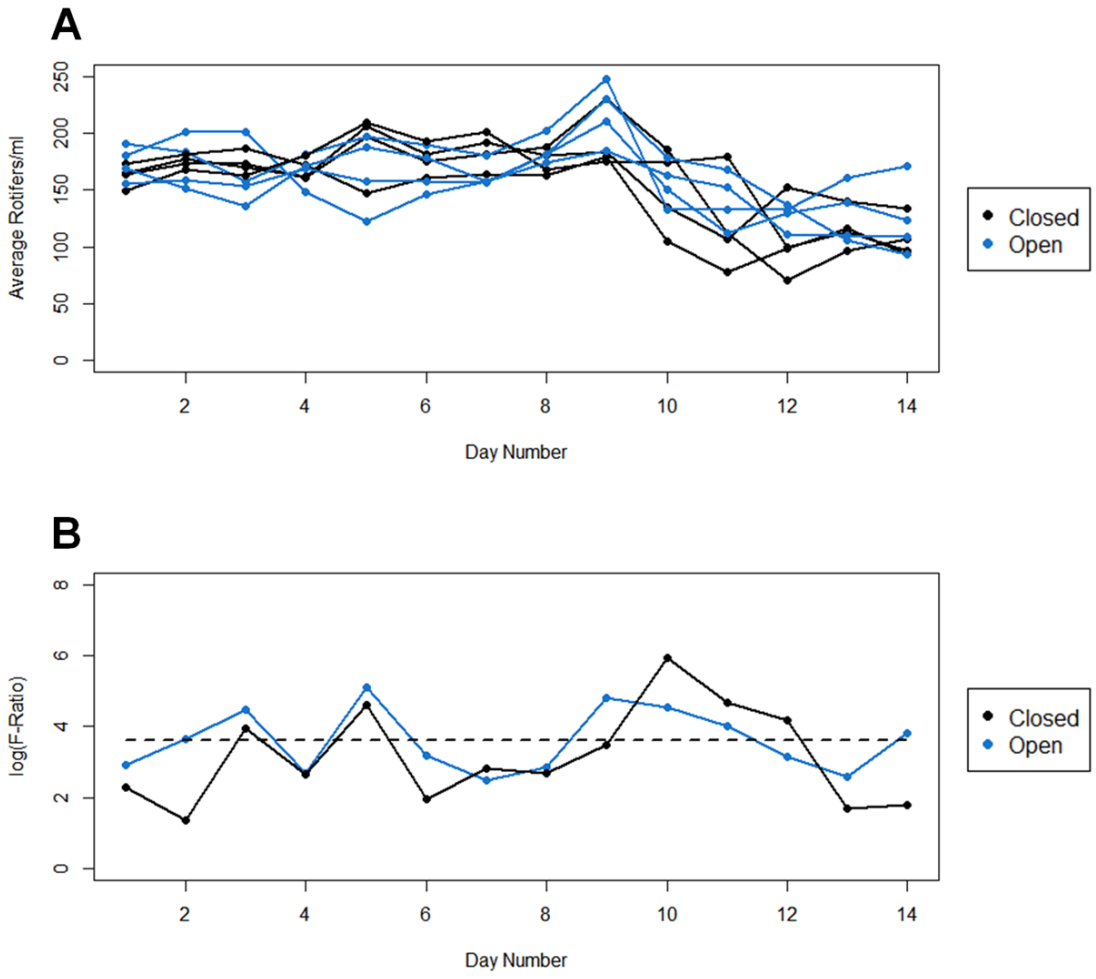

Figure 16: Pre-harvest trajectories in Experiment 2 for the A) 1 day treatment and 10 day treatment. Each line represents the average population density of rotifers (number/ml) vs. time. Blue markers represent open microcosms and black markers represent closed microcosms. B) Experiment 2 ANOVA results for comparison among colonies with counts as the response. F-ratios are plotted for each day during the pre-harvest period for. Blue markers represent open microcosms and black markers represent closed microcosms. The dashed line represents the significance threshold for a nominal p=0.05 test with Bonferroni correction for 14 tests.

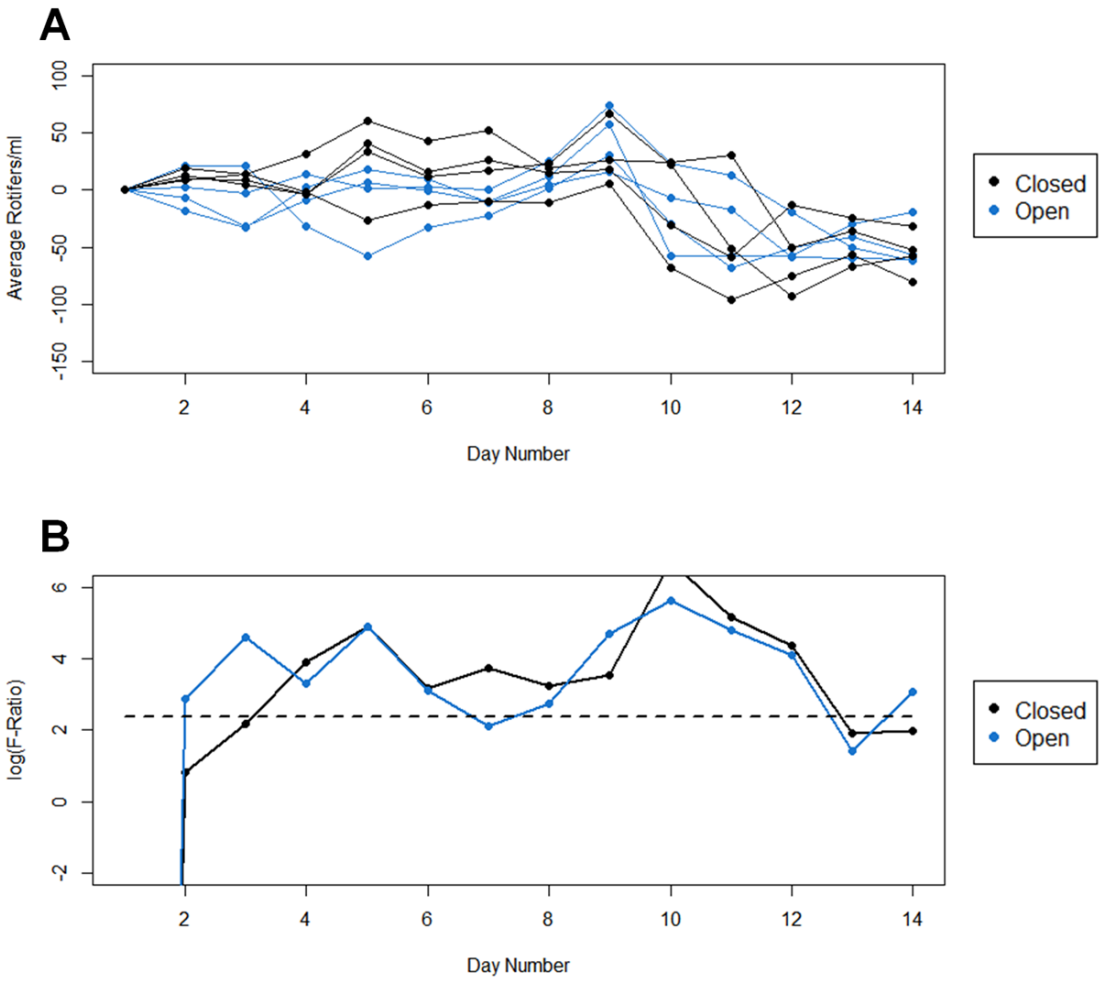

Figure 17: Pre-harvest trajectories in Experiment 2 for the A) 1 day treatment and 10 day treatment. Each line represents the average population density of rotifers (number/ml) vs. time. Averages have been adjusted for initial mean density. Blue markers represent open microcosms and black markers represent closed microcosms. B) Experiment 2 ANOVA results for comparison among colonies with adjusted average population density of rotifers (number/ml) as the response. F-ratios are plotted for each day during the pre-harvest period for. Blue markers represent open microcosms and black markers represent closed microcosms. The dashed line represents the significance threshold for a nominal p=0.05 test with Bonferroni correction for 14 tests.

Appendix A

Various food levels were used to determine a feeding level that will sustain a rotifer colony at 100 rotifers per ml. Six 18L PVC microcosms were filled with de-ionized water and brought up to salinity of 30 ppt using Instant Ocean. Fifty watt immersion heaters were placed into the microcosms and the temperature was set to 24C. Approximately 2,000,000 rotifers were added to each bucket. Each bucket received a 50% water change every other day. Colonies were fed RG complete daily at rates (ml/d) of: 40, 20, 10, 5, 3, and 1. Rotifers were counted daily.

Based on these results, I determined that 20ml per day would be a reasonable feeding level to maintain colonies and expect the resulting population density to be roughly 100 rotifers per ml.

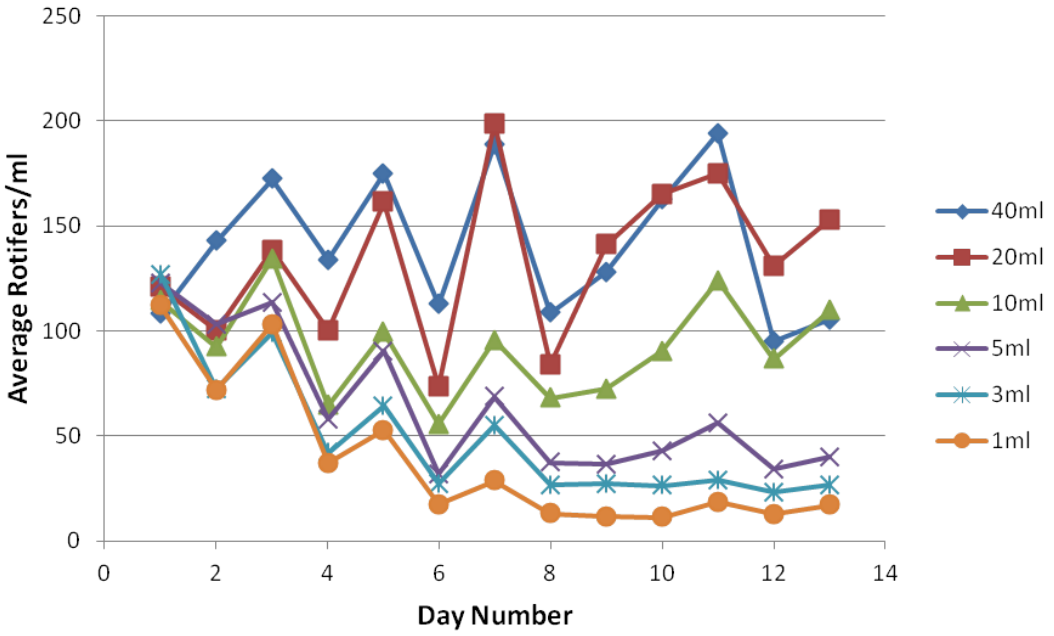

Figure A.1: Average rotifers/ml vs. day number. Each line represents counts for microcosms at each feeding rate.

Appendix B

Long-established microcosms develop a substantial epifaunal community on the wall of the microcosms. This experiment was used to determine the impact of this community. Rotifers were set up in four 18L microcosms as in Appendix A. Temperature was held at 24C and the salinity was kept constant at 30ppt. Each microcosm received a 25% water change daily. Rotifers were counted and fed 20ml of food once a day. Four microcosms were established for several weeks to allow them to develop a substantial biofilm.

To evaluate the impact of the biofilm community on rotifer growth, I removed the water from all four microcosms. I cleaned the film out of two of the microcosms and left the film in the other two intact. I then refilled the microcosms and added rotifers.

From these results, I concluded that the biofilm significantly impacts rotifer growth and for all subsequent experiments I replaced the microcosms weekly with clean containers.

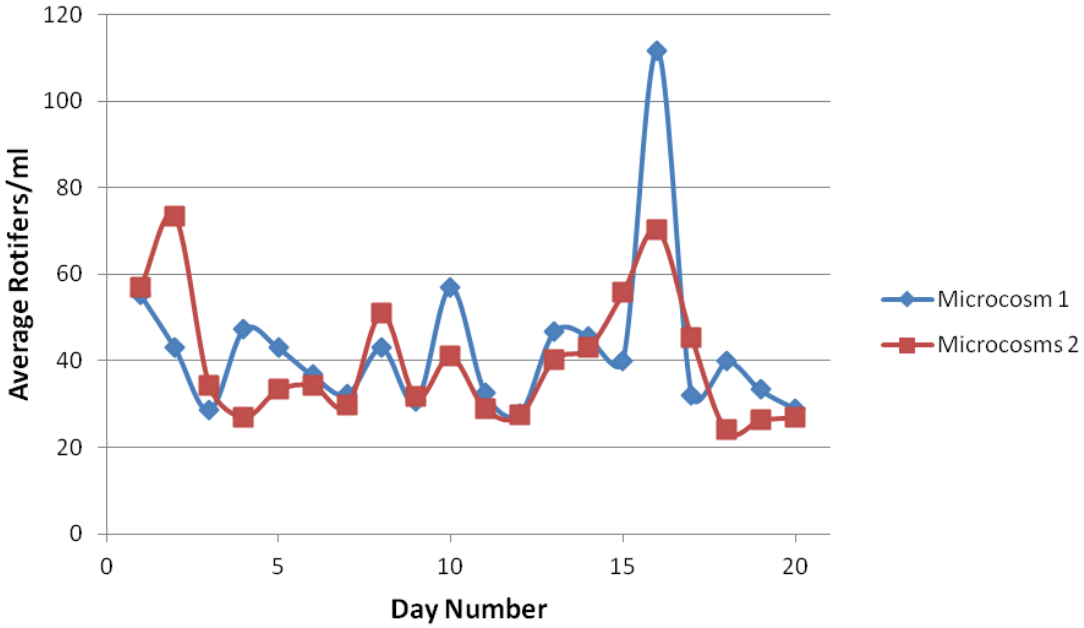

Figure B.1: Average rotifers/ml vs. day for microcosms with algae already established.

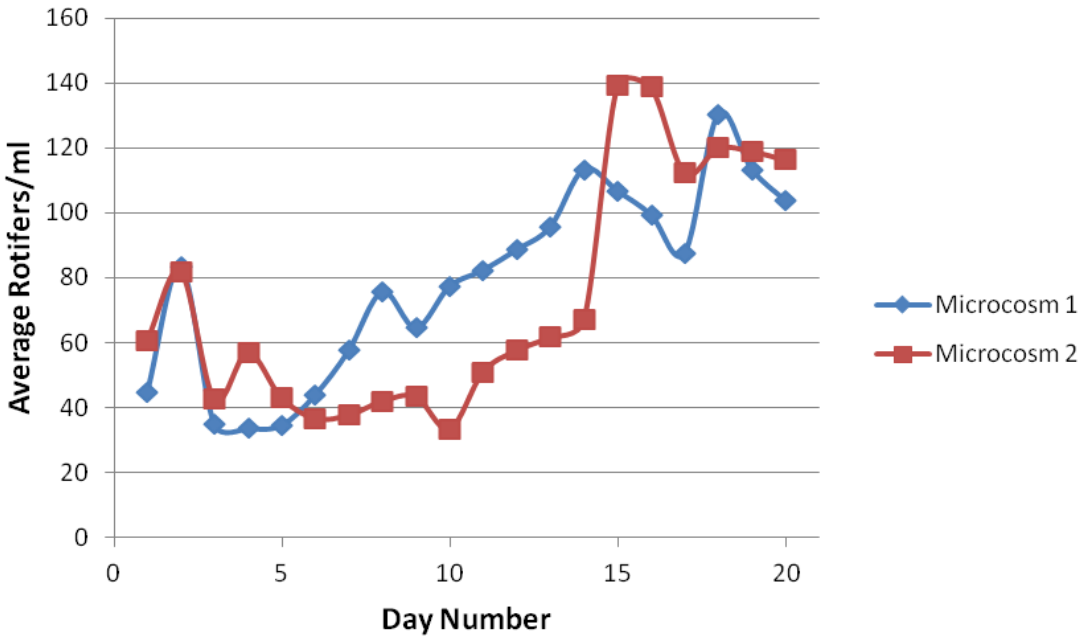

Figure B.2: Average rotifers/ml vs. day for clean microcosms.

Appendix C

In this experiment, I evaluated the rate at which Artemia survive and mature over a range of food densities. These trials were conducted in 18L microcosms with artificial seawater at 30ppt and 24C. To set them up, I hatched Artemia from cysts and estimated the density of nauplii using three 1ml counts. Based on these counts, I seeded 11 microcosms with one of four target numbers of nauplii (ranging from 40,000 to 267,000). Artemia were fed RG complete 1x per day using feeding rates ranging from 3ml/day to 20ml/d.

I monitored growth and maturation with daily counts and visual observations for two weeks. Juvenile stages were monitored using 3, 1ml samples. Adult and juvenile stages were monitored by counting the total number in three 1L samples. I noted the first appearance of adult Artemia for each food / initial density combination and estimated the total number of adults alive at the end of the experiment.

Based on these results, I concluded that 20 ml of RG complete per day is sufficient to allow *Artemia* to mature within a week. I used these results (and the results of Appendix D) to estimate the initial numbers of nauplii and adult Artemia to stock in Experiment 1.

Food (ml/day)	Initial Nauplii	Days Until Juveniles/Adults	Average Number of Juveniles/Adults
3	40,000	N/A	N/A
3	40,000	N/A	N/A
3	160,000	N/A	N/A
12	40,000	6	16.75
12	160,000	7	9
12	160,000	7	10.25
12	267,000	N/A	N/A
20	40,000	5	3
20	160,000	6	14.5
20	267,000	5	3
20	267,000	6	1

Table C.1: Results for Artermia maturation experiment. Column one contains ml of food each microcosm received each day. Column two is the initial number of nauplii each microcosm was seeded with. Columns three and four contain number of days till the first juvenile/adult stages were seen and the average number juveniles/adults observed on the final day of the experiment for each microcosm. N/A indicated that no juveniles/adults were seen.

Appendix D

Sixty petri dishes were used to hold one adult brine shrimp and an assigned number of rotifers. Foam insulation was used to float the petri dishes in two different tanks held at two different temperatures. One tank was held at 24C and the other was held at 28C. Thirty petri dishes were assigned to each temperature treatment. The foam insulation was broken up into three columns of ten petri dishes. Columns held at 24 were A-C and the columns at 28C were D-F. Rotifers in the petri dishes ranged from 10 rotifers to 100 rotifers. There were six replicates of each and one of each replicate in columns A-F. The position of each replicate in the columns was randomized. The purpose was to assess the rate at which brine shrimp consume or kill rotifers at different concentrations. After three different time periods the brine shrimp were removed carefully from the replicates and the remaining living rotifers were counted. The rotifers were either counted after 15, 30 or 60 minutes. Each time slot had one replicate from each of the rotifer levels from both of the temperature treatments.

This experiment shows that as time and initial rotifer concentration increases, so does the number of rotifers killed (Figures D.1, D.2). Between the two temperature treatments, 28C generally showed an increased killing rate for most rotifer levels (Figure

D.3). However, the rate of removal is not simply proportional to the number of rotifers present, suggesting that there is a limit to the rate at which they are killed by *Artemia*.

Based on this experiment, I estimate that a single Artemia would kill 700 rotifers per day. Assuming that I have 100 rotifers / ml and a total volume of 18L there are 1.8 million rotifers. Assuming that rotifers are capable of doubling in 1day, I need 2500 Artemia to kill 1.8 million rotifers and hold the rotifer population in check.

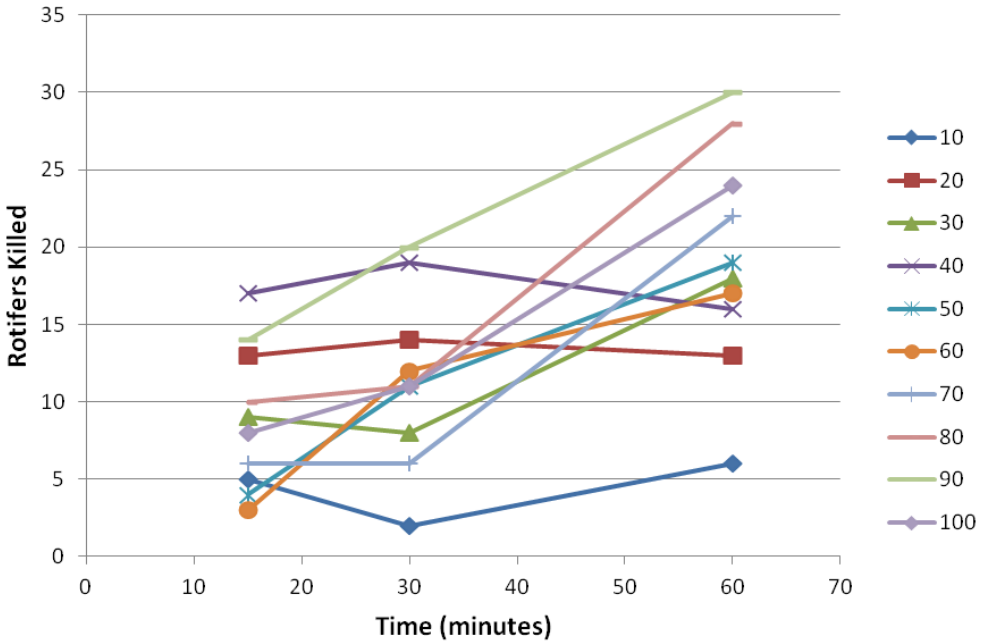

Figure D.1: Rotifers killed vs. time for the 24C treatment. Each line represents a different initial starting rotifer concentration. Each point on the line represents the average number of rotifers killed after 15, 30 or 60 minutes.

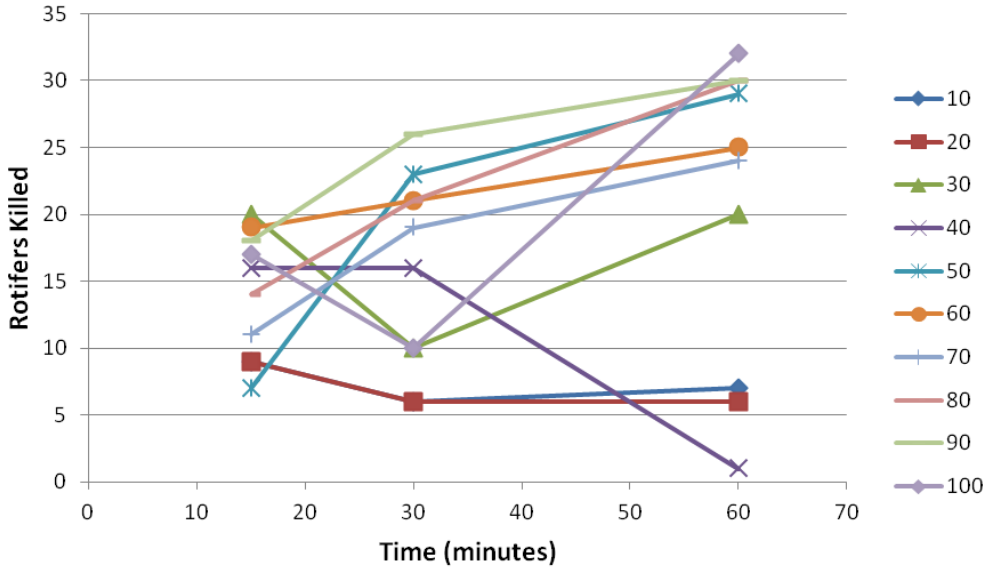

Figure D.2: Rotifers killed vs. time for the 28C treatment. Each line represents a different initial starting rotifer concentration. Each point on the line represents the average number of rotifers killed after 15, 30 or 60 minutes.

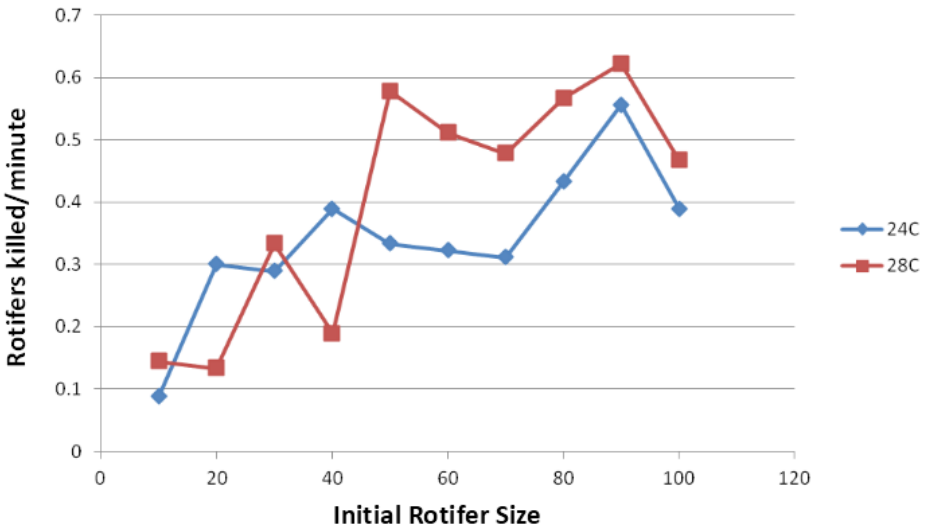

Figure D.3: Average rate of rotifer kill over time (rotifers per min) as function of initial rotifer density.

Appendix E

This pilot experiment consisted of eighteen 18L microcosms with both rotifers and brine shrimp. Six microcosms were kept in a water bath held at 24C, six were in a bath held at 30 and six were kept in water bath the cycled between 24C-30C over a 14 day period. All microcosms were kept at 30 ppt and received equal amounts of oxygen. Lids were partially kept on the microcosms at all times except when water was being removed/added or it was a feeding time. Standard daily water changes were done on each bucket.

This experiment was designed to include the same acclimation period, harvest period, passive recovery period and active recovery period as the three main experiments described in the body of my thesis. I used the data from the acclimation period to explore an explanation as to why my microcosms in experiment one diverge right after initial seeding.

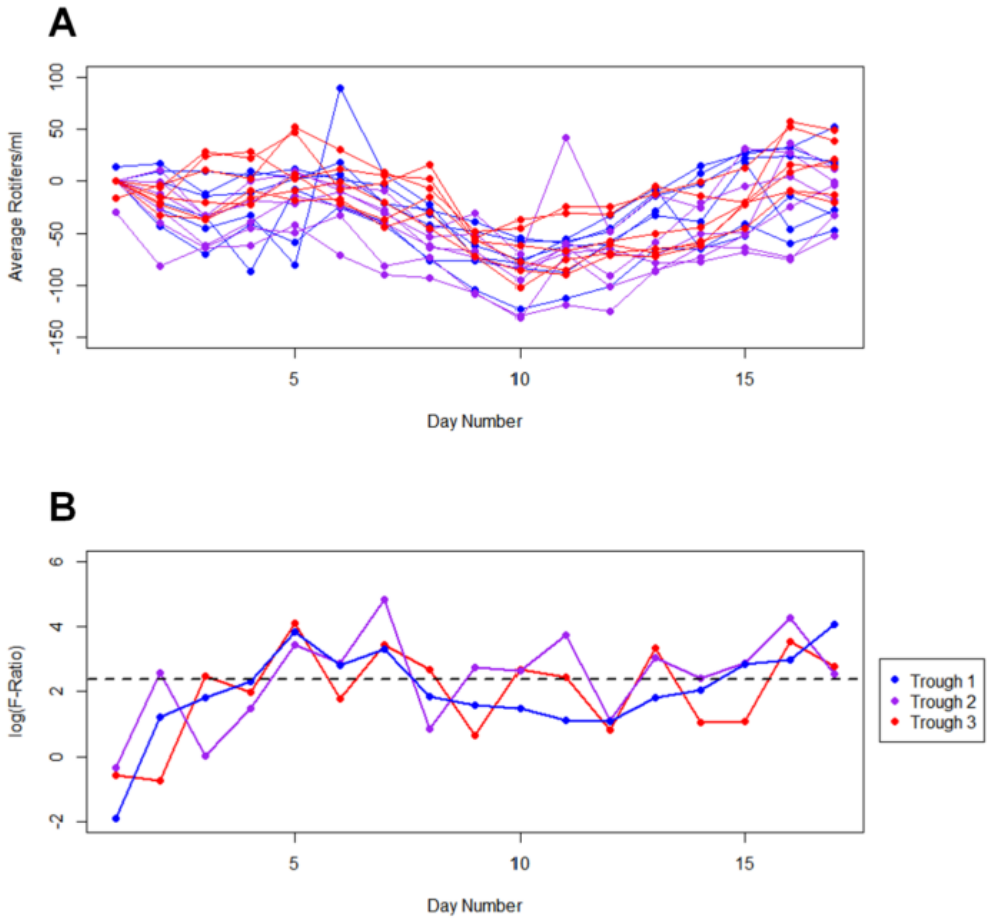

Figure E.1: Pre-harvest population densities vs. time in the Pilot Experiment for A) trough 1 (blue), trough 2 (purple), and trough 3 (red). Each line represents the daily average density of rotifers (number/ml) for a different replicate within each recovery treatment. Averages have been adjusted for initial mean density. B) Pilot Experiment ANOVA results for comparison among colonies with adjusted average population density of rotifers (number/ml) as the response. F-ratios are plotted for each day during the pre-harvest period for trough 1 (blue), trough 2 (purple), and trough 3 (red). The dashed line represents the significance threshold for a nominal p=0.05 test with Bonferroni correction for 27 tests.

Appendix F

This experiment was intended to test whether the failure of all rotifer colonies in Experiment 3 held at 500ml was due to water volume, a contaminant, or some other factor. To do so, three microcosms were filled with 1000ml of artificial seawater and three more were filled with 500ml of artificial seawater. Freshly acquired rotifers from Reed Mariculture were then added to all microcosms at an initial density of 100 rotifers per ml. Water conditions, daily counts, feeding rates and maintenance were all identical to those in Experiment 3.

All of the 500ml colonies crashed within two weeks, while all of the 1L colonies remained at roughly 100 rotifers per ml. From this I concluded that the 500ml volume is not conducive to rotifer growth. Rotifers require modest water movement, which is provided by aeration. I suspect that the water movement provided by aerating the half-full flasks (roughly 2 inches deep) was insufficient to permit rotifer growth.

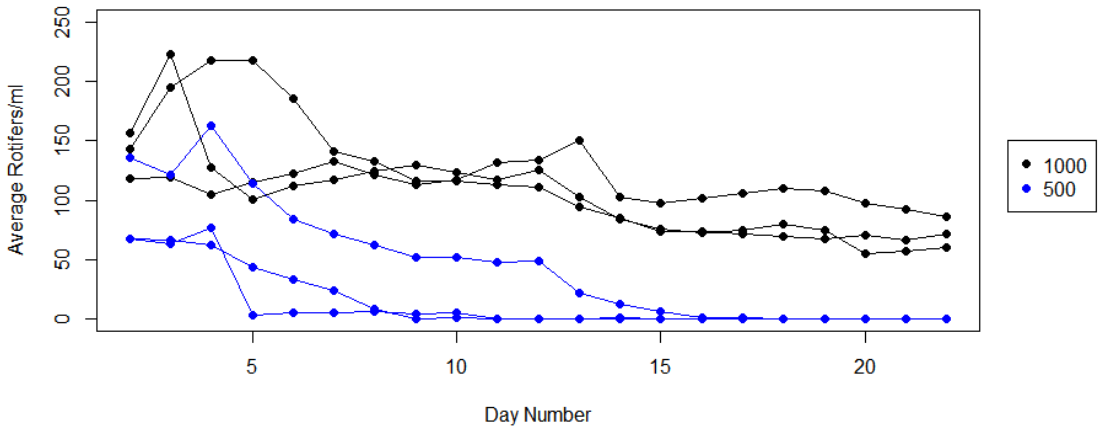

Figure F.1: Post-recovery trajectories in Experiment 2 for the 1 day treatment and 10 day treatment. Each line represents the average population density of rotifers (number/ml) vs. time. Black markers represent 1000ml microcosms and blue markers represent 500ml microcosms.

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