

**EFFECTS OF DISTURBANCE, COMPETITION AND  
PRODUCTIVITY ON DYNAMICS OF INDUCIBLE TROPHIC  
POLYMORPHISM IN *TETRAHYMENA VORAX***

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POLYMORPHISM IN *TETRAHYMENA VORAX***

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## SUMMARY

Inducible trophic polymorphism enables organisms to alter their trophic level when facing environmental changes, and therefore can ameliorate the intensity of competition. The freshwater ciliated protist *Tetrahymena vorax* was found to have three distinct phenotypic morphs with two trophic levels. Its carnivorous macrostomes consume intraspecific competitors and its bacterivorous pyriform microstome morph and tailed microstome morph indiscriminately. Cannibalism here indicates an extreme case of niche differentiation and resource utilization via phenotypic plasticity and significantly affects the dynamic equilibrium of *T. vorax*'s three morphs. By manipulating productivity level, disturbance frequency and the presence or absence of an interspecific competitor species *Colpodium striatum*, I demonstrated the dynamic transformations of *T. vorax*'s three morphs and endeavored to explain the underlying mechanisms. In this study, I also tested some classic assumptions about phenotypic tradeoffs in *T. vorax* and hence clarified some misunderstandings and proposed novel hypotheses.

# CHAPTER 1

## INTRODUCTION

Ecological communities are described as assemblages of interacting species populations within defined geographic areas, and biotic interactions between or within species can lead to changes in organism fitness (Lang & Benbow 2013). Species coexisting in the same spatial region at the same trophic level often encounter intense conflicts due to overlapping resource requirements (Chesson 2000). Direct and indirect interaction between two or more organisms striving for the same resources is defined as competition (Odum 1953). Intraspecific competition happens between individuals of the same species, while interspecific competition occurs between two or more species (Lang & Benbow 2013). The effects of competition on species' abundances make it a rather important element in constructing communities (Violle *et al.* 2010).

In some exceptional cases, predation can occur between competitors, which can significantly affect competition intensity. While it is apparent that interguild predation may alleviate interspecific competition to a certain extent, intraguild predation can also decrease exploitative competition at the expense of smaller individuals because it is likely to be size-specific and asymmetrical (Holomuzki *et al.* 2010). The occurrence of intraguild predators can significantly affect community structure because the predator–prey interactions can complicate the existing competitive interactions, causing possible consequences such as species' exclusion, trophic niche shifts, and cascading interactions in food webs (Polis & Holt 1992, Thompson & Gese 2007).

An organism's potential ability to produce various phenotypes with different fitness



is defined as phenotypic plasticity (DeWitt et al. 1998). Phenotypic plasticity enables the alteration of behavior, morphology, and/or physiology in individuals, which can profoundly affect the population dynamics between interacting species (Banerji & Morin 2009). As examples of phenotypic plasticity, trophic polymorphisms enable organisms to change their trophic level so as to have two or more phenotypic morphs of the same species exploiting different food niches. The occurrence of inducible trophic polymorphisms can lead to significant changes in both number and types of interactions in a food web. Intraguild predation, for instance, is sometimes a consequence of inducible trophic polymorphisms, which can greatly complicate the existing species interactions (Kopp & Tollrian 2003, Banerji & Morin 2009).

Past studies on cichlid fishes (Swanson *et al.* 2003), salamanders (Jefferson *et al.* 2014), sparrows (Maney *et al.* 2005), among other animals as well, indicate that inducible trophic polymorphism is a common phenomenon among many species. Wimberger (1994) suggested that intraspecific trophic divergences caused by inducible trophic polymorphism often lead to foraging specialization and alterations of relative foraging efficiencies, and therefore influence potential intraspecific niche differentiation that helps species to explore vacant niches in their native environment. Through radical changes in morphology, physiology, metabolism or behavior, life history of a species may undergo significant alterations in response to environmental variation and relative abundances of other coexisting species or dynamics between its phenotypic morphs. Among the wide range of species in which inducible trophic polymorphism may take place, these transformation trajectories can be reversible or irreversible (Banerji & Morin 2009).

Inducible trophic polymorphisms are often involved in inducible offenses, which

can enhance organism's feeding ability on certain prey types and may require morphological or/physiological changes. For example, enlarged jaw musculatures and differently shaped keratinized mouthparts in tadpoles of the frogs *Spea* spp carnivorous morph enable it to consume larger prey than its omnivorous morph, indicating that its morphological divergence should be complementary with its trophic divergence (Pfennig & Murphy 2002).

With regards to abiotic causes, disturbance was often considered to be a major factor influencing species coexistence. Disturbances disrupt community and population structures through direct changes in resources or physical environment, or indirect influence on species with different responding strategies (Resh *et al.* 1988). Removal of, or damage to existing individuals caused by disturbance can sometimes provide opportunities for inferior competitors to prosper, because of the potential reduction of average resource utilization and release of resource in community (Cornell & Lawton 1992, Jiang & Patel 2008). Different levels of disturbances can renew limited resource and create patterns of spatiotemporal heterogeneity, and therefore alter species composition and abundances in communities (Levin & Paine 1974).

Theories and former studies suggested that top-down and bottom-up forces usually act on communities simultaneously, indicating that population dynamics of intraguild predator and prey could be affected by productivity levels and resource utilization efficiencies (Morin 1999, Thompson & Gese 2007). Competition is presumed to be the determining factor of community patterns at low productivity levels, where superior competitors should become dominant because of more access to resources and more efficient energy transfer between trophic levels. In high productivity systems, however,

individuals with better predation-avoiding strategies that are predicted to be superior competitors, because sufficient resources can alleviate competition and therefore guarantee that predation should be the major influence on community structure (Bohannon & Lenski 2000, Banerji & Morin 2009).

Laboratory microbial model systems are ideal subjects to address ecological questions that have proven difficult or impossible to study through field systems. They often enable strict experimental control and easy replications, and ensure long-term data acquisition in short time spans, as delayed responses in long-lived organisms often turn out to be rapid and evident in microbe with short generation times (3–48h) (Kaunzinger & Morin 1998, Twagilimana *et al.* 1998, Jessup *et al.* 2004). In this study, the focal species *Tetrahymena vorax* is a free-swimming freshwater ciliated protozoan (Smith *et al.* 2012). *T. vorax* has been characterized as an ideal experimental subject for studying inducible trophic polymorphism because of its small size (60–250 µm in length) and short generation time (~6 hours) make it possible to assess the long-term consequences of inducible trophic polymorphism in a relatively short time span. *T. vorax* comprises three distinct morphs in its isogenic populations: pear-shaped, bacterivorous pyriform microstomes; elongated, bacterivorous tailed microstomes; and greatly enlarged, oval-shaped, carnivorous macrostomes (Banerji & Morin 2009, Banerji 2011).

During the transformation from microstomes to macrostomes, individuals of *T. vorax* experience an oral replacement process: an original small oral apparatus in the microstome is completely resorbed and reformed into a large mouth; posterior to the oral apparatus, a pharyngeal pouch forms at the same time to function as a reservoir site for captured ciliate prey (Buhse 1966). The transformation process from early stage

microstomes into macrostomes often occurs in the presence of another ciliate competitor species, and enables transformed *T. vorax* macrostome individuals to consume ciliate competitors including microstome conspecifics, which alters their original trophic level. Turning into a predatory morph enables macrostomes to optimize their food–resource utilization by occupying a different food niche than their original competitors (Grønlund *et al.* 2002, 2013, Kopp & Tollrian 2003, Banerji & Morin 2009). What triggers the transformation from microstomes into macrostomes is assumed to be an inducible cue, in this case being stomatins containing ferrous ions and nucleic acids released by current competitor and potential prey ciliate species, such as *Tetrahymena thermophila*, *Tetrahymena pyriformis*, or *Colpidium striatum* (Buhse 1966, Grønlund *et al.* 2011).

Few previous studies have focused on interactive effects of competition, disturbance and productivity on *T. vorax* population dynamics. To better understand how polymorphism affects a species' reaction to different environmental factors in flux, this project created multiple scenarios to explore possible outcomes of *T. vorax* exposed to different levels of productivity and disturbance, in the absence or presence of interspecific competitors, to explore the effects of competition, disturbance and productivity on population and morph dynamics of the polymorphic freshwater ciliated protozoan *Tetrahymena vorax*.

Although empirical evidence suggests that carnivorous macrostomes prefer interspecific competitor ciliates to conspecific microstomes as prey, cannibalism seems unavoidable in *T. vorax* populations when food resources are limited (Grønlund *et al.* 2002). Though Grønlund *et al.* (2013) mentioned that tailed microstomes should be absent in axenic solutions, what triggered the emergence of tailed microstome and the

transforming mechanism and tradeoffs between the two prey forms (*i.e.* pyriform and tailed microstomes) remained unclear. In this study, I also specifically examined each morph's swimming speed to test the hypothesis that tailed microstomes should be less susceptible to the cannibalistic macrostome predators compared to pyriform microstomes, because its fully developed tail might provide a better swimming ability (Banerji & Morin, 2009).

## CHAPTER 2

### MATERIALS AND METHODS

In addition to *Tetrahymena vorax*, the bacterivorous protist species *Colpidium striatum* was also used in this experiment as additional interspecific competitors so to assess growth rate of *T. vorax* populations under different conditions. Both species are free-swimming ciliated protozoans from freshwater systems, and their short generation times enabled data collections of multigenerational population dynamics (Holyoak & Sachdev 1998, Jiang *et al.* 2009). Each experimental species was purchased commercially—*T. vorax* from American Type Culture Collection (Manassas, Virginia, USA), and *C. striatum* from Carolina Biological Supply (Burlington, North Carolina, USA). The experimental species were maintained in 250 mL enclosed glass jars with 100 mL aqueous medium, which initially contained 0.55 g of crushed protozoan pellets (Carolina Biological Supply, Burlington, North Carolina, USA) per liter of deionized water, and was inoculated with the bacterium *Serratia marcescens* as the prey (Price & Morin 2004). Stock cultures were prepared 2 weeks prior to the experiment. Experimental microcosms were created in the same manner as were the stock cultures. Protozoan medium solutions and 250 mL covered glass jars were autoclaved, and the bacterium prey *S. marcescens* was inoculated 24 hours before the addition of 30 individuals of *T. vorax* into each microcosm. Both stock cultures and microcosms were kept in incubators with a consistent 22 °C setting under 12-hour light-dark daily cycle.

### *Manipulation of competition, disturbance and productivity*

The main experiment consisted of a three-way factorial design, including 3 growth medium concentrations, 4 disturbance frequencies, and the presence or absence of a competitor species *Colpidium striatum*, so to find out how productivity, disturbance and interspecific competitors affect population abundance and morph composition of *T. vorax*. The ciliated protist *C. striatum* was chosen to be the competitor species because of its common presence in natural freshwater systems, and its morphological distinctness from *T. vorax* ensured consistent discriminations between the two coexisting species. Low, intermediate, and high growth medium concentration initially contained 0.022, 0.55, and 1.1g of crushed protozoan pellets (Carolina Biological Supply, Burlington, North Carolina, USA) per liter of deionized water, respectively. Disturbance was created with a Branson ultrasonics SLPe digital sonifier cell disruptor (Fisher Scientific, Pittsburgh, Pennsylvania, USA) at 60% amplitude for 20 seconds. This treatment induced density-independent mortalities without causing extra adverse effects on the microbial communities (Jiang & Patel 2008, Violle *et al.* 2010). A gradient of disturbance frequencies was carried out in the order of 1) no disturbance, 2) low disturbance, where disturbances were imposed every six days, 3) intermediate disturbance, where disturbances were imposed every three days, and 4) high disturbance, where disturbances were imposed every two days.

Each treatment was consisted of three replicates, therefore this study comprised of a total of seventy-two microcosms. Abundances of *Colpidium striatum* and each morph of *T. vorax* in each treatment were estimated daily through examining a randomly selected 0.3 mL sample microscopically for three weeks (Banerji & Morin 2009).

### *Collection of swimming speeds*

Because it was not realistic to capture the motion of individuals only during predation, in this study, I chose the fastest individuals in *T. vorax* populations and assumed that the mean swimming speed can represent their swimming potentials in predation (macrostome) or anti-predation (microstomes). 80 consistently free-swimming *T. vorax* individuals of each morph were videotaped using digital camera device attached to a microscope at 20X magnification, and their swimming speeds were estimated through the Olympus cellSens Standard software (Olympus Corporation, Shinjuku, Tokyo, Japan).

### *Statistical analyses*

To summarize the effects of competition, disturbance and productivity on the responses of populations of *T. vorax*, I used SPSS Statistics V21.0 (IBM Corporation, Armonk, New York, USA) and ran a three-way analysis of variance (ANOVA) test with post-hoc Tukey's HSD (honest significant difference) test on abundance of *T. vorax*'s population on 21<sup>st</sup> sampling day of the experiment. I also ran three-way multivariate analysis of variance (MANOVA) tests on daily and final-day abundances of the morphs of *T. vorax*'s. Effects of each independent factor and interactions between factors on each morph were tested via three-way ANOVA with post-hoc Tukey HSD as well.

To test how different factors influenced the morph structure within *T. vorax* population, I used diversity index to represent *T. vorax*'s phenotype composition. The evenness of *T. vorax* population can be described by Pielou's evenness index as follow:

$$H' = - \sum p_i \ln p_i \quad (\text{eq 1})$$

$$H'_{\max} = - \sum 1/S \ln (1/S) = \ln S \quad (\text{eq 2})$$

$$J' = H'/H'_{\max} \quad (\text{eq 3})$$



where  $J'$  represents how even a population/community is; higher  $J'$  value indicates less variation in proportions between phenotypes in the population.  $H'$  is the number derived from Shannon diversity index (eq 1) and  $H'_{\max}$  is the maximum possible value of  $H'$  when each morph is equal in the population;  $p_i$  represents the proportion of the  $i$ th phenotype individuals in final *T. vorax* populations;  $S$  is the number of morphs in *T. vorax* population, which is 3 in this study, hence the value of  $H'_{\max}$  equals to  $\ln 3$ .

A three-way ANOVA and post-hoc Tukey HSD test on *T. vorax*'s final-day Pielou's Evenness Index was used to examine the morph composition in the population.

In consideration of the significant variances between active and passive individuals, swimming speed data of the 20 (25%) fastest pyriform microstome, tailed microstome and macrostome individuals were selected and put into a one-way ANOVA test to determine if there were differences in swimming speeds between the morphs of *T. vorax* during predation.

## CHAPTER 3

### RESULTS

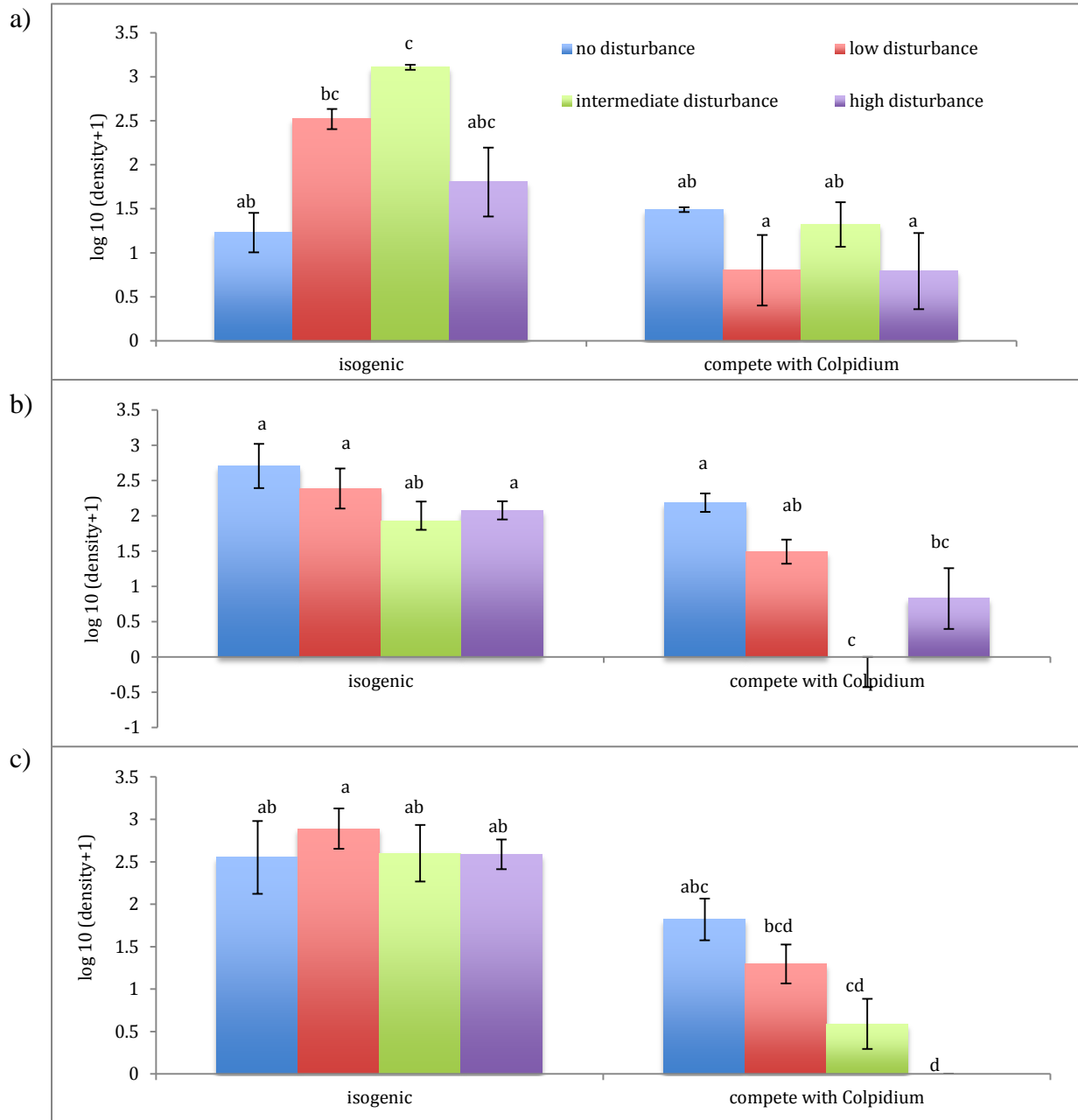
#### Effects of factors on *T. vorax* population size

Based on data from the final-day sampling, when *T. vorax* populations had achieved equilibrium, ANOVA test results indicate that competition and disturbance have significant effects on *T. vorax*'s population size (Three-way ANOVA, *Table 1*,  $p < 0.001$ ).

**Table 1.** Results of three-way ANOVA test for effects of productivity, disturbance and competition on population abundance of *T. vorax* from last sampling day.

	df	Mean Sq	F	P value
competition	1	31.121	145.467	<0.001
disturbance	3	1.575	7.363	<0.001
productivity	2	0.152	0.712	0.496
competition*disturbance	3	2.132	9.967	<0.001
competition* productivity	2	0.793	3.707	0.032
disturbance* productivity	6	1.486	6.945	<0.001
competition*disturbance* productivity	6	0.338	1.579	0.174
Error	48	0.214		

As seen in *Figure 1*, *T. vorax* population abundance can be significantly decreased by presence of the interspecific competitor *Colpidium striatum* compared to that of isogenic *T. vorax* population, suggesting that intraspecific competition has significantly negative effects on *T. vorax* population. Meanwhile, negative effects of disturbance differ significantly on *T. vorax* population size between isogenic and competition groups, which is consistent with the ANOVA result that reveals the interaction between competition and disturbance (*Table 1*,  $p < 0.001$ ). Effects of productivity level on *T. vorax* population density also differ significantly between isogenic and competition groups, which is supported by the term showing significant interaction between competition and



**Figure 1.** Mean final *T. vorax* population sizes ( $\pm$ SE) at (a) low (b) intermediate and (c) high productivity levels (density: #cells/mL). Post-Hoc Tukey HSD results displayed as letters above columns, groups not sharing the same letters are significantly different.

productivity in ANOVA test (*Table 1*,  $p < 0.05$ ). The fact that effects of disturbance on *T. vorax* population differ with different productivity treatments proved significant disturbance and productivity interaction term in ANOVA as well (*Table 1*,  $p < 0.001$ ).

### Effects of factors on *T. vorax* morphs

Three-way MANOVA analysis on data from final-day sampling showed that competition and disturbance have significant effects on *T. vorax* morphs (Three-way MANOVA, *Table 2*,  $p < 0.001$ ).

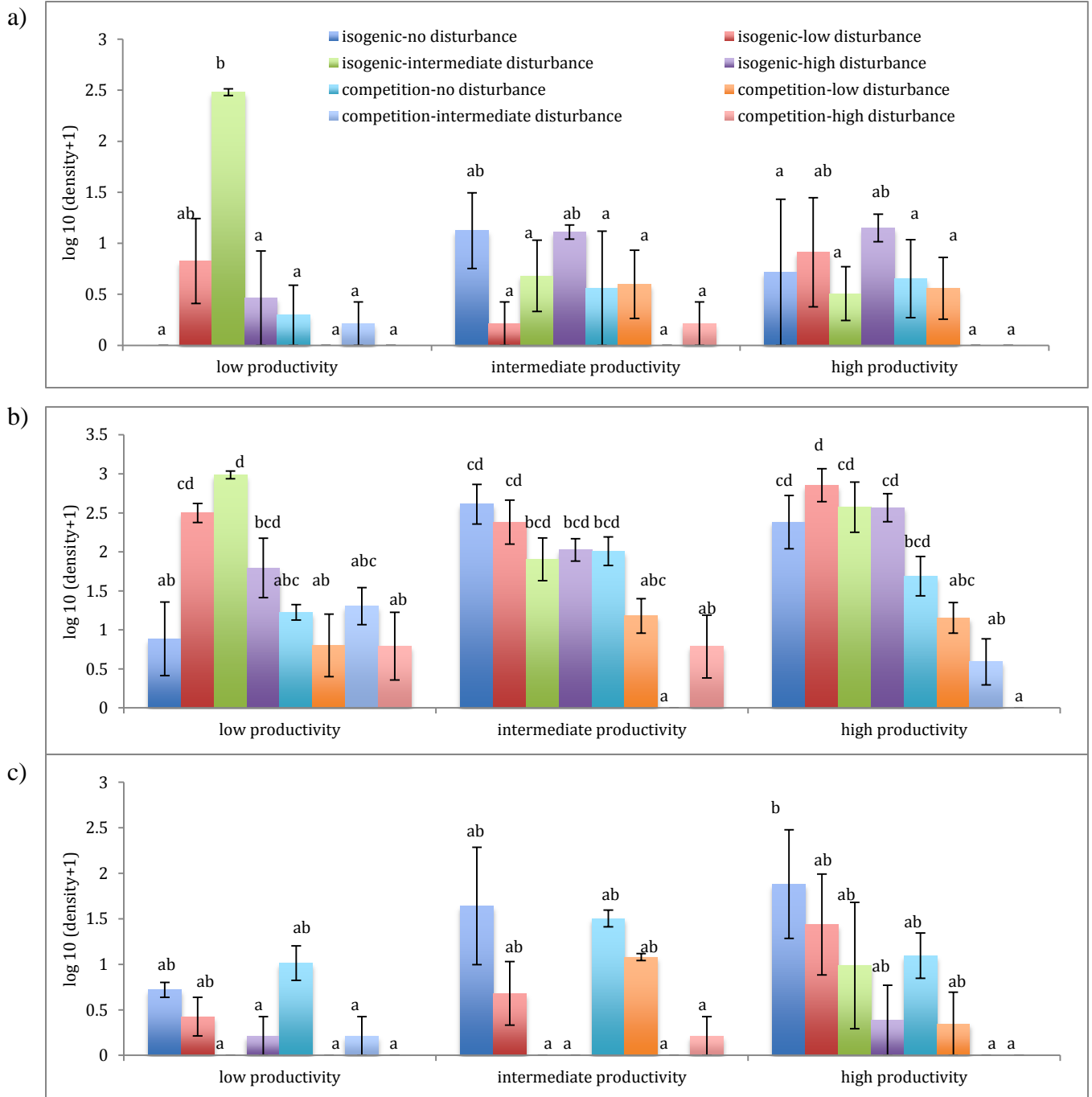
**Table 2.** Results of three-way MANOVA test summarizing effects of productivity, disturbance and competition on *T. vorax*'s morph abundances from last sampling day.

	df (Hypothesis, Error)	Pillai's Trace	F	P value
competition	3, 46	0.785	56.147	<0.001
disturbance	9, 144	0.726	5.111	<0.001
productivity	6, 94	0.184	1.585	0.160
competition* disturbance	9, 144	0.582	3.853	<0.001
competition*productivity	6, 94	0.328	3.071	0.009
disturbance* productivity	18, 144	0.757	2.698	0.001
c*d*p	18, 144	0.490	1.563	0.077

(In c\*d\*p: c: competition; d: disturbance; p: productivity)

Competition can decrease pyriform microstome abundance under most circumstances. Effects of disturbance on pyriform microstome density are different between competition and productivity groups, indicating the existence of interactive effects between disturbance and competition, and disturbance and productivity (*Figure 2*, Three-way ANOVA, *Table 3*,  $p < 0.05$ ).

Both competition and disturbance can significantly decrease tailed microstome population size. Differences between disturbance treatments increase when interspecific competition is present, which suggested the interaction between competition and



**Figure 2.** Mean final *T. vorax* abundances ( $\pm$ SE) of (a) pyriform microstome (b) tailed microstome (c) macrostome (density: #cells/mL). Post-Hoc Tukey HSD results are displayed as letters above columns, groups not sharing the same letter are significantly different.

**Table 3.** Results of three-way ANOVA test summarizing effects of productivity, disturbance, and competition on each morph of *T. vorax* from last sampling day.

morph	factors	df	Mean Sq	F	P value
pyriform microstome	competition	1	6.290	20.773	<0.001
	disturbance	3	0.085	0.280	0.840
	productivity	2	0.006	0.020	0.980
	competition*disturbance	3	1.068	3.528	0.022
	competition*productivity	2	0.236	0.779	0.465
	disturbance*productivity	6	1.176	3.885	0.003
	c*d*p	6	0.733	2.421	0.040
	Error	48	0.303		
tailed microstome	competition	1	31.709	143.254	<0.001
	disturbance	3	0.950	4.292	0.009
	productivity	2	0.220	0.992	0.378
	competition*disturbance	3	2.124	9.596	<0.001
	competition*productivity	2	0.827	3.736	0.031
	disturbance*productivity	6	1.584	7.155	<0.001
	c*d*p	6	0.297	1.344	0.257
	Error	48	0.221		
macrostome	competition	1	1.056	3.762	0.058
	disturbance	3	5.284	18.817	<0.001
	productivity	2	1.255	4.470	0.017
	competition*disturbance	3	0.047	0.166	0.919
	competition*productivity	2	1.498	5.335	0.008
	disturbance*productivity	6	0.322	1.147	0.350
	c*d*p	6	0.179	0.639	0.698
	Error	48	0.281		

(In c\*d\*p: c: competition; d: disturbance; p: productivity.)

disturbance. Tailed microstome abundance differences between isogenic and competition groups are minimum at intermediate productivity level and bigger at low productivity level, while tailed microstome densities between competition groups at high productivity level differed greatest, indicating the interaction of competition and productivity on tailed microstome population. Disturbance treatments created more significant differences in isogenic groups at low productivity level while higher productivities offset the impacts of

disturbance; in groups with interspecific competitors, productivity influence effects of disturbance on tailed microstome just the opposite way, as between disturbance treatments there is no significant difference in tailed microstome abundance at low productivity level, however, tailed microstome population densities differed significantly between disturbance treatments at intermediate and high productivity levels. Both facts supported the significant disturbance and productivity interaction term in ANOVA test (Figure 2, Table 3,  $p < 0.05$ ).

Macrostome's densities can be significantly decreased by disturbance treatments or increased by higher productivity levels. The non-significantly negative effects of interspecific competition on macrostome population abundance become even more negligible at higher productivity level, suggesting the interaction between competition and productivity on macrostome population, which is consistent with the significant competition and productivity term in ANOVA test (Figure 2, Table 3,  $p < 0.05$ ).

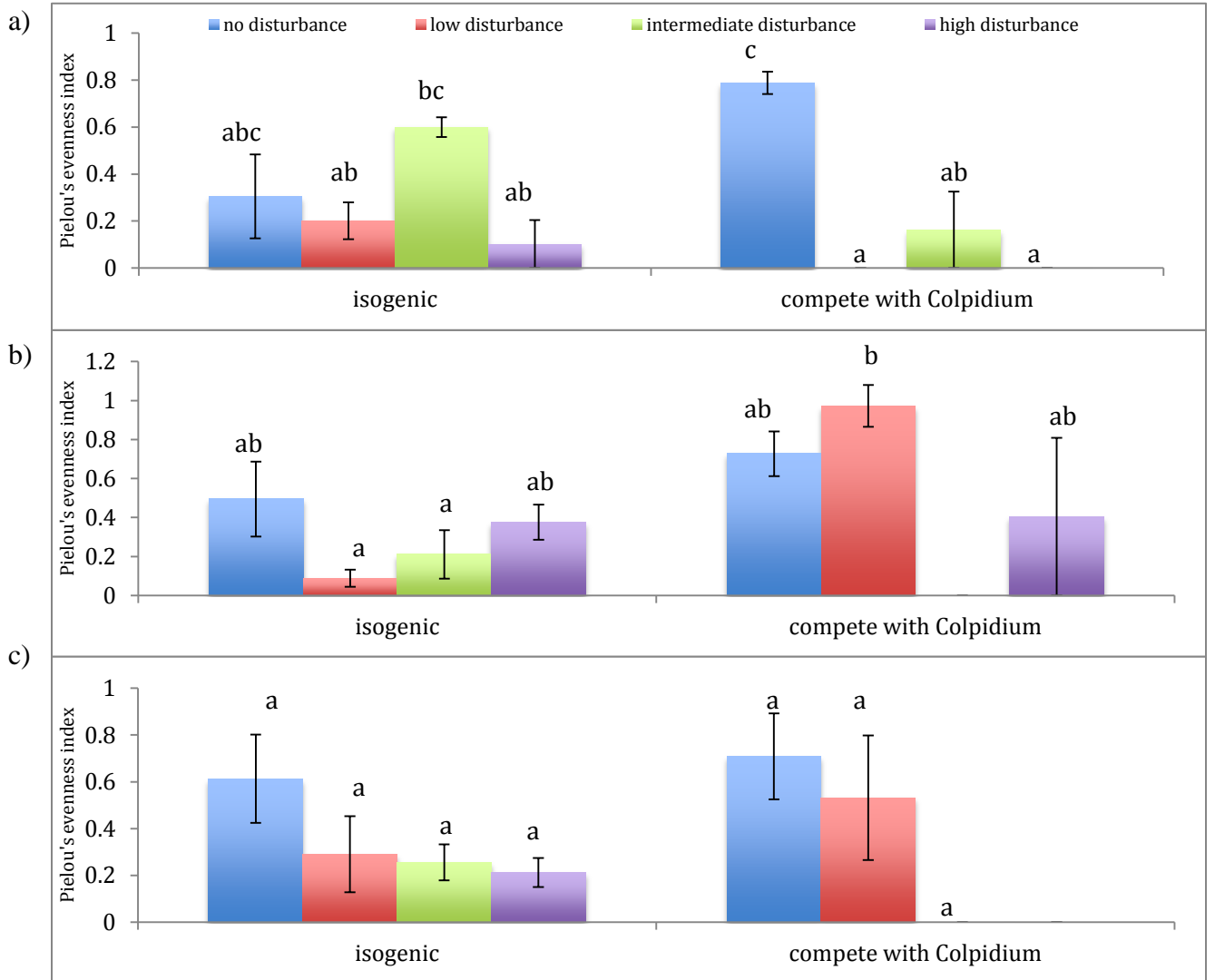
**Table 4.** Results of three-way ANOVA test summarizing effects of productivity, disturbance and competition on *T. vorax* morph's evenness from last sampling day.

	df	Mean Sq	F	P value
competition	1	0.121	1.999	0.165
disturbance	3	0.454	7.482	<0.001
productivity	2	0.129	2.125	0.133
competition*disturbance	3	0.203	3.349	0.029
competition*productivity	2	0.118	1.943	0.157
disturbance*productivity	6	0.120	1.983	0.092
competition*disturbance*productivity	5	0.147	2.430	0.052
Error	39	0.061		

As seen in Figure 3, Pielou's evenness index of *T. vorax* population differs between disturbance treatments, and the differences between disturbance treatments are significant in treatments with interspecific competition, unlike that in isogenic populations, revealing

that disturbance and interaction between competition and disturbance on *T. vorax* phenotypic dynamics are significant, which is consistent with ANOVA test results

(Figure 3, Three-way ANOVA, Table 4,  $p < 0.05$ ).



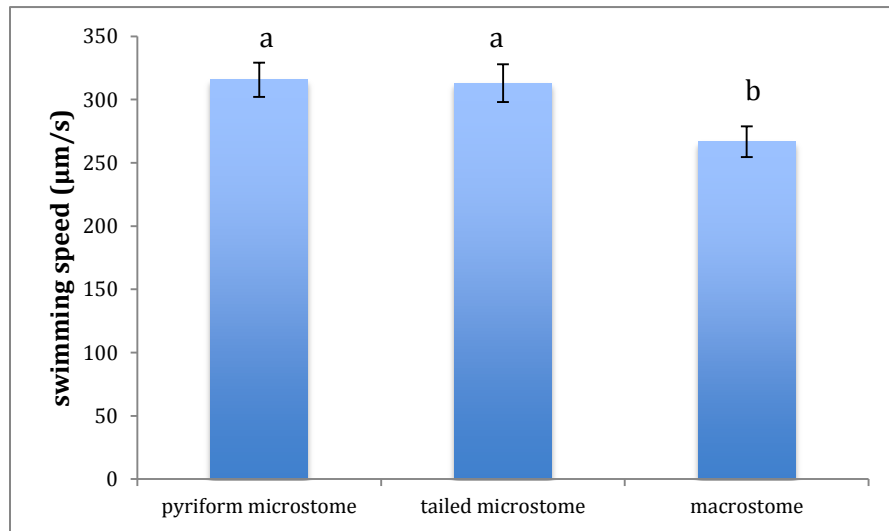
**Figure 3.** Mean final *T. vorax* population evenness (±SE) at (a) low (b) intermediate and (c) high productivity level. Post-Hoc Tukey HSD results are displayed as letters above columns, groups that do not share the same letters are significantly different. *T. vorax* went extinct in intermediate-productivity-intermediate-disturbance and high-productivity-high-disturbance treatments, therefore there was no valid evenness index in either group.



Repeated measures test results on daily morph dynamics of *T. vorax* population are mostly consistent with other statistical tests and will not be described here (Three-way MANOVA repeated measure test, Appendix A, Table 5).

### Swimming speed difference between three morphs of *T. vorax*

From the original sampling pool comprising of 80 free-swimming individuals of each morph, swimming speed ranged from 48.47–442.23  $\mu\text{m/s}$  in the pyriform microstome group, 78.67–458.83  $\mu\text{m/s}$  in the tailed microstome group, and 70.39–395.45  $\mu\text{m/s}$  in the macrostome group. Due to the substantial variations of swimming speed between active and passive individuals, 25% fastest swimming individuals were selected from each morph group for the final statistical test, which narrowed down the swimming speed ranges to 199.02–442.23  $\mu\text{m/s}$  in the pyriform microstome group, 251.38–458.83  $\mu\text{m/s}$  in the tailed microstome group, and 191.62–395.45  $\mu\text{m/s}$  in the macrostome group.



**Figure 4.** Mean swimming speed ( $\pm\text{SE}$ ) of 20 most active *T. vorax* individuals of three morphs. Tukey HSD test results are displayed as letters above columns, groups sharing the same letter are not significantly different from each other.

Results of this modified random sampling test reveal that pyriform microstome individuals have a mean swimming speed of  $286.63(\pm 13.07)$   $\mu\text{m/s}$ ; tailed microstomes have a mean swimming speed of  $288.74(\pm 12.87)$   $\mu\text{m/s}$ ; and *T. vorax* macrostomes' mean swimming speed was  $247.17(\pm 10.46)$   $\mu\text{m/s}$ . The One-way ANOVA and Tukey test results suggest swimming speed of each morph differ significantly, though swimming speed of pyriform microstome is not statistically different from that of tailed microstome, the difference between swimming speed of pyriform and tailed microstome is significant (One-way ANOVA,  $F=4.145$ ,  $p=0.21$ , *Figure 4*).

## CHAPTER 4

### DISCUSSION

Among different forms of polymorphisms, trophic polymorphism can be an especially complicated topic to study due to its direct impact on intraspecific niche variation, and far-reaching influences on population dynamics when cannibalism is present (Svanbäck & Persson 2004, Banerji & Morin 2009). Traditional studies on polymorphisms have focused on transformation processes at the species level, or differences and trade-offs between morphs (Buhse 1966, Smith 1993, Zera & Harshman 2001, Maney 2005). During recent years, more researchers began to look into the role of polymorphism in population and community ecology (Werner & Peacor 2003, Bolnick *et al.* 2011, Violle *et al.* 2012), and how environmental factors such as productivity (Kassen *et al.* 2000, Werner & Peacor 2006), disturbance (Agrawal & Spiller 2004), and competition (Bourke *et al.* 1999, Svanbäck *et al.* 2008, Bolnick *et al.* 2010) may affect its mechanisms and dynamics, but there were rare prior works studied effects of different factors simultaneously. In this study, I explored the effects of competition, disturbance and productivity on population and morph dynamics in the polymorphic freshwater ciliated protozoan *Tetrahymena Vorax*, and found that competition and disturbance can decrease its population abundance while higher productivities can reduce effect size from competition and disturbance.

#### **Effects of competition on *T. vorax***

The presence of *Colpidium* had a significant effect on the overall *T. vorax* population abundance. A former study suggested that *Colpidium* should increase the size

of *T. vorax* population at low productivity level but decrease it at higher productivity levels (Banerji & Morin 2009), which was consistent with the results in undisturbed treatments from this study. However, the abundance differences caused by interspecific competition were found to be not significant, which could be explained by the equilibrium that *T. vorax* population had reached after long-term no-disturbance treatments, comparing to the relatively short-period observations in the former study (3 weeks vs. 1 week). However, the presence of *Colpidium* can significantly decrease *T. vorax* population abundance in disturbance treatments (Appendix C). The fact that *Colpidium* decreased population abundances of *T. vorax* exclusively in disturbed treatments may be caused by inefficiency of energy transfer between trophic levels (Banerji & Morin 2009). Macrostome turned out to be the only phenotypic morph not affected by competition, while the two bacterivorous morphs (*i.e.* pyriform and tailed microstomes) were both affected by competition significantly. Compared to feeding directly upon the bacteria prey, consumption of *Colpidium* or interspecific microstomes provided carnivorous macrostome with less available energy. In the absence of predation, *Colpidium* was proved to be a superior competitor to *T. vorax* microstomes in exploitative competition (Price & Morin 2004). Therefore, *T. vorax*'s overall efficiency of energy transfer at population level can be significantly reduced by the presence of *Colpidium*. Hence *Colpidium* can consequently decrease the population sizes of *T. vorax* in treatments where disturbances were imposed. The more significantly negative effects of *Colpidium* on *T. vorax* population at the high productivity level can be explained by the same "inefficient energy transfer" theory as well, along with a plausible assumption that *Colpidium* might outgrow its average size when treated with high productivities

compared to that at lower productivity levels, so to become inedible to macrostomes, which brings lower energy return rates for macrostomes and the entire *T. vorax* population (Banerji & Morin 2009).

### **Effects of disturbance on *T. vorax***

Disturbances in the natural environment such as flood and hurricane can bring out unpredicted consequences by interrupting existed dynamics of organisms, breaching original community structures and sometimes destroying an entire ecosystem (Resh *et al.* 1988). There was no former study focused specifically on how disturbance would affect population dynamics of *T. vorax*. Similar works had been done before induced disturbance into experimental designs only to find out how effects of other factors such as community assembly (Jiang & Patel 2008) or competition (Violle *et al.* 2010) on community dynamics would be impacted by disturbance. However, among the three tested factors in this study, disturbance had significant effects on not only the overall population size of *T. vorax*, but also the abundance of both macrostome and tailed microstome populations. What was interesting was that disturbance turned out to have no significant effect on overall population size of *T. vorax* in the absence of interspecific competitors, which suggested the ability of *T. vorax* to sustain its isogenic population regardless of different frequencies of disturbance impositions. Disturbances also failed to cause any significant difference in overall population abundance of *T. vorax* at low productivity level in the presence of *Colpidium*. As a matter of fact, *T. vorax* managed to exclude *Colpidium* at the low productivity level when the highest frequency levels of disturbance (every two days) were imposed, which was supposed a result of collaborations between microstome and macrostomes in *T. vorax* population. On the

other hand, the pattern was reversed in intermediate disturbance treatments at intermediate productivity level and high disturbance treatments at high productivity level, as *T. vorax* became the victim that was exterminated (Appendix B).

The interaction between disturbance and productivity in the presence of *Colpidium* varied between treatments with different combinations of both factors. High disturbance treatments actually increased abundance of each morph in *T. vorax* populations compared to that in intermediate disturbance treatments at the intermediate productivity level, and maximized overall competitiveness of *T. vorax* at the low productivity level, where *Colpidium* was driven to extinction. So why did high disturbances annihilate *T. vorax* with high productivities? One explanation could be that, as mentioned above, it is likely that *Colpidium* with higher productivities will become inedible to *T. vorax* macrostomes, therefore different productivities may alternate the foraging preference or life strategy in macrostomes. If macrostomes cannot consume interspecific prey *Colpidium* at the high productivity level, the two possible outcomes should be that either macrostomes feed exclusively on conspecific microstomes, which should jeopardize the overall population abundances of *T. vorax*, or macrostomes should transform reversely back to microstomes, which are inferior competitors to *Colpidium* in exploitative competition on bacteria preys. Both situations could lead to the extinction of *T. vorax* population. The same extinction situation in intermediate disturbance treatments at the intermediate productivity level could also be explained by complicated combinations of all factors with a similar pattern, which remained to be uncovered through more detailed follow-up studies by successor researchers.

### **Effects of productivity on *T. vorax***

Although productivity did not have a significant effect on long-term overall population size or phenotypic composition within *T. vorax* populations, the focal phenotype macrostomes turned out to be significantly more abundant with higher productivities. Macrostome is known to be “high-maintenance” morph compared to microstome morphs because of the change of trophic level makes energy transfer processes less efficient. However, the expensive, sometimes risky trade-offs should be evolutionary meaningful: direct consumptions of potential competitors can be the optimal competition strategy, and keeping intraspecific competition under control is also in favor of the survival of the entire population. Meanwhile, the relatively rapid reversibility between morphs in protozoan species also ensured timely adjustments of phenotype compositions in *T. vorax* population whenever necessary (Kidder 1941). All of these made macrostome an exceptionally important morph in *T. vorax* polymorphisms. Susceptibility to low productivities in macrostomes therefore makes productivity a main factor cannot be ignored in *T. vorax* studies.

Given that the high productivity level is 50 times higher than low productivity level, I was surprised to see different productivity treatments only had caused so little differences in abundance and structure of *T. vorax* population. *T. vorax* was only found in a natural freshwater pond once and later found out to be not a superior competitor comparing to many other common ciliated protozoans (Banerji & Morin 2009), my study proved that its insufficient ability in resource utilization and energy transfer can be a possible reason.

### **Between phenotypic morphs of *T. vorax***

The dominance of tailed microstomes in *T. vorax* population over the two other morphs in every treatment could easily lead to a plausible assumption that tailed microstomes possess higher fitness than the two other morphs, especially the similarly sized pyriform microstome. Due to the visible difference in shape, some might propose that the elongated tail in a tailed microstome should provide them with extra thrust and therefore obtain a higher swimming speed compared to untransformed pyriform microstomes. There are conspicuous advantages to having a faster swimming speed: more rapid access to common resources is important in exploitative competition situations with interspecific and intraspecific competitors; quicker avoidance from a generalist predator indirectly increases relative mortality in other competitor populations, therefore helping the faster swimmer to survive apparent competition (Lang & Benbow 2013). In my study, however, this hypothesis was not supported, as statistical test results stated clearly that there was no significant difference in fastest swimming speed between pyriform and tailed microstomes, meaning tailed microstomes may not be able to evade a cannibalistic macrostome better than pyriform microstomes do. Hours of observation inspired me to come up with a new, untested hypothesis: since macrostomes appeared to be mostly passive filter feeders when no interspecific prey was present, their success or failure in taking in a microstome was more related to its shape rather than its swimming speed; it might have been the elongated length and “slippery” tail in a tailed microstome that made it more difficult to be captured and more likely to escape even after being engulfed compared to a pyriform microstome. Former studies, however, did detect faster swimming speeds and more successful turns in macrostomes when treated with stimulation from



filtrate of interspecific competitors, and these significant improvements in a macrostome's swimming ability would greatly enhance its prey capturing behavior, and therefore may affect predator–prey interactions and eventually population abundances and community structure (Grønlén et al. 2010, 2011, 2013).

In this study, it was also interesting to find out how each morph react to different impacting factors differently. Population abundance of macrostomes remained unaffected by interspecific competition, but somehow turned out to be significantly impacted by productivity levels, which had no effect on population abundance of microstome phenotypes. The “good-for-nothing” pyriform microstome turned out to be the only morph not susceptible to disturbance, which keeps its population unharmed and stable under conditions that make tailed microstome and macrostome populations fluctuate (Table 3). It appears that the tradeoffs between *T. vorax*'s morphs might not be in the form of apparent characters like swimming ability, but in the form of more subtle properties such as different responses towards different environmental changes. Therein lies the advantage of a polymorphic species in interspecific competition: diversity of phenotypes means higher niche breadth at population level, which can sometimes convert into higher fitness of species under certain conditions (Svanbäck & Persson 2004).

### ***Conclusion***

Results of my study on the focal species *T. vorax* revealed how competition, disturbance and productivity impact its overall population abundances as well as its phenotypic dynamics respectively. Of the three phenotypic morphs, pyriform microstome is mainly influenced by the presence of interspecific competitors, tailed microstome can

be significantly inhibited by both disturbance and interspecific competition, while macrostome turns out to be most susceptible to low productivity and high disturbances. Different outcomes in different experimental treatments suggest inducible trophic polymorphism in *T. vorax* to be effective in sustaining its populations in laboratory conditions. However, the extremely rare existence of *T. vorax* in nature can suggest the insufficiency of inducible trophic polymorphism as a living strategy to keep the species from extinction. My investigation of mechanisms and consequences of inducible trophic polymorphism in *T. vorax* may shed light on studies of how population dynamics, community structures and evolutionary processes of some species can be affected by unusual phenomenon such as individual specializations, niche partitioning, intraguild predation and/or even cannibalism in aspects of variable settings, and how these consequences can be considered in conservation of species with low viability in the wild.

## APPENDIX A

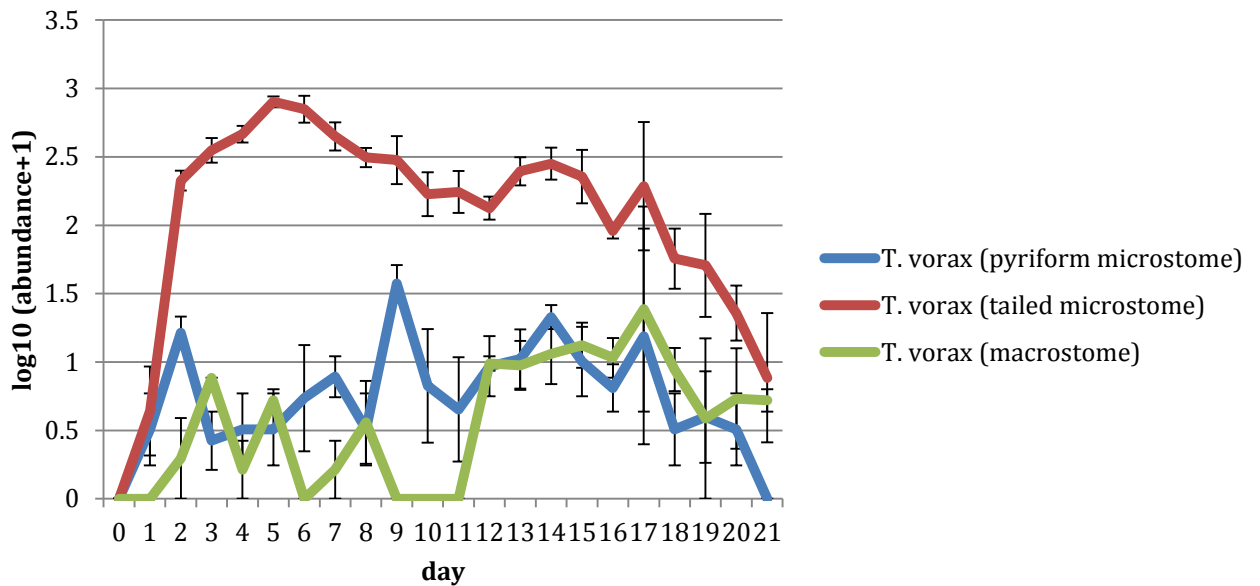
Table 5. Results of three-way MANOVA repeated measures test summarizing effects of productivity, disturbance and competition on daily abundances of *T. vorax*'s morph.

	Wilks' Lambda	F	Hypothesis df	Error df	P value
productivity	0.167	22.239	6.000	92.000	<0.001
competition	0.274	40.728	3.000	46.000	<0.001
disturbance	0.520	3.845	9.000	112.103	<0.001
productivity*competition	0.317	11.916	6.000	92.000	<0.001
productivity*disturbance	0.382	2.941	18.000	130.593	<0.001
competition*disturbance	0.543	3.556	9.000	112.103	0.001
productivity *disturbance*competition	0.365	3.102	18.000	130.593	<0.001

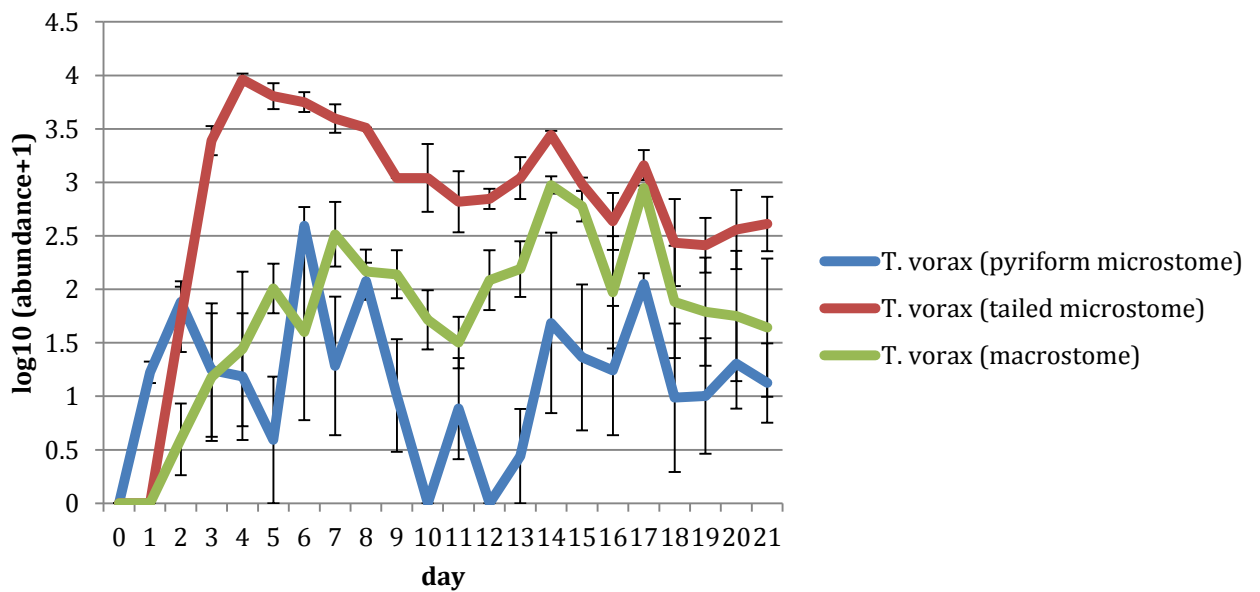
## APPENDIX B

### POPULATION DYNAMICS OF *T. VORAX* & *COLPIDIUM* $\pm$ STANDARD ERROR

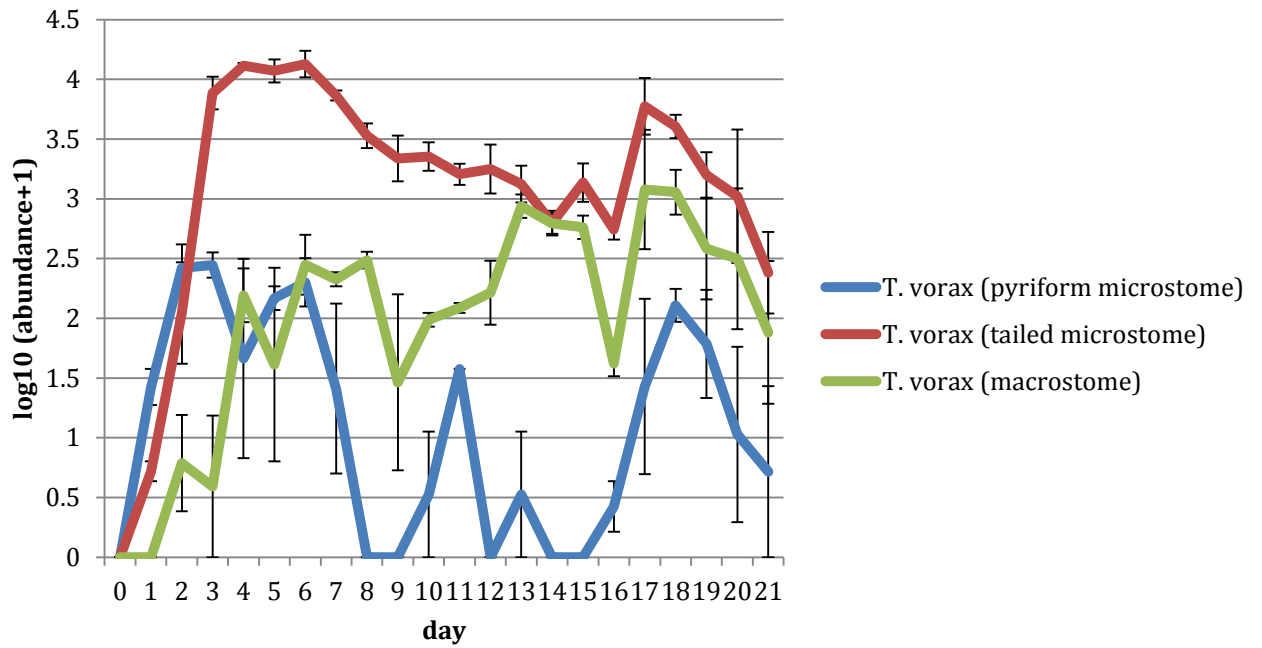
#### 1. low productivity, isogenic, no disturbance



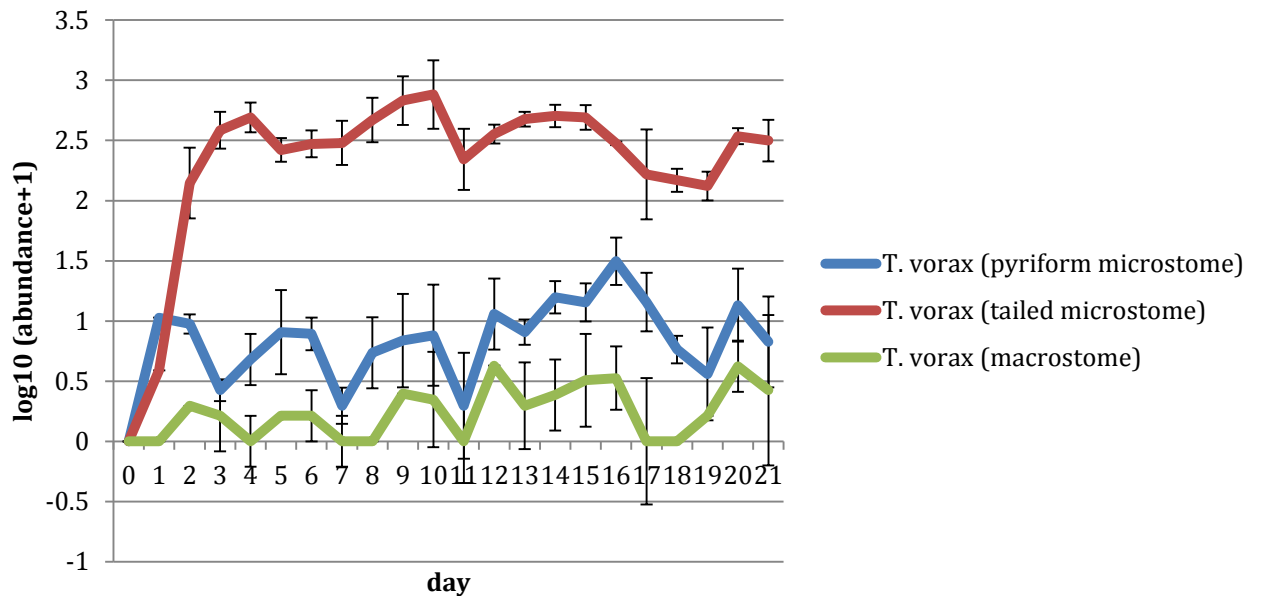
#### 2. intermediate productivity, isogenic, no disturbance



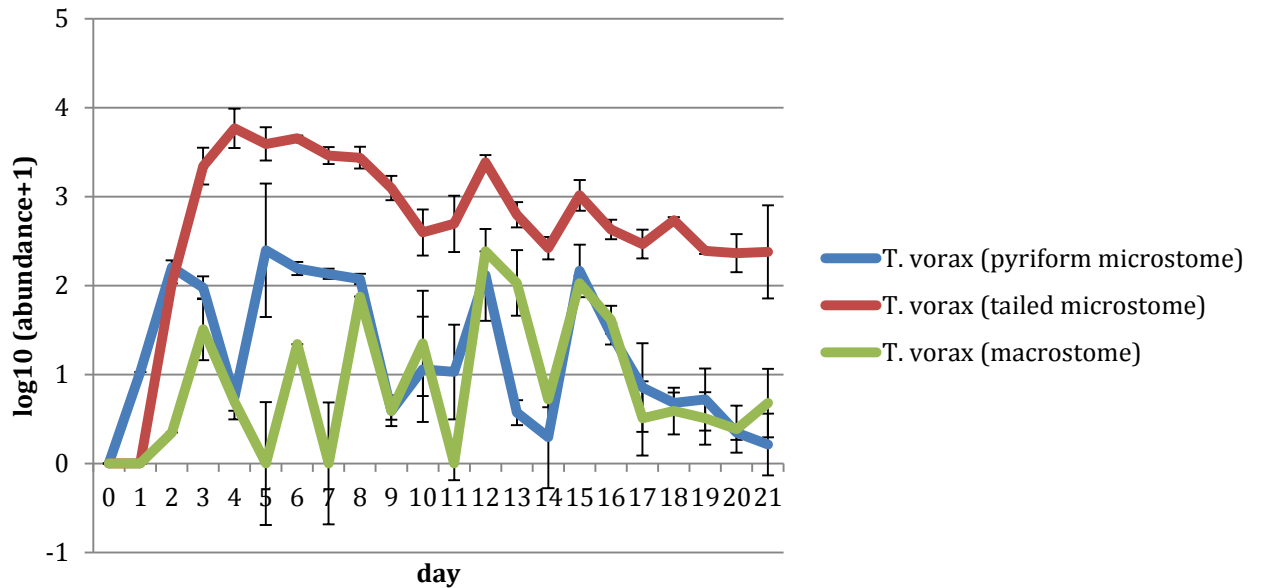
### 3. high productivity, isogenic, no disturbance



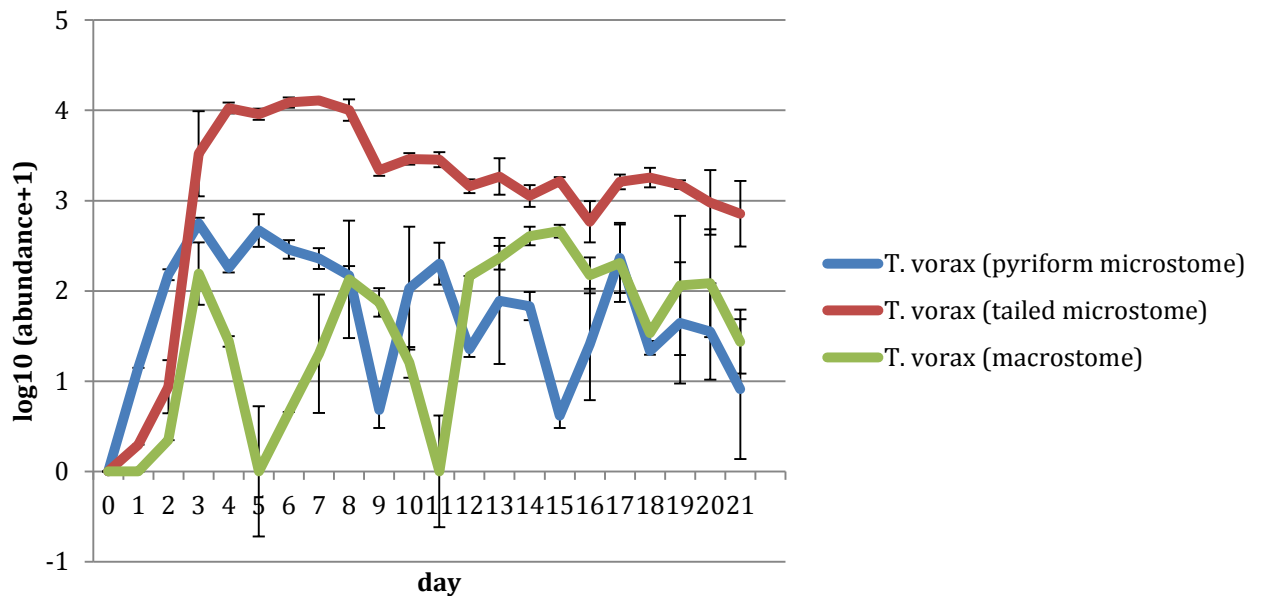
### 4. low productivity, isogenic, low disturbance



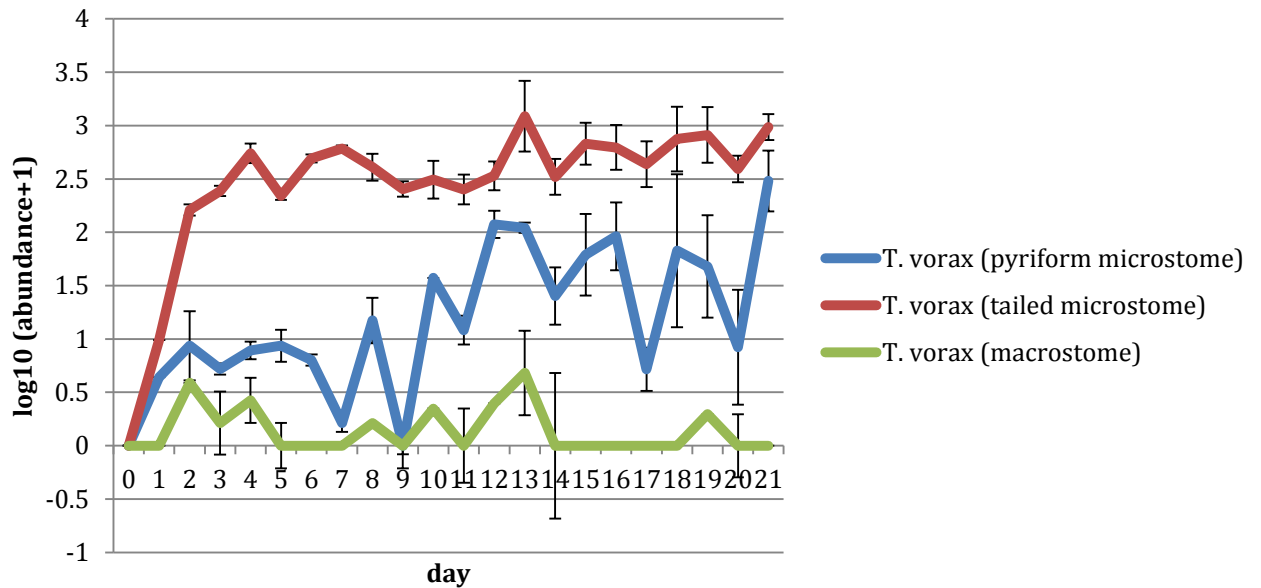
## 5. intermediate productivity, isogenic, low disturbance



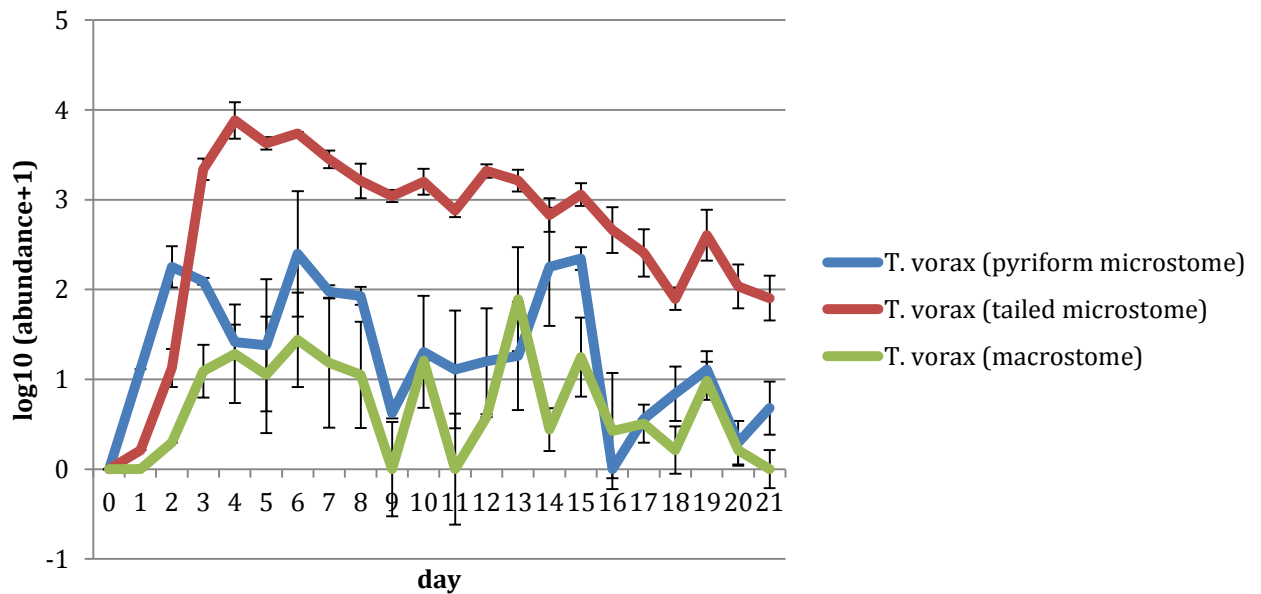
## 6. high productivity, isogenic, low disturbance



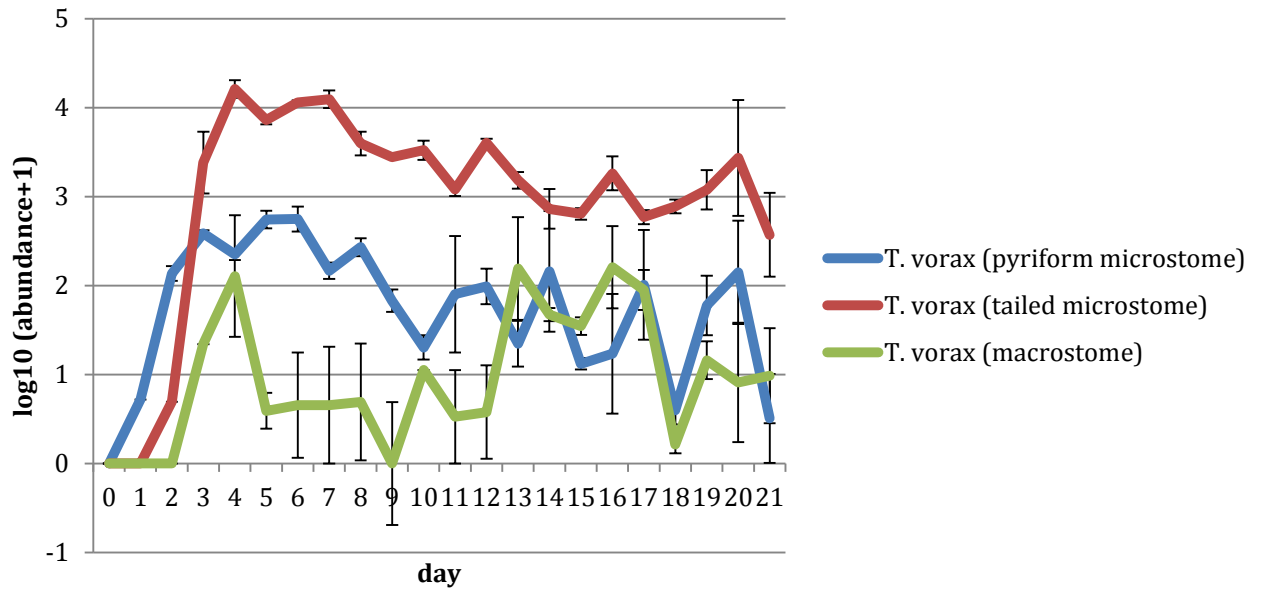
## 7. low productivity, isogenic, intermediate disturbance



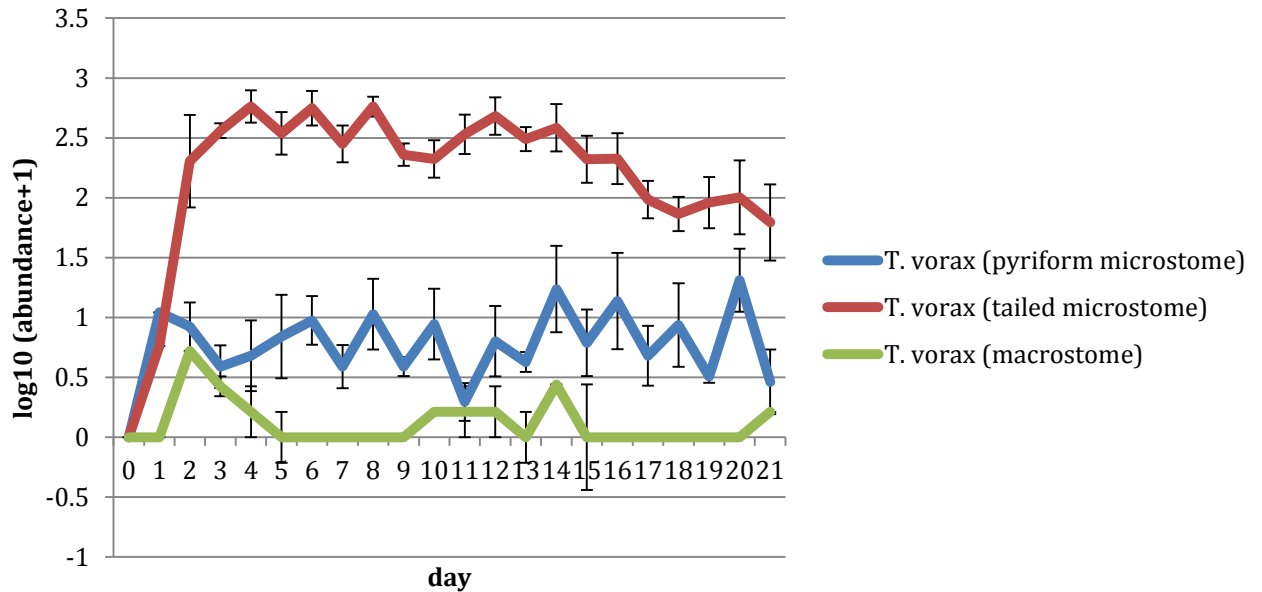
## 8. intermediate productivity, isogenic, intermediate disturbance



## 9. high productivity, isogenic, intermediate disturbance

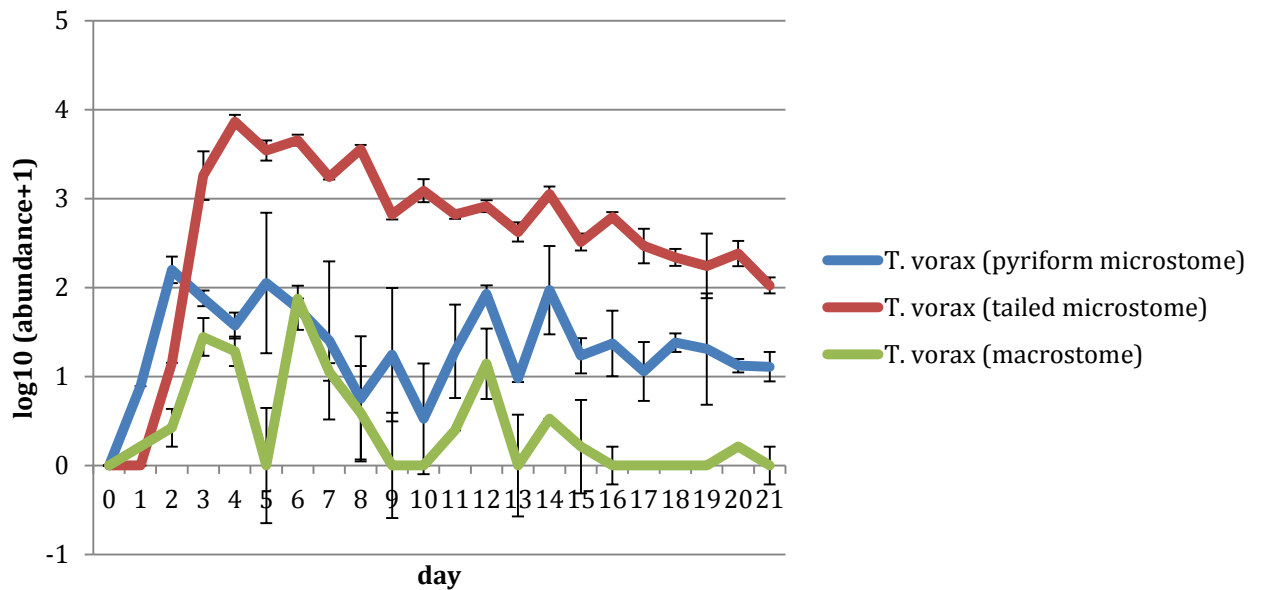


## 10. low productivity, isogenic, high disturbance

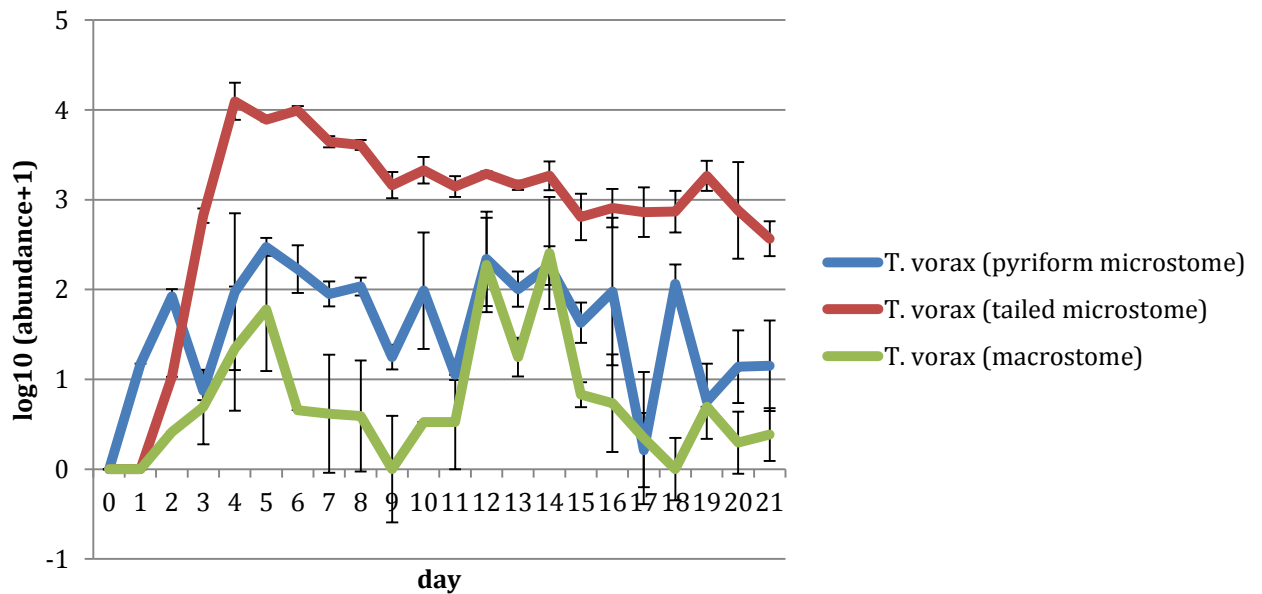




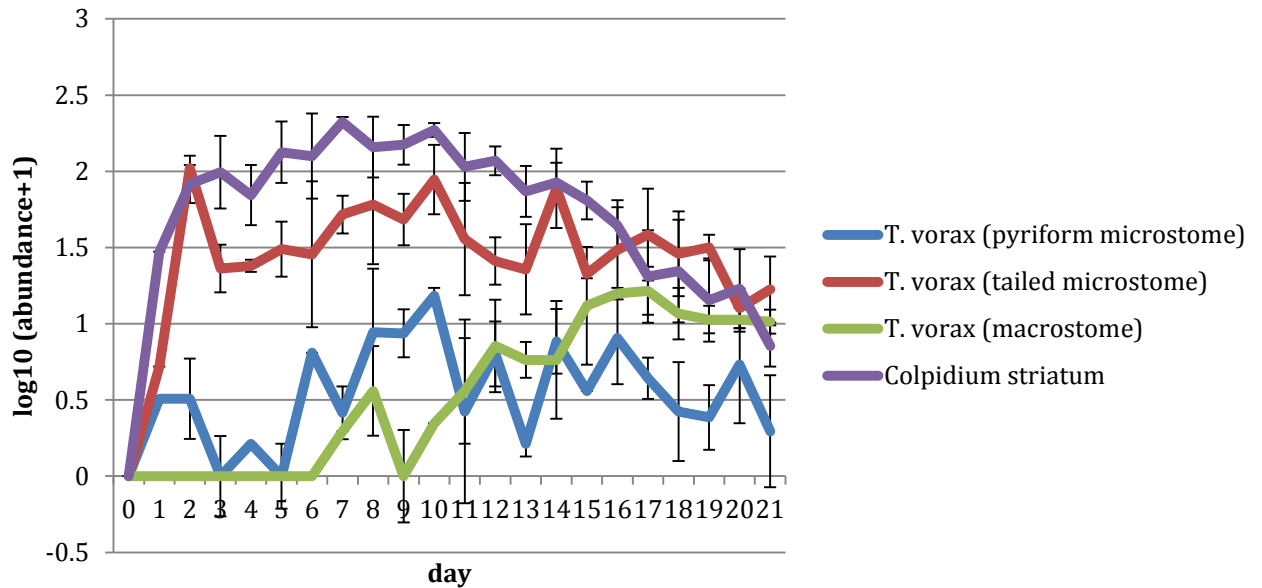
## 11. intermediate productivity, isogenic, high disturbance



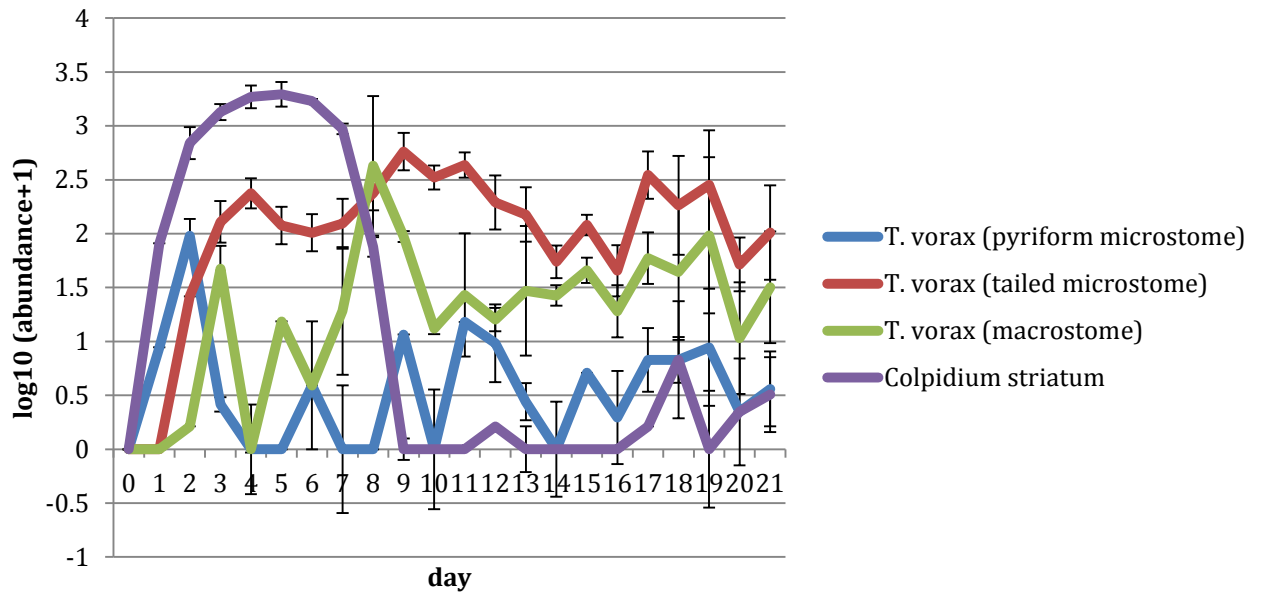
## 12. high productivity, isogenic, high disturbance



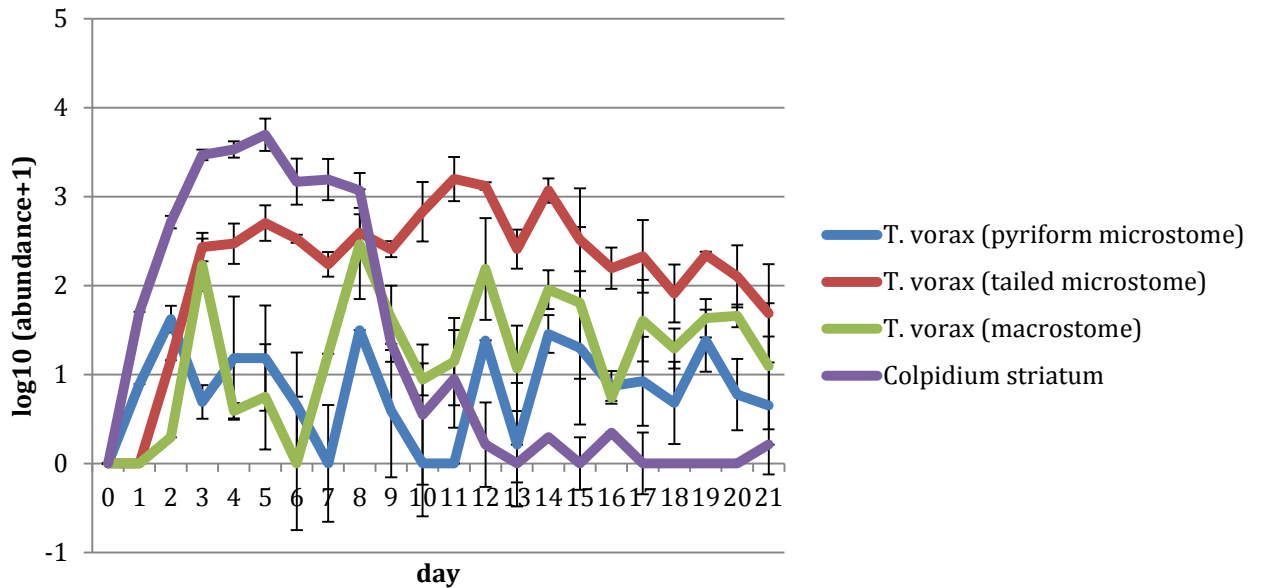
### 13. low productivity, compete with Colpidium, no disturbance



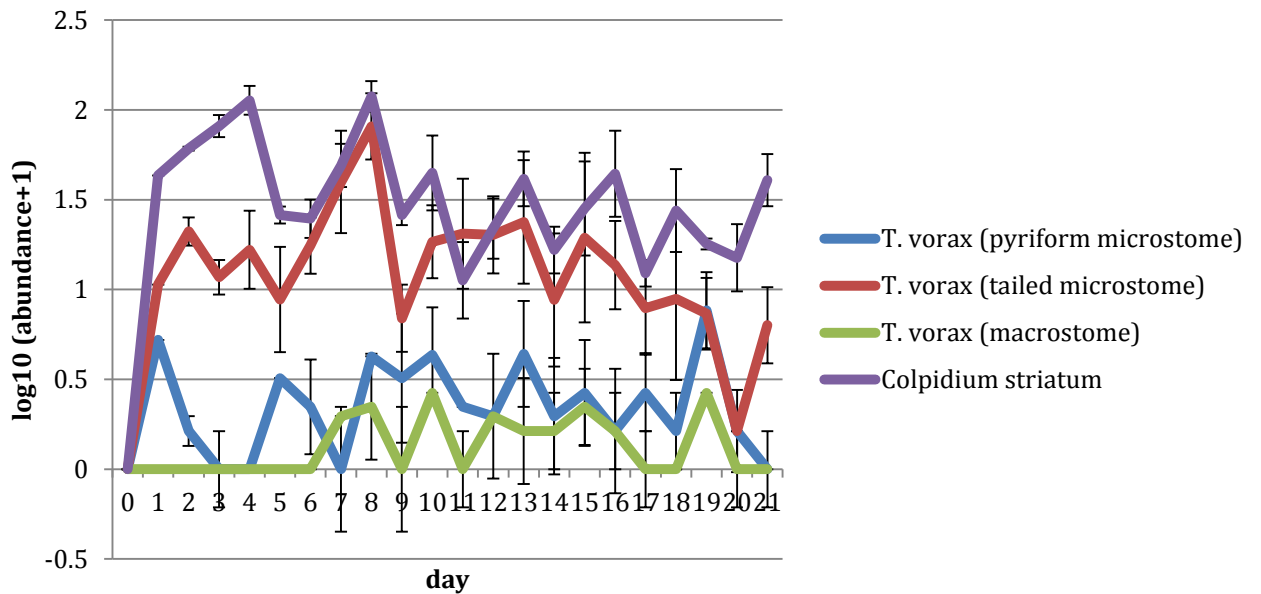
### 14. intermediate productivity, compete with Colpidium, no disturbance



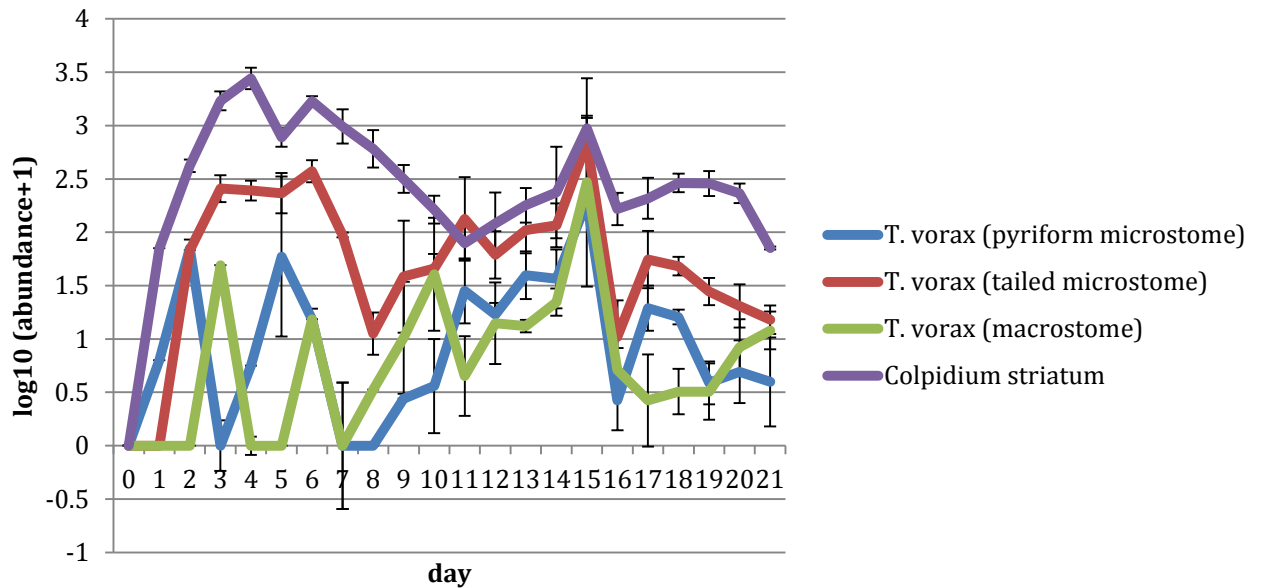
### 15. high productivity, compete with Colpidium, no disturbance



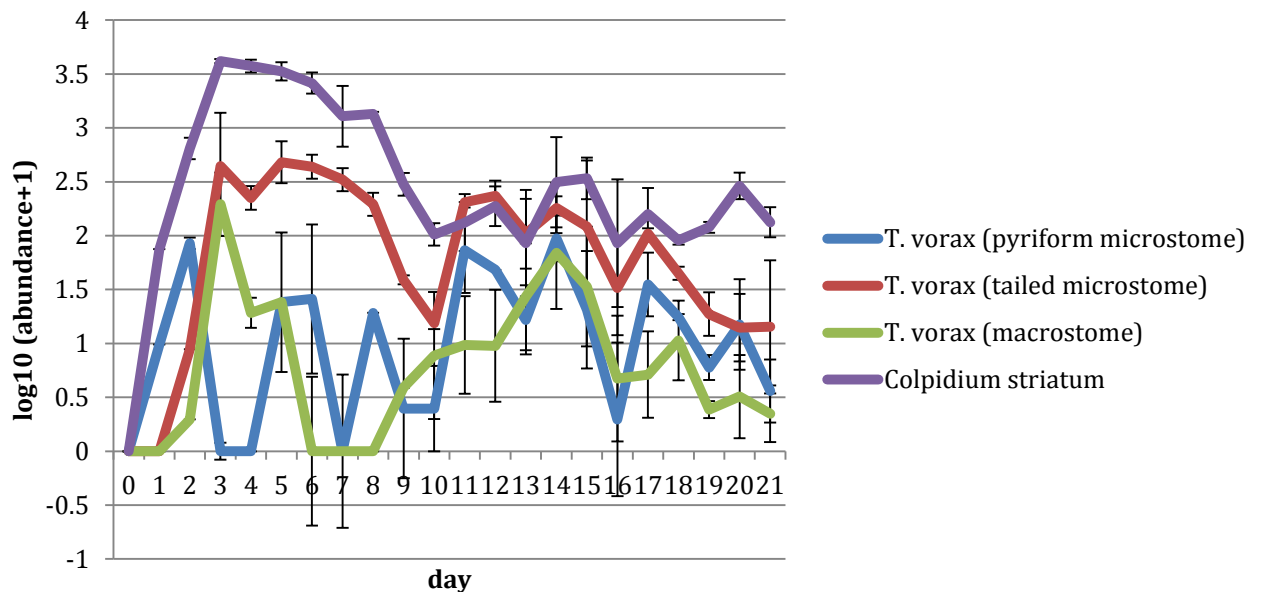
### 16. low productivity, compete with Colpidium, low disturbance



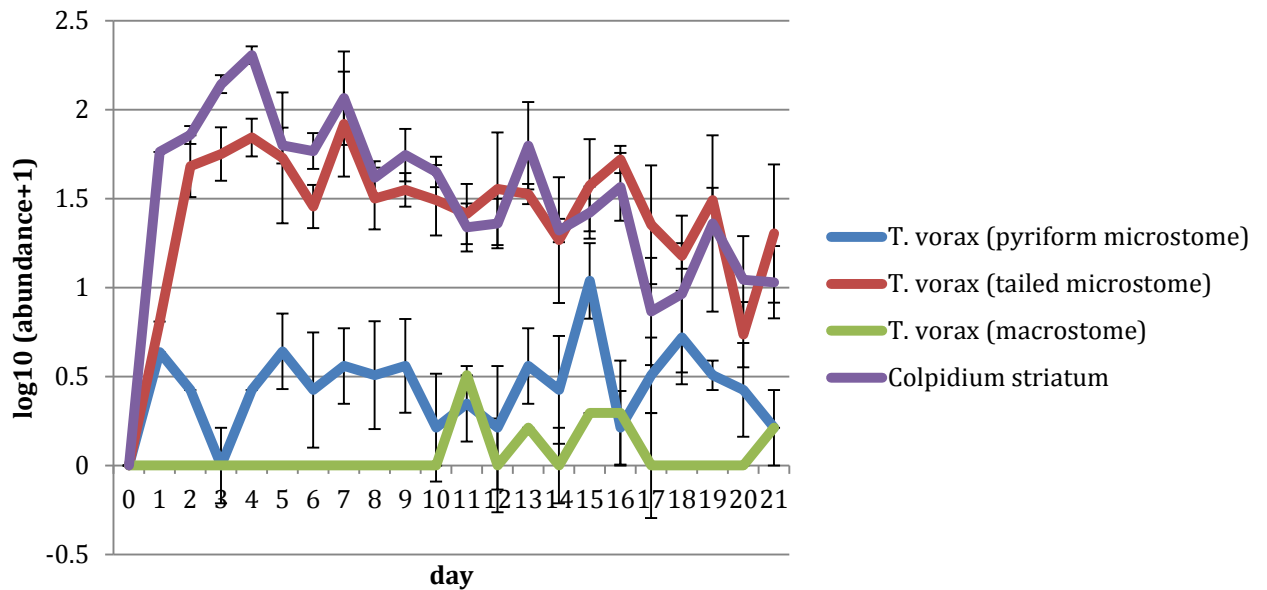
### 17. intermediate productivity, compete with Colpidium, low disturbance



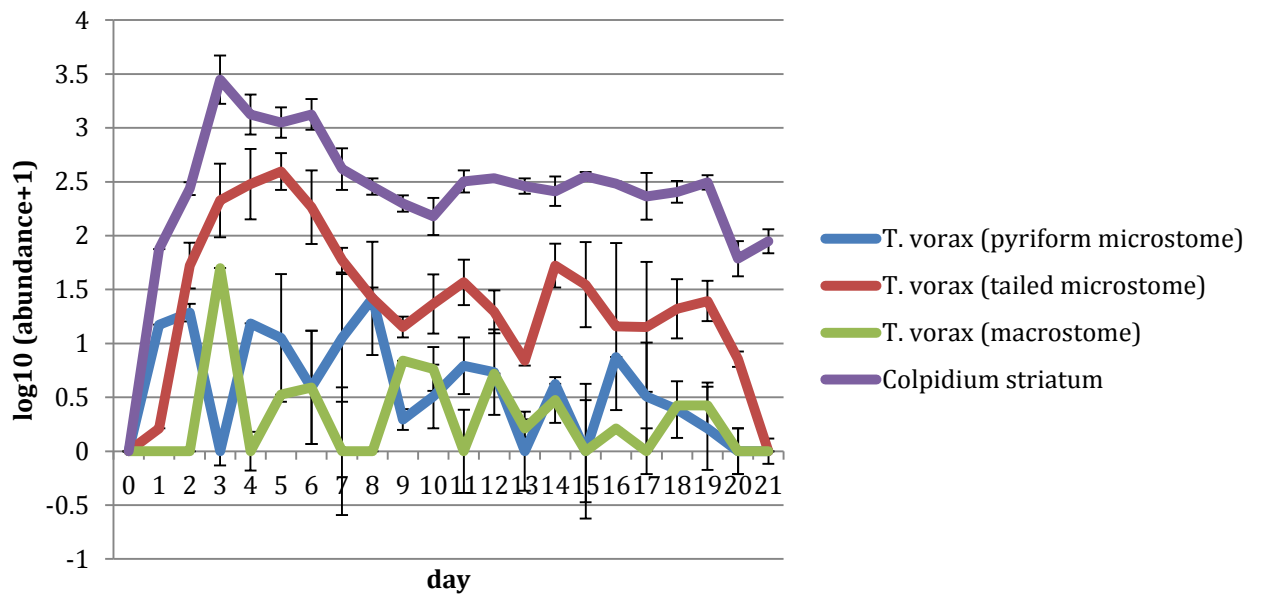
### 18. high productivity, compete with Colpidium, low disturbance



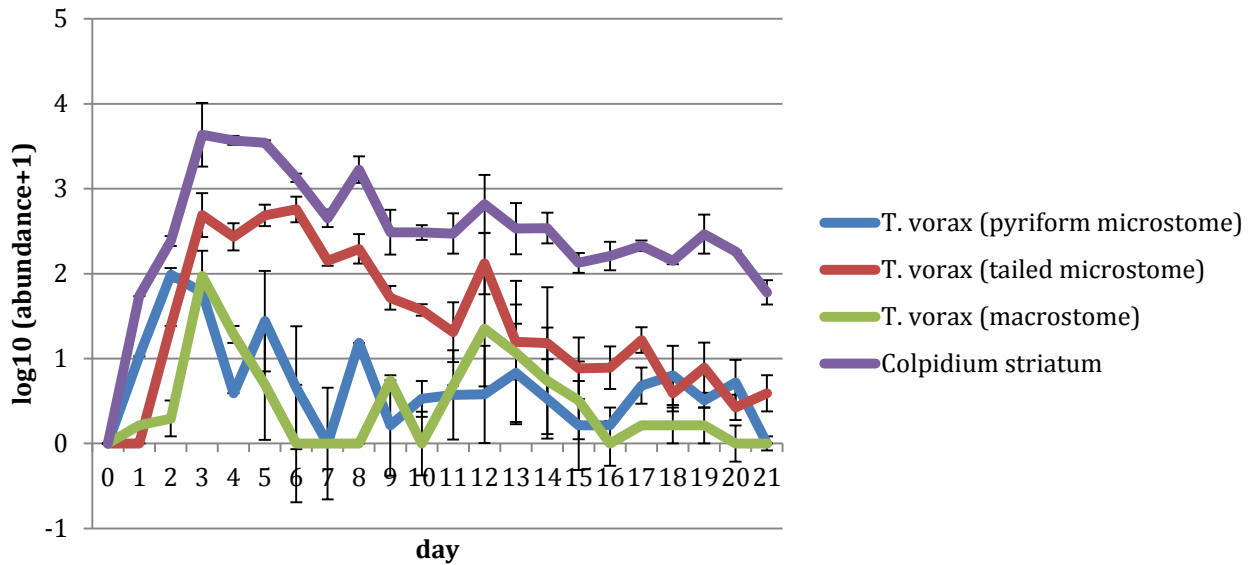
## 19. low productivity, compete with Colpidium, intermediate disturbance



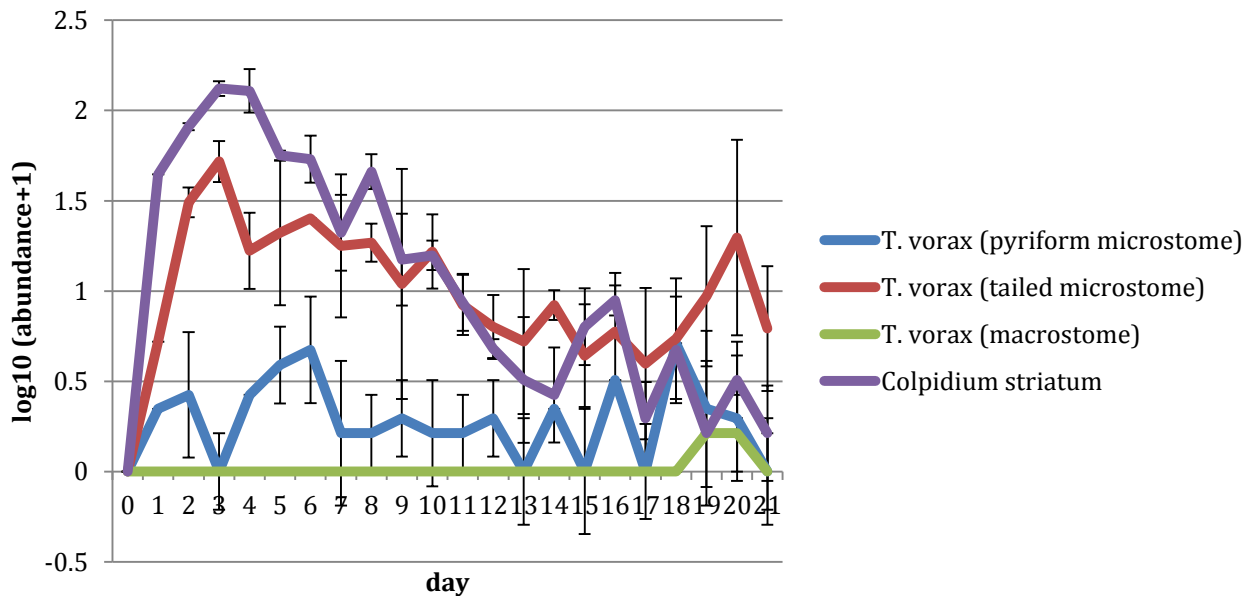
## 20. intermediate productivity, compete with Colpidium, intermediate disturbance



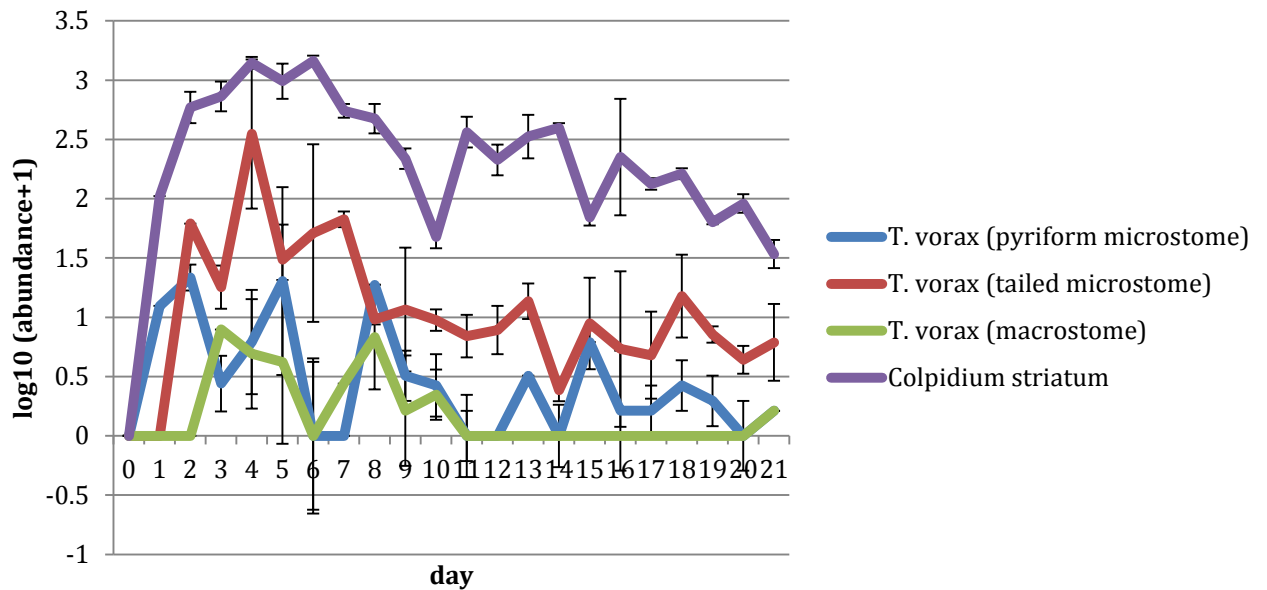
## 21. high productivity, compete with Colpidium, intermediate disturbance



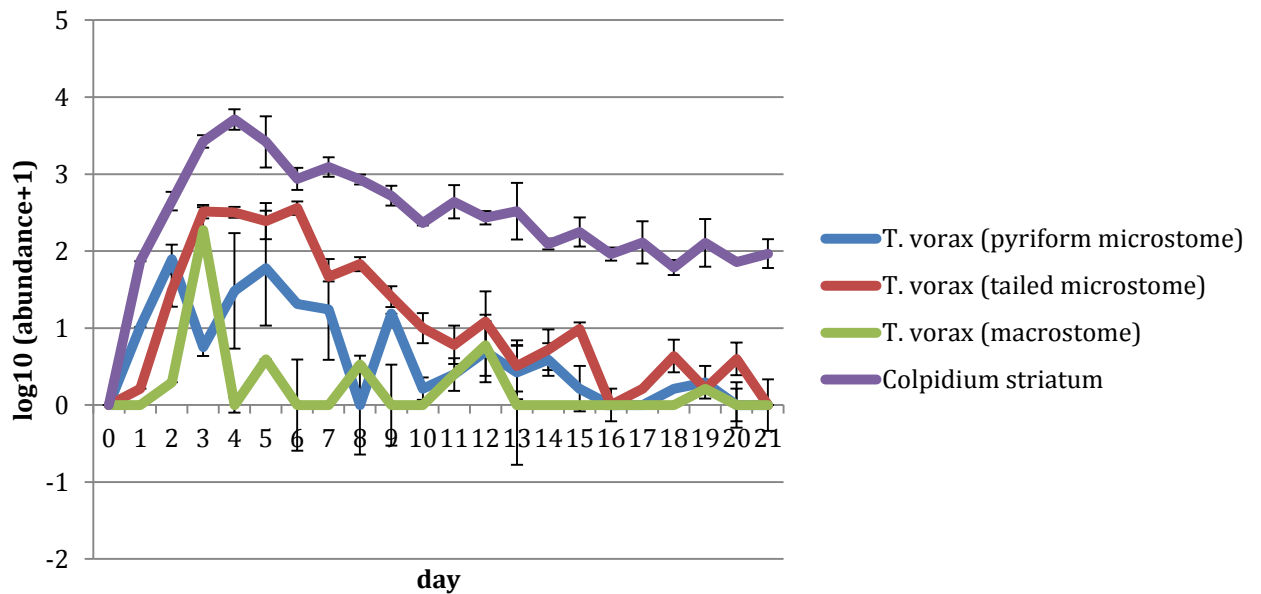
## 22. low productivity, compete with Colpidium, high disturbance



### 23. intermediate productivity, compete with Colpidium, high disturbance

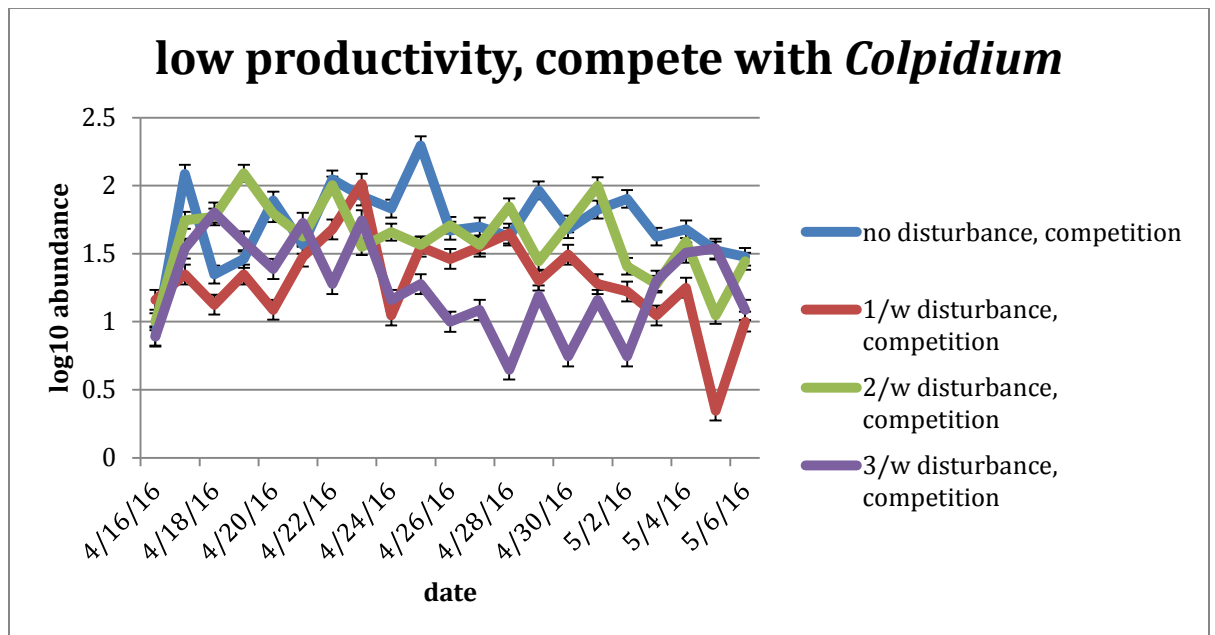
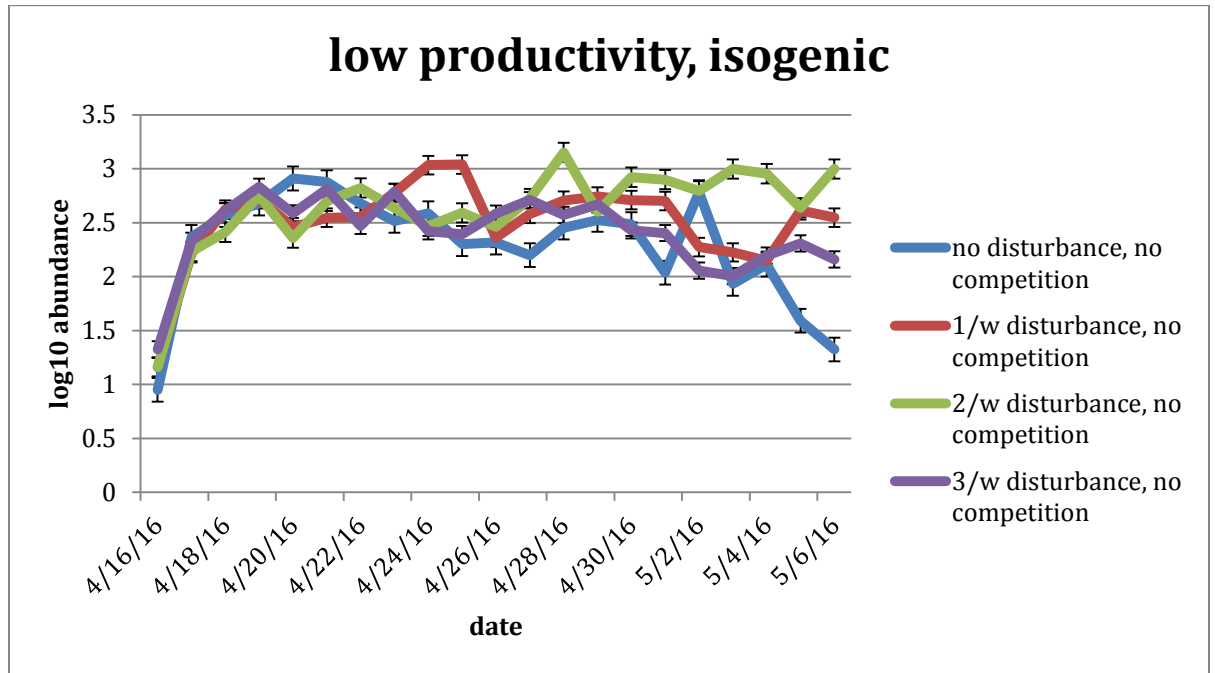


### 24. high productivity, compete with Colpidium, high disturbance



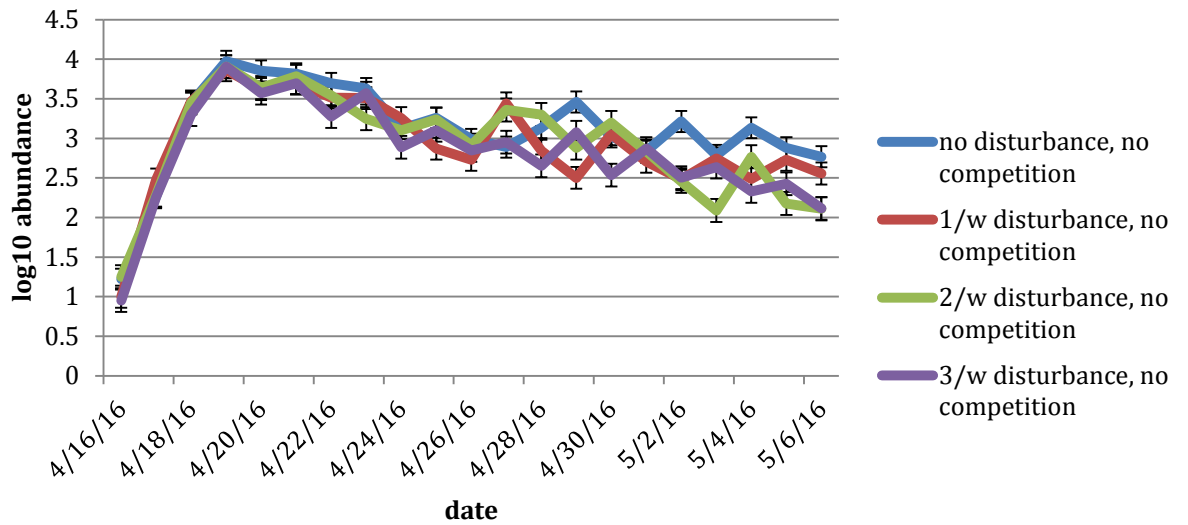
## APPENDIX C

### POPULATION DYNAMICS OF *T. VORAX* & *COLPIDIUM* $\pm$ STANDARD ERROR

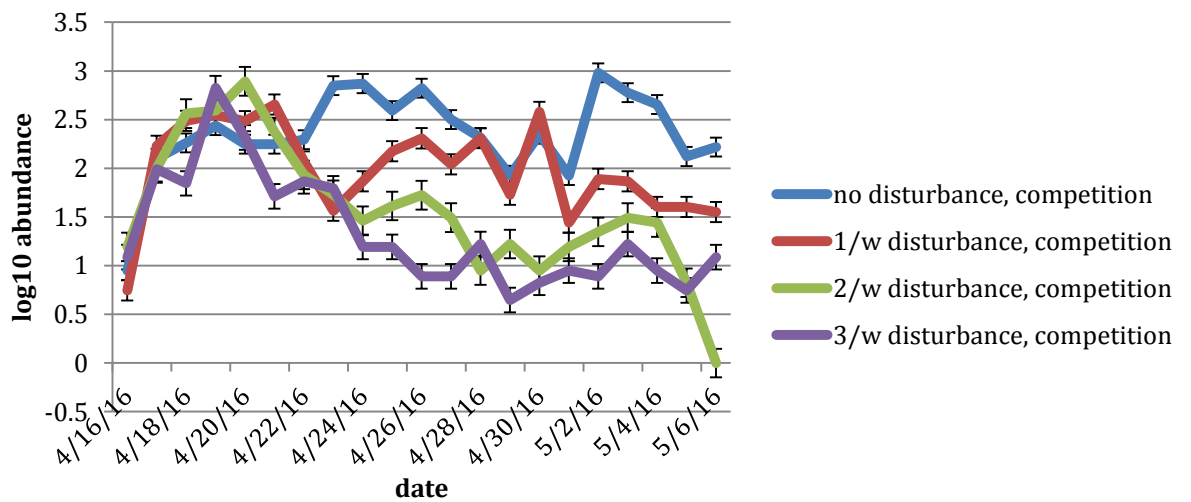




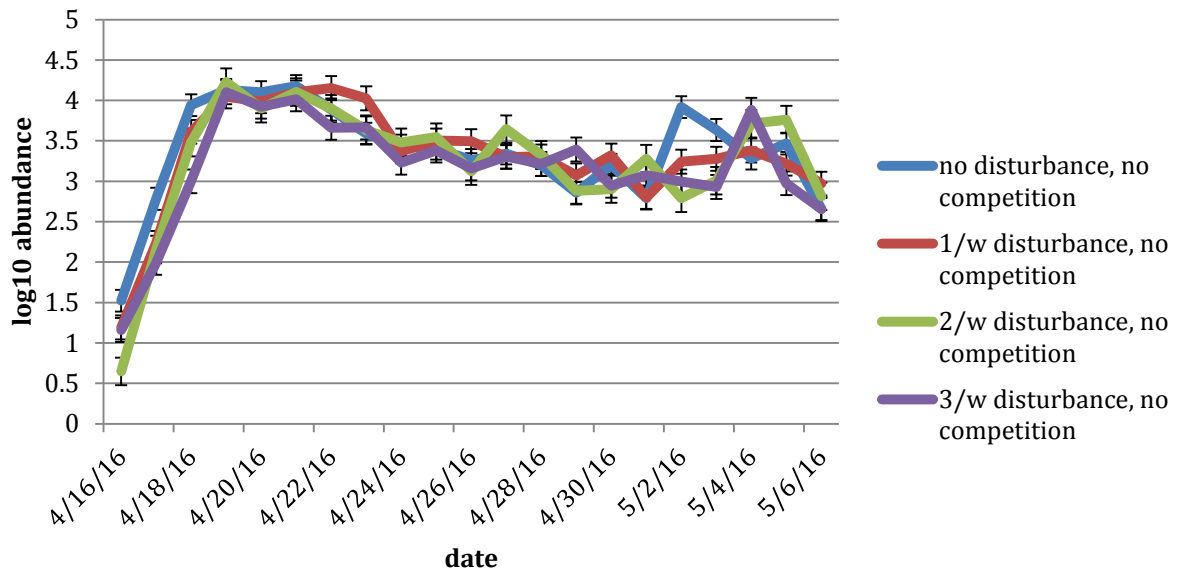
### medium productivity, isogenic



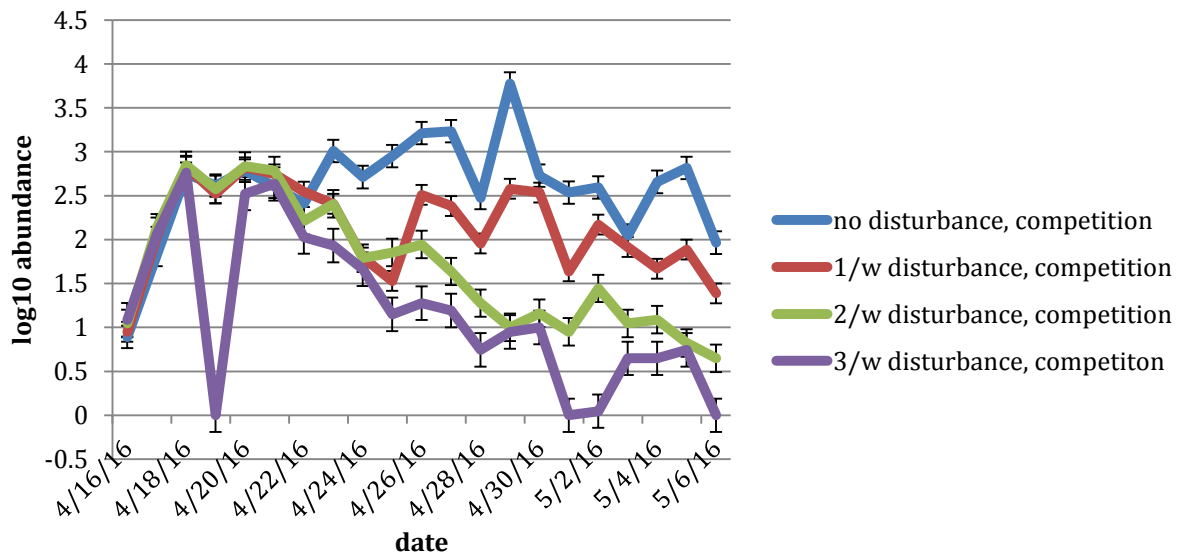
### medium productivity, compete with *Colpidium*



## high productivity, isogenic



## high productivity, compete with *Colpidium*



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