Mechanisms of Invertebrate Dispersal of Purple Pitcher Plant (*Sarracenia purpurea*) Inquilines

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Abstract

The inquiline invertebrate metacommunity living in the leaves of the purple pitcher plant (Sarracenia purpurea) consist of individual aquatic populations living separate from one another, but connected through dispersal. The dispersal of invertebrates between the pitchers is necessary for the colonization of new pitcher environments. I hypothesized that the protozoan and rotifers of these communities disperse between pitchers passively through the wind and rain rather than using pitcher-frequenting animals as vectors. Water and detritus were added to forty cleaned out pitchers (20 real, 20 artificial). Half of the pitchers were covered with a fine mesh restricting animal access to the pitchers and half were left open. This study tracked the richness, abundance, and diversity of the organisms that appeared in the pitchers over a two week period. There was no difference between the richness ($p=0.61$), abundance ($p=0.30$), or diversity ($p=0.51$) between the covered and open pitcher communities suggesting that the invertebrates disperse aerially and that animals play no important role as dispersing vectors. There was lower richness ($p<0.01$), abundance ($p<0.01$), and diversity ($p<0.01$) in the artificial pitchers than the real because there was no oxygenation of the water in the artificial tubes, as in the pitchers. It was concluded that the abiotic, passive means of dispersal that these organisms practice stabilizes the otherwise fragile inquiline community living in the pitchers.

Introduction

Community composition and assemblage are key to the study of community ecology. It is often assumed that the various environmental spaces or landscapes of environments are uniform throughout or are continuous. Rather, some populations can be seen as living in metapopulations, dispersed among smaller enclosed populations. Hanski suggests that an environment may consist of several populations that exist in a series of separate patches of habitats (Hanski 1998; Hanski & Ovaskainen 2000). The study of metacommunities therefore concerns migration between the discrete habitat patches.

Aquatic habitats are good examples of metapopulations since they are isolated patches, separated by land. Aquatic invertebrate zooplankton and protozoa have surprisingly fast dispersal rates as well as a high dispersal capacity to new freshwater habitats (Louette & Meester 2005). The invertebrates' eggs as well as the invertebrates themselves often are able to enter desiccation tolerant resting stages. This permits them to safely enter the terrestrial habitats that separate their aquatic habitats allowing for their migration. The most common theories on how zooplankton migration occurs among freshwater communities are either animal vectors such as birds, amphibians, and insects or abiotic vectors such as wind or rain (Cáceres & Soluk 2002). One study found that dispersal rates were constant within new habitats regardless of the animal contact suggesting that freshwater invertebrates disperse primarily via abiotic vectors (Cáceres & Soluk 2002). The overall success of a zooplankton species in

migrating to a new community is not how quickly it can disperse but whether that species can sustain a population once it has arrived.

The aquatic inquiline communities living in the leaves of the pitcher plant *Sarracenia purpurea* are a good model for testing migration and dispersal in metapopulations. The pitcher plants produce new cup shaped leaves in the summer growing season in northern latitudes that survive for a year or longer. The leaves fill up with between 0 and 50 ml of rainwater, providing an aquatic habitat for an inquiline community of bacteria, protozoa, and small invertebrate animals. Small insects are attracted to the leaves, fall in, and drown in the water. These prey are then broken down by the community living in the leaf and the plant absorbs the nitrogen released into the water by the community. The plant therefore depends upon the presence of this community in order to receive the nutrients that the bog soil cannot provide (Miller and Kneitel 2005). The various inquilines living within the plant include larval dipterans such as the pitcher plant mosquito (*Wyeomyia smithii*), a sarcophagid (*Fletcherimyla fletcheri*), and a midge (*Metriocnemus knabi*); the pitcher plant mite (*Sarraceniopus gibsoni*) and a bdelloid rotifer (*Habrotrocha rosa*) are also found exclusively in *S. purpurea* (Miller and Kneitel 2005). Various other species of protozoa and bacteria are commonly found in the pitchers. A complex food web is present among these invertebrates in which the invertebrates feed on bacteria, the detritus, and each other (see Miller and Kneitel 2005).

It is known that the inquilines of pitcher plants can show up within days of the leaf's opening, but little is known about the means of the invertebrate migration. Since migration and dispersal are so vital to these populations, this study will try to find the means by which the inquilines are dispersed among the pitcher communities. The various members of the metacommunity have different methods of dispersal. For example the pitcher plant dipterans (*Wyeomyia smithii* and *Metriocnemus knabi*) disperse their eggs as airborne adults via oviposition and the sarcophagid (*Fletcherimyla fletcheri*) and the pitcher plant mite (*Sarraceniopus gibsoni*) actively crawl into pitchers (Miller and Kneitel 2005). Not much is known on the means of protozoa or rotifer migration. The two most common hypotheses are dispersal through aerial means or dispersal through animal vectors. When testing newly created pitcher plant environments I expect to find a pattern similar to Cáceres & Soluk's (2002) study on newly created pond environments. I hypothesize that passive aerial dispersal will be the most common form of dispersal. In this study, I prevent animals from visiting cleaned out pitchers with a fine mesh. If wind and rain are the primary means by which the inquilines disperse between pitchers, then I expect to find no difference in the population composition in the covered pitchers versus similar open pitchers over a period of two weeks.

Materials and Methods

The purple pitcher plant, *Sarracenia purpurea* is found in bogs ranging from Canada to Florida (Miller and Kneitel 2005). This experiment was carried out at Forest Service Bog on the property of the University of Notre Dame Environmental Research Center in northern Wisconsin (UNDERC 46' 13" N by 89' 32" W). Forest Service Bog is a sphagnum dominated bog amidst a new-growth forest that has not been disturbed since extensive logging up to 80 years ago. This experiment was conducted in the month of June.

Experimental Design

I chose ten plots of two proximate healthy pitcher leaves of similar size in the bog. The plots were at least 5m apart from one another. At the time of experimentation, there were not enough pitchers that had newly opened that year so in order to be consistent, all pitchers used were at least a year old. All of the pitchers' contents were removed and I washed out each pitcher with sterile water multiple times. I also placed two sterile 50mL centrifuge tubes amongst the chosen pitcher leaves in each of the plots to act as synthetic pitcher leaves since one cannot be certain that all of the invertebrate organisms or eggs were washed out of the actual pitcher leaves.

The contents of the pitchers and tubes were prepared in the laboratory. I measured out 40 aliquots of 25mL sterile water in separate vials and I added about 0.2g of detritus material to each. The detritus consisted of deceased nocturnal dipterans that I had collected in the area using a New Jersey Light Trap over two

consecutive nights. The vials of detritus and water were vigorously shaken in order to fully mix them and were randomly added to the cleaned out pitchers and tubes. All the pitchers and tubes therefore had the same contents at the beginning of the experiment.

At each plot, I covered the opening of one of the pitcher leaves and one of the sterile tubes with a fine 1mm mesh secured by a rubber band; the other leaf and tube in the plot were left open. Therefore, each of the ten blocks, had a covered pitcher, an open pitcher, a covered centrifuge tube and an open tube.

Sampling

The experiment began on June 9, 2007 and ran for two weeks. The pitchers were each sampled four times: 2, 4, 6, and 14 days after the experiment was set up. There was no precipitation between the set up and the first three sampling sessions but there were 30.5mm of rain between the third and fourth sampling session.

The invertebrate richness and abundance of each pitcher were determined from 100 μL samples in a Palmer cell counter with a compound microscope (x100). Protozoa were identified to genus (as best possible) and rotifers were counted as all were the same species, *Habrotrocha rosa*.

The percent dissolved oxygen in each of the pitchers and tubes was measured at the end of the two week sampling session using a YSI Model 55 Handheld Dissolved Oxygen System.

Laboratory Experiment

I filled 8 beakers (50 mL) with 25mL of sterile water and 0.2g of detritus material. The beakers and contents were autoclaved in order to sterilize them. Four sterilized beakers were placed in a clean upside down fish tank (30x51x25cm) to create a closed environment. Another 50mL beaker filled with 25mL of established pitcher plant pitcher fluid taken from pitchers in Forest Service Bog. The pitcher fluid was sampled under a microscope to make sure there were invertebrates present. I added 0.2g of detritus to the pitcher plant fluid and all invertebrate predators (*Wyeomyi smithii*) were removed from the fluid. The other four sterilized beakers were placed alone under a fish tank in the same manner as a control. The closed environments were allowed to sit for 12 days undisturbed. After this time, all of the beakers were sampled once in the same manner as in the field experiment.

Statistical Testing

I performed a repeated measures ANOVA to see if there was a change over time in the species richness of the pitchers over the 4 sampling sessions using two independent variables: the container (pitcher or tube) and covering (covered or open). Invertebrate abundance was log transformed to meet ANOVA assumptions. I determined the diversity of invertebrate taxa in each pitcher or tube for each sampling session using the Shannon H diversity index. Because the diversity data did not conform to the assumptions of parametric tests and this was

not remedied with transformations, I performed a non-parametric Kruskal-Wallis test comparing effect of the container and the covering with diversity. I ran 2 other Kruskal-Wallis tests looking at the effect of just the container and the effect of just the covering on diversity as post-hoc tests. I used a student t-test to compare the dissolved oxygen of the pitchers verses the centrifuge tubes. Using student t-tests, I also tested whether the number rotifers and *Colpoda* found in pitchers varied based on whether they were covered or uncovered. I chose *Colpoda* and rotifers for these tests because of my confidence in my identification of these taxa during sampling. All statistics were calculated using SYSTAT 12 (2007) and Microsoft Excel (2002).

Results

During the first time sampling, 3 pitchers were found empty because of a hole in the pitcher. In these cases, a new pitcher in proximity to the plot was cleaned out in the same manner as the others and fresh water and detritus were added. Sampling of these pitchers continued as normal, and their results were counted in with the data.

Various species of invertebrates were successful in dispersing to the pitchers during the two weeks of sampling. This was marked by a very significant increase in the species richness in all the pitchers over time (table 1). Figure 1 shows the trend of increasing richness over the four sampling periods. While the covering treatment (covered or open) had no significant effect on

species richness over time (table 1, figure 1), the container (pitcher or tube) had a highly significant effect on species richness over time (table 1).

After the pitchers were colonized for two weeks, in the fourth sampling session (the most abundant, rich, and diverse), the covering of the pitchers also had no significant effects on abundance (table 2) or diversity (table 3). Only the container had a significant effect on abundance and diversity, as well as richness (figure 2). There was always significantly lower abundance (table 2), species richness (table 1), and diversity (table 3) in the tubes than in the pitchers. Whether the pitcher was covered or open had no significant effect on the abundance, richness, or diversity whatsoever.

The dissolved oxygen of the water found in the pitchers was significantly higher than the dissolved oxygen of the water found in the tubes after two weeks ($t=6.59$, $p<0.01$, Mean pitcher DO = 41.71%, Mean tube DO = 12.6%) (figure 3).

The covering had absolutely no significant effect on the number and presence of either rotifers ($t=-0.65$, $p=0.52$) or *Colpoda* ($t=0.96$, $p=0.34$).

Discussion

I hypothesized that if abiotic factors such as wind and rain were the primary means of invertebrate dispersal between pitchers, then the pitchers that were covered would have equal richness, abundance, and diversity than the open pitchers after two weeks. However if insects and other animals acted as vectors, dispersing the invertebrates among the pitchers, then the pitchers that were open

would have a significantly higher richness, abundance, and diversity than the covered. While there was a definite increase in the species richness over the course of this study (figure 1), the results of this study showed no significant differences between the richness, abundance, and diversity of the open and covered pitchers (figure 2). This suggests that animals, in fact, do not play a major role in the dispersal of the microscopic pitcher plant inquilines. While most of the community may be dispersed aerially, perhaps some specific taxa rely on animal vectors. For two specific invertebrates, there was no difference in the presence and abundance of *Colpoda* or bdelloid rotifers between the covered and open treatments. These pitcher inquilines do not rely on the aid of larger animals in order to colonize.

Animal Vectors

There has been evidence that suggests animals act as vectors for other aquatic systems. Aquatic animals have been captured with samples of zooplankton and protozoa on their bodies (Maguire 1959; Maguire 1963). However, these were found on larger animals such as birds, amphibians, and large insects such as dragonflies and wasps: animals that do not frequent pitchers. No studies found any protozoa or zooplankton on the animals that would frequent pitchers such as mosquitoes or other dipterans (Maguire 1959). It would appear that the larger organisms found with hitchhiking aquatic invertebrates play a significant role in zooplankton and protozoan dispersal to larger aquatic

environments such as lakes, ponds, and the bogs in which pitcher plants reside. However there is much evidence supporting that these larger animals do not act as vectors dispersing invertebrates between limnetic habitats. Wind and rain are the primary means for microscopic invertebrate dispersal for these larger habitats as well (Cáceres & Soluk 2002; Cohen & Shurin 2003). It is not surprising that animals play a minimum role in the ecosystems of the smaller isolated aquatic habitats of the pitcher plant.

Passive Dispersal

Maguire (1963) noted that there is a surprisingly high abundance of invertebrates floating around in the atmosphere. He considered that rain washes out the dust and organisms from the air and took cultures from rain water to measure the abundance of invertebrates floating around in the air column above. In some places in Texas, Maguire deduced that there was as much as one *Colpoda* cyst that was washed to every 2.8 square inches (7.1 cm) the ground during a rainfall. Other studies have noted that proximity to a source population positively correlates with aquatic invertebrate distribution rates (Cohen 2003; Cáceres & Soluk 2002). If the invertebrates disperse independently from animal movement, then they become airborne from the source and must use the wind or rain to be moved to another environment. This likely explains the high dispersal capacities of freshwater invertebrates (Louette & De Meester 2005); they don't rely on the movement patterns of another party. Therefore one can assume that in a bog with

a high abundance of pitcher plant individuals in close proximity, such as my study site, there is an abundance of pitcher plant invertebrate that have become airborne from thriving pitcher communities, available for colonization. With this possibility, it would make sense that the amount of biota floating in the air would colonize pitchers quickly, regardless if animals were acting as vectors. It would be interesting to test this by comparing invertebrate cultures from rainwater collected in the midst of pitcher plant communities with cultures from rainwater collected at varying distances from the pitcher plant communities. One could also test how much proximity to a pitcher plant with a healthy inquiline community affects the rate and amount of invertebrate dispersal to another pitcher plant. This would also test whether the protozoa community is specific to the pitchers or if they have dispersed from larger bodies of water.

I performed the laboratory experiment to confirm that it is indeed abiotic factors that disperse the invertebrates from pitcher to pitcher. The experiment showed negative results. After letting it sit for two weeks, the only organism that dispersed from the pitcher fluid vial to the other vials in the experimental chamber was an aquatic type of mold. While there were still invertebrates in the pitcher fluid vial, none had dispersed to the others. Nothing was present in the vials after two weeks in the control chamber. These results go against those found in the field experiment suggesting that animals are entirely responsible for pitcher plant community dispersal. However, this experiment was not a good representation of

the outside environment. This experiment was carried out in small containers with no airflow and little light. If it was performed again with an agent causing airflow, better light, and with synthetic rain, I believe the results will be more consistent with the results of the field study in that these organisms can disperse aerially.

Error in Experimental Design

Real and artificial pitchers were not found to be equivalent in this study. I used them so that I could be sure that their environment had no invertebrate organisms at the beginning, since I could not be sure that I washed out all of the invertebrate community from the plant pitchers. There was overall significantly lower species richness, abundance, and diversity in the tubes than in the pitchers (figure 2). This may be due to the fact that by the end of the experiment, bacterial metabolic activity in the tubes resulted in hypoxic environments in comparison to the oxygen rich pitchers (figure 3). Prior to the study, I did not take into account the fact that through photosynthesis, the pitchers oxygenate the water they hold, allowing for a much more productive community (Miller & Kneitel 2005). Therefore the harsh, low oxygen environments of the tubes allowed few stable populations of invertebrates to thrive in them. In the future if trying to begin with sterile environments, I could find some means to oxygenate these tubes without being to disruptive to the community structure or clean out pitchers with a mild antibiotic or alcohol in order to fully remove the community without killing the

plant. I also could autoclave the detritus and water before adding them to the pitchers to make sure that the detritus was sterile.

There may have been some slight error in the experimental design because the mesh coverings of the "covered" treatments were not fully successful in keeping out invertebrates. When sampling from the covered pitchers, I noticed that some of the covered tubes had dipterans floating in the tube that I did not add. Also by the end of two weeks, many of the covered pitchers, as well as the open pitchers, had sarcophogid larvae present. The mesh still repelled most of the animals that approached the pitchers and this probably had a small effect on the data. However, in the future, a better means for repelling animals should be found such as a larger mesh covering that covers the entire pitcher rather than just the opening.

During data collection, the pitchers were not sampled destructively; I did not clean out the pitchers every time I sampled. Therefore, if an organism was able to disperse to the pitcher, it had to compete with the already present organisms to establish a viable population that would be counted during sampling. This was probably a problem with the poorly oxygenated tubes: organisms were arriving but to an unsuitable environment. This resulted in an inaccurate survey of dispersal richness and diversity. The large amount of detritus in the pitchers may have also prevented dispersed organisms from establishing populations. Looking at other studies it appears that more detritus was added to the pitchers at

the beginning of my study than is usually found in the pitchers (Kneitel & Miller 2002; Kneitel & Miller 2003; Hoekman 2007). Kneitel & Miller (2003) point out that higher basal resources (detritus, in this case), promote higher abundances of organisms in pitcher plants. Therefore it appears that in many of the pitchers, there was an early colonizer that quickly multiplied in the excess resources and created an enormous population. As this population reached its carrying capacity (K), other arrivals to the community were unable to succeed because there were already too many of the first colonizer. This goes against the findings of Hoekman (2007) and Kneitel (2002) who found that with more resources comes higher richness but much more detritus with higher surface area was added to the pitchers in this study than in their studies. However, these two studies had natural predators present (*Wyeomyia smithii*), which may have kept the abundances of protozoa at bay, allowing for more diversity. A similar result was found in which treatments of pitchers with detritus with higher surface area had lower richness (J. Goedhart unpublished data). This would explain why in some pitchers sampled, there were over 70,000 individuals of one genus of protozoa found in a 100 μL sample and only one or two individuals of another. Another explanation is the paradox of enrichment (Rosenzweig 1971) in which an enrichment of resources in a stable ecosystem can result in decreased diversity and production. The excess detritus added lowered the species richness and overall diversity of some of the pitchers and tubes sampled and less should be added in future experiments.

Figure 1 shows that there is a definite increase in the richness of the pitchers over the sampling session, but the average richness does not fully asymptote as time goes on, it keeps increasing. This suggests that there was a higher potential for species richness in the pitchers and that the experiment was not carried out for a long enough time period. Due to time constraints, I had to stop sampling after two weeks but sampling should have continued until the richness of the pitchers stopped increasing.

Conclusion

This study found that animal vectors have no influence on the dispersal of pitcher plant inquilines to new environments. Abiotic factors such as wind and rain seem to be the most prominent means by which members of the pitcher plant community migrate between pitchers. However, various factors such as dissolved oxygen or abundance of organisms already in the pitcher can severely impact the ability for a dispersed protozoan to develop a stable population. It may appear that the community of each individual pitcher is highly unstable since they are not physically connected and the metapopulations therefore depend wholly on migration and colonization success. This study has shown that the aerial method of dispersal on which each individual pitcher community depends on is quite stable and little would be able to disrupt the migration of these organisms to new pitcher habitats. If these communities were dependant upon animal vectors for dispersal, then factors such as extinctions and droughts would have detrimental

effects upon the pitcher inquiline populations, rendering them highly unstable. The abiotic means of invertebrate migration is a connecting force for all these individual unstable environments and it stabilizes the metacommunity of pitcher plant inquilines as a whole.

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Appendix

Tables

Table 1: Results of a Repeated Measures ANOVA comparing the container (tube or pitcher) and the opening (open or covered) with time.

	Source	SS	df	Mean Squares	F-ratio	p-value
Between Subjects	Container	71.56	1	71.56	61.52	<0.01
	Opening	0.31	1	0.31	0.26	0.61
	Container*Opening	0.76	1	0.76	0.65	0.43
	error	41.88	36	1.16		
Within Subjects	Time	49.47	3	16.49	34.77	<0.01
	Time*Container	21.57	3	7.19	15.16	<0.01
	Time*Opening	0.52	3	0.17	0.36	0.77
	Time*Opening*Container	0.97	3	0.32	0.68	0.56
	error	51.23	108	0.47		

Table 2: Results of an ANOVA test comparing the effects of the container (tube or pitcher) and the opening (covered or open) on abundance from the fourth sampling session.

Source	Type III SS	df	Mean Squares	F-ratio	p-value
Container	97.50	1	97.50	8.21	<0.01
Opening	13.37	1	13.37	1.13	0.30
Container*Opening	5.79	1	5.79	0.49	0.49
Error	427.71	36	11.88		

Table 3: These are the results of the non-parametric Kruskal-Wallis test looking at the effects of the container and opening on the diversity (Shannon H) of the pitchers from the fourth sampling session A non-parametric Kruskal-Wallis test on how the opening and the container individually effect the diversity of the pitcher plant community were done as a post-hoc to the first Kriskal-Wallis test.

	Group	Count	Rank Sum	p-value
Kruskal-Wallis	Pitcher,Covered	10	258	< 0.01
	Pitcher,Uncovered	10	328	
	Tube,Covered	10	129	
	Tube,Uncovered	10	105	
Post hoc Kruskal-Wallis	Pitcher	20	586	< 0.01
	Tube	20	234	
	Covered	20	387	0.51
	Uncovered	20	433	

Figures

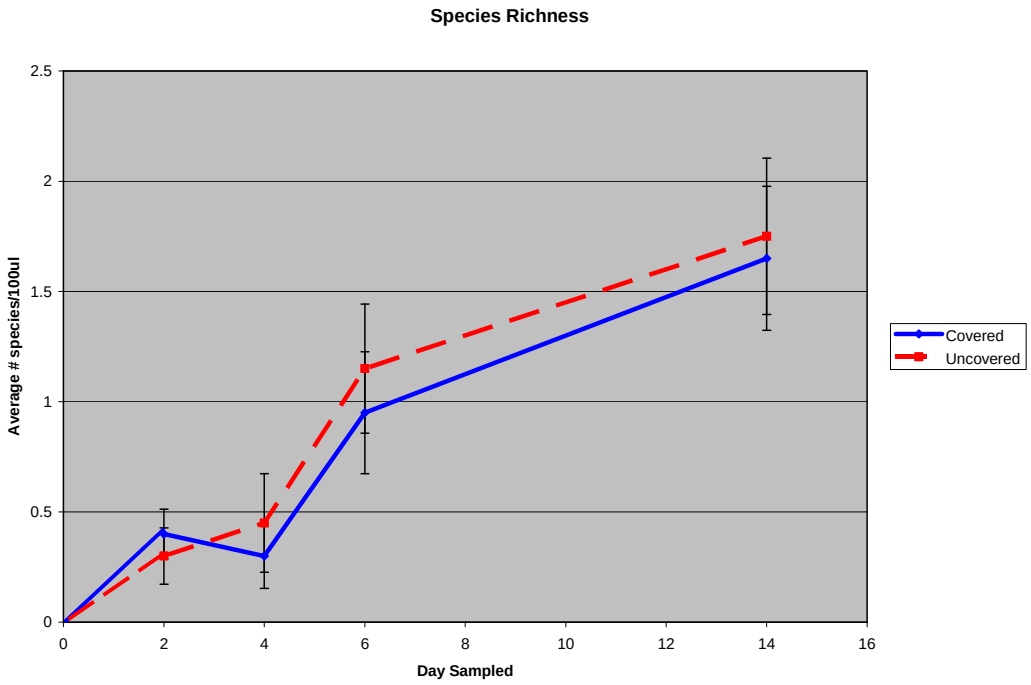

Figure 1: The increase of species richness in all the pitchers over time.

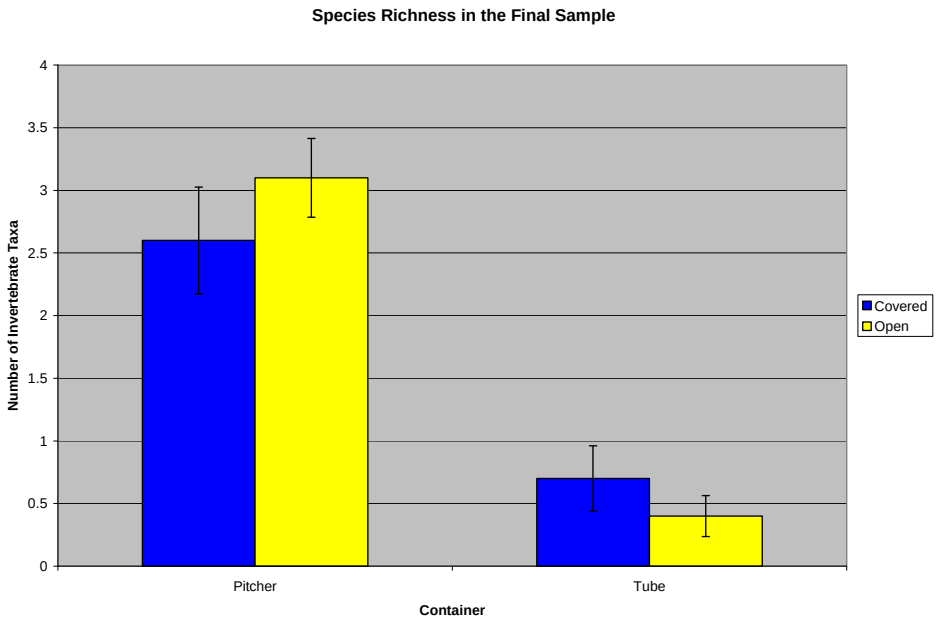

Figure 2: Species richness found in the pitchers in the final sampling session.

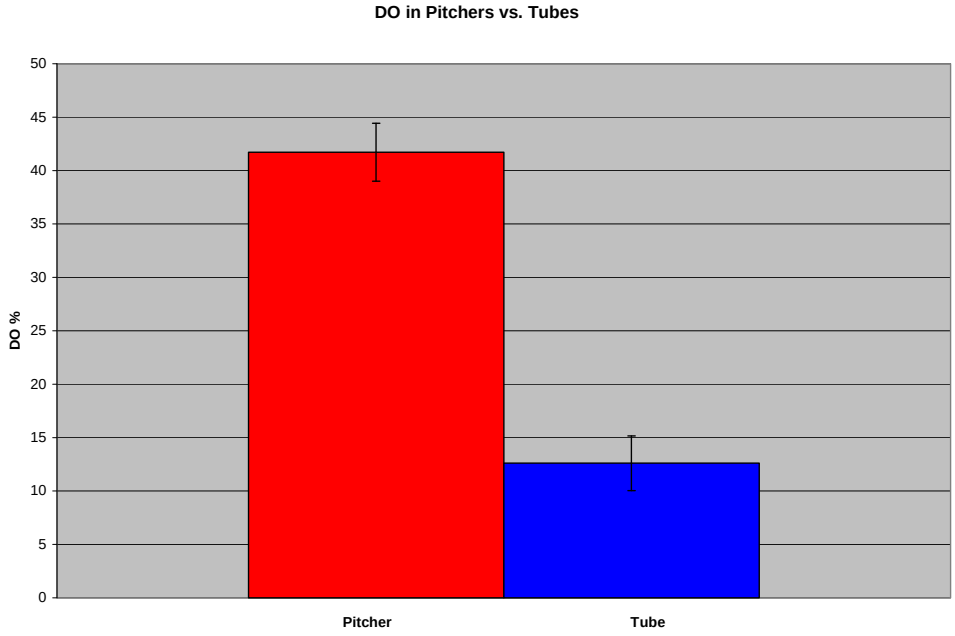

Figure 3: The dissolved oxygen the pitchers and tubes after two weeks.