MACUB

IN VIVO

The Publication of the Metropolitan Association of College and University Biologists

Spring 2019 Volume 40, Issue 3

Monmouth University

will be hosting the

52nd ANNUAL MACUB CONFERENCE
Saturday, October 26, 2019

Instructions for Authors

IN VIVO is a peer-reviewed journal that is published three times yearly during the Fall, Winter, and Spring. Original research articles in the field of biology in addition to original articles of general interest to faculty and students may be submitted to the editor to be considered for publication. Manuscripts can be in the form of a) full length manuscripts, b) mini-reviews or c) short communications of particularly significant and timely information.

Articles can be submitted electronically to *invivo@mec.cuny.edu* or mailed as a printed copy (preferably with a diskette that contains the file) to the Editorial Board at Medgar Evers College. All submissions should be formatted double spaced with 1 inch margins. The title of the article, the full names of each author, their academic affiliations and addresses, and the name of the person to whom correspondence should be sent must be given. As a rule, full length articles should include a brief abstract and be divided into the following sections: introduction, materials and methods, results, discussion, acknowledgments and references. Reviews and short communications can be arranged differently. References should be identified in the text by using numerical superscripts in consecutive order. In the reference section, references should be arranged in the order that they appeared in the text using the following format: last name, initials., year of publication. title of article, journal volume number: page numbers. (eg. - [1]Hassan, M. and V. Herbert, 2000. Colon Cancer. *In Vivo* **32:** 3 - 8). For books the order should be last name, initial, year of publication, title of book in italics, publisher and city, and page number referred to. (eg. - Prosser, C.L., 1973. *Comparative Animal Physiology*, Saunders Co., Philadelphia, p 59.). Abbreviations and technical jargon should be avoided. Tables and figures should be submitted on separate pages with the desired locations in the text indicated in the margins.

In Vivo (Brooklyn) is published by
The Metropolitan Association of College and University Biologists
Brooklyn, NY
ISSN 2639-2658
url: https://macub.org/

In This Issue:

Save the Date
The 2019 MACUB Conference will be at Monmouth University
Oct. 26, 2019

Call for Manuscripts

Publish your manuscripts in *In Vivo*
Follow the Instructions for Authors on the inside cover and submit your manuscripts electronically to the Editorial Board at invivo@mec.cuny.edu

Dietary Overlap of Two Species of Sunfish, *Lepomis auritus* and *Lepomis macrochirus*, in a Devegetated Suburban Lake.

Linda A. Lalicata[1*], Joseph W. Rachlin[2] and Barbara Warkentine[3]

[1]Bronx Community College, New York, NY 10453
[2]Lehman College, New York, NY 10468
[3]SUNY Maritime College, New York, NY 10465
[*]Corresponding author – linda.lalicata01@bcc.cuny.edu

Abstract

The focus of the following study was to explore dietary overlap in two species of sunfish, *Lepomis auritus* (Linnaeus, 1758), redbreast sunfish, and *Lepomis macrochirus* Rafinesque, 1819, bluegill sunfish, co-occurring in a suburban lake in Putnam County New York, Lake Mahopac, approximately eighty kilometers north of New York City. The stomach contents were examined from both species and identified to the lowest practical taxa. To estimate the dietary similarities between the two types of sunfish, calculations were done using the Schoener % overlap index ($\alpha i = 1 - 1/2\sum[pxi - pyi]$). These two species were chosen because little research has been done to compare them. Lake Mahopac was selected because of its lack of aquatic vegetation, due to the introduction of *Ctenopharyngodon idella*, grass carp, as a biological control for *Myriophyllum spicatum*, Eurasian watermilfoil. Bluegills prefer still waters with abundant aquatic plants where they can hide and feed; redbreasts are most frequently found in slow-moving streams but also inhabit lakes and ponds. Changes in habitat complexity, such as what has occurred in Lake Mahopac, can alter feeding strategies. Previous studies on the diets of the two species indicated that there could be possible dietary overlap, which could lead to potential competition for resources. After calculating the Schoener % overlap index it appears that there is no dietary overlap between the two populations, with $\alpha i = 0.299$.

Introduction

Overlap is common between different species of sunfish[1-3] and is often the product of habitat structure[4]. Some species are more adaptable to different habitats than others and will abandon preferred habitats if the change will be more profitable[1]. Bluegill sunfish normally forage in vegetated waters, but Werner *et al.* found in their 1981 study that bluegills can adapt to different habitats based on learning mechanisms and sampling[5]. The diets of *Lepomis auritus* (Linnaeus, 1758), redbreast sunfish and *Lepomis macrochirus* Rafinesque, 1819, bluegill sunfish, were compared in this study to see if there is overlap in prey intake of the two species in the altered ecosystem. The lack of submerged vegetation is due to the introduction of *Ctenopharyngodon idella*, grass carp, which was used as a biological control for aquatic weed beds. The diets of the two species are similar. Redbreasts ingest a range of prey items, including gastropoda, bivalves, crustaceans, aquatic and terrestrial insects, insect larvae, and occasionally small fish[6]. It has been documented that bluegill forage on zooplankton[7], macroinvertibrates[8-10], as well as on small fish[11]. There has been little research

done on the comparison between these two species, dietary or otherwise, which is why they were chosen for this study. This study was conducted under unique circumstances. Typically Lake Mahopac would have ample aquatic flora and weed beds, which is the preferred habitat of the bluegill[12,13]. Since the plant life was diminished to the point of almost complete eradication bluegill were forced to survive in open waters, which is more optimal for redbreasts. Would this alteration to the habitat influence a change in dietary repertoire for the bluegill and possibly cause overlap between the two species?

Collection Site

Lake Mahopac is a natural, freshwater lake approximately 80 kilometers from New York City. It has a circumference of 10.46 kilometers with a mean depth of 8.84 meters. The deepest section is approximately 18.29 meters. There is an inlet on the northeast side of the lake and an outlet on the southwest side. Bluegill and redbreast sunfish were collected from two sites on the lake. Since much of the shoreline of Lake Mahopac is privately owned points along the shore were limited.

Site A is located at 41°22'18.51"N 73° 45'05.76"W; Site B is 41°22'21.16"N 73° 44'55.43"W (Figure 1). These sites were selected because they offered ideal conditions in which to use an umbrella net and for the placement of a minnow trap. Other sites proved unsuitable because after numerous tries, no fish were captured. There was great difficulty obtaining large enough numbers of both species. The methods that garnered adequate numbers of redbreasts did not provide an equal amount of bluegill Open-water fishing yielded no additional bluegill specimens, whether the method of collection was the umbrella net, minnow trap, or hook and line, despite numerous attempts over the years of study. Electro-fishing was rejected because of the stresses it places upon fish populations[14,15]. There had been indications during the time of collection that fish populations had declined in Lake Mahopac, most probably due to the change in their habitat[16]. It would not have been prudent to further depress fish numbers of already impacted populations.

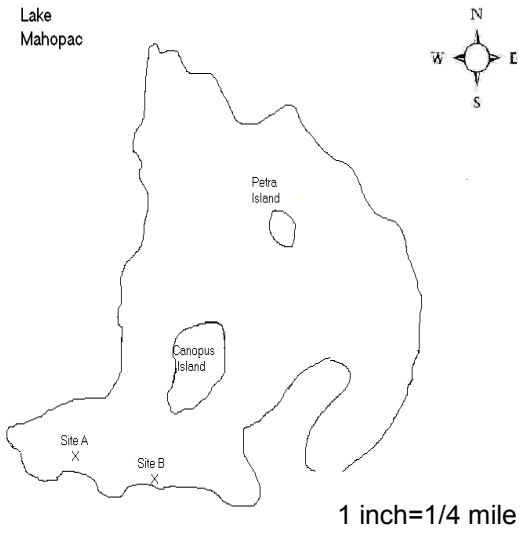


Figure 1: Lake Mahopac
Site A. 41°22'18.51"N 73°45'05.76"W
Site B. 41°22'21.16"N 73°44'55.43"W

Materials and Methods

Sunfish tend to stay mainly in the littoral zone[18], therefore collection sites were close to the periphery of the lake. Fish were collected using an umbrella net and minnow trap at different times of the day and night, since redbreast sunfish are diurnal feeders[19]. The umbrella net allows the capture of several fish at one time making it an effective method. The fish trap was utilized where the use of an umbrella net was difficult to maneuver and therefore inefficient. Bait (bread, dog food, cat food, dried fish flakes) were placed inside the trap to lure fish. The trap was modified to better allow fish entry; flexible wire was used to hold the openings in place so the fish could swim in but not out. The trap was set in water depth of about 0.457 meters and was removed at approximately fifteen minute intervals to collect the trapped fish; the trap was then put back into place. Fish would enter the trap quickly, hence the fifteen-minute intervals.

Although larger fish could not escape, it was discovered that smaller fish could swim out sideways. Ideally collection continued until at least ten fish were collected for that day but unfortunately some days yielded less to none of that number. A total of 111 fish (74 redbreasts and 43 bluegill) were collected from 2008 to 2010 (Table 1).

Collected fish were euthanized humanely using MS222 (tricaine methane sulphonate) to anaesthetize the fish before the addition of 10% formaldehyde. The fish remained in 10% formaldehyde for approximately one to two weeks according to standard museum protocol before transfer to 75% ethanol. The fish were then sexed, weighed, and measured before removal of the stomach (Table 1). Stomachs were removed from the preserved fish by cutting from the anus to the start of the pectoral fin. Small cross cut incisions were made upwards of approximately one inch for easier removal. The alimentary canal is then detached from the body by cutting the large intestine and the esophagus. The stomach is identified and cut away from the alimentary canal at the sphincters. It is blotted dry with paper toweling and weighed to the nearest tenth of a gram. It is then opened by cutting lengthwise across the base. The two sides are spread apart to remove the stomach contents by carefully scraping the tissue using dissecting picks and flushing with water or alcohol. The stomach contents were then preserved in 75% alcohol until they could be examined and identified. Stomach contents were separated into the lowest practical taxa (Table 2) and placed in a Petri dish and examined under a dissecting microscope for enumeration. Many of the prey items were identified by body parts and not intact items. In this case the identifications were based upon wing venation, tarsal segments, and head capsules. In cases where individuals could not be reconstructed, the animals were counted based upon the head capsules. The individuals were put into vials containing 75% alcohol. These specimens were used in calculating the Schoener % overlap index. This index has the following form: ($\alpha_i = 1 - 1/2\sum [p_{xi} - p_{yi}]$), where p_{xi} and p_{yi} are the proportions of the ith items in x and y (the two species that are being compared). A value higher than approximately 0.60 is considered to be biologically meaningful and would indicate possible competition between the groups in question[20]. Since there is no software that can be used to calculate the Schoener % overlap index they were done manually using a Texas Instruments TI-34 II Explorer Plus calculator.

Results

The Schoener % overlap index was calculated for the two species to determine if there is dietary overlap between the redbreast and bluegill

Table 1: Mean length and mass of redbreast (*Lepomis auritus*) and bluegill (*Lepomis acrochirus*) sunfish from Lake Mahopac

Fish Species (Lake Mahopac)	Sex	Number of Fish	Mean Standard Length In Centimeters ± SD	Range	Mean Total Mass in Grams ± SD	Range
Redbreast	Female	52	9.8 ± 1.5	7.2 – 13.5	32.5 ±16.8	13.4 – 93.9
Redbreast	Male	22	11.1 ± 2.1	8.2 – 17.0	47.5 ± 29.4	13.0 – 119.3
Bluegill	Female	24	9.9 ± 2.1	6.8 – 13.9	39.1 ± 25.2	8.3 – 8.7
Bluegill	Male	19	9.5 ±1.9	6.7 – 13.2	30.3 ± 20.5	7.6 – 69.7

Table 2: Number and proportion of prey taxa removed from redbreast sunfish (*Lepomis auritus*) and bluegill sunfish (*Lepomis macrochirus*) stomachs.

Taxa	#	Prop.	#	Prop.
Annelida				
Oligochaeta	3	0.003	1	0.001
Crustacea				
Hydracarina	11	0.011	9	0.011
Cladocera				
Daphniidae	12	0.012	5	0.006
Sididae	81	0.082	205	0.248
Anostraca				
Chirocephelidae	-		393	0.475
Amphipoda				
Hyalellidae	16	0.016	17	0.020
Decapoda				
Cambaridae	1	0.001	-	-
Isopoda				
Asellidae	34	0.034	7	0.008
Ostracoda				
Myodocopidae	1	0.001	18	0.022
Bivalvia				
Sphaeriidae	2	0.002	2	0.002
Gastropoda				
Planorbidae	4	0.004	-	-
Valvatidae	2	0.002	2	0.002
Undetermined	1	0.001	2	0.002
Unionoida				
Unionidae	1	0.001	2	0.002
Coleoptera				
Scarabidae	11	0.011	4	0.005
Dytisicidae	1	0.001	-	-
Elmidae	1	0.001	2	0.002
Hydrophilidae	1	0.001	-	-
Diptera				
Chironomidae	460	0.464	86	0.104
Ceratopogonidae	23	0.023	4	0.005
Dixidae	3	0.003	1	0.001
Empididae	1	0.001	-	-
Psychodidae	2	0.002	-	-
Phoridae	-	-	2	0.002
Undetermined		-	1	0.001
Trichoptera				
Hydropsychidae	11	0.011	-	-
Lepidostomatidae	36	0.036	11	0.013
Ephemeroptera				
Heptageniidae	5	0.005	2	0.002
Ephemerellidae	2	0.004	-	-
Undetermined	1	0.001	-	-
Odonata				
Calopterygidae	-	-	3	0.004
Coenagrionidae	2	0.002	2	0.002
Hymenoptera				
Formicidae	193	0.195	9	0.011
Arachnidae				
Araneae	3	0.003	1	0.001
Naviculales				
Naviculaceae	11	0.011	37	0.045
Eggs, Undetermined	55	0.055	-	-

sunfish in Lake Mahopac with a result of 0.299, which indicates that there is no dietary overlap between redbreasts and bluegills. Although there were similarities in the prey items consumed, bluegill showed a preference for crustaceans, such as cladocerans and anastroca, whereas redbreast sunfish consumed larger amounts of aquatic larval insects (Table 2).

Discussion

Overlap is common between different species of sunfish[1-3] and is often a product of habitat structure[4]. Some species are more adaptable to different habitats than others and will abandon preferred habitats if the change will be more profitable[1]. Bluegill sunfish normally forage in vegetated waters, but Werner *et al.* found in their 1981 study that bluegills can adapt to different habitats based on learning mechanisms and sampling[15]. The Schoener % overlap index was calculated for the two species populations as a whole with a result of 0.299, indicating that there is no meaningful dietary overlap. Bluegill diets include insect larvae (such as chironomidae and trichoptera), crustaceans (such as amphipoda and daphnia), as well as gastropoda[10] (Table 2). These organisms were frequently found in the stomachs of the sampled bluegill with cladocera making up a large percentage, about 25% of the overall intake. Redbreast sunfish are primarily insectivores, occasionally consuming crustaceans and gastropods, as well as other food items[21]. Insects were the primary food source for the sampled redbreasts, with chironomidae being consumed most frequently (Table 2).

Since few if any papers have been written specifically on competition between redbreasts and bluegills it is impossible to compare these results with

those of other researchers. Since there is no meaningful dietary overlap between the two species, competition for resources appears unlikely. Whether or not the lack of aquatic plants had any impact upon the findings is impossible to say at this time; future research to compare the two species when the lake is in a more vegetated state could shed more light on this question.

References

[1]Werner, E.E. and D.J. Hall, 1979. Foraging efficiency and habitat switching in competing sunfishes. Ecology **60(2):** 256-264.

[2]Osenberg, C.W., E.E. Werner, G.G. Mittelbach and D.J. Hall, 1988. Growth patterns in bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) sunfish: environmental variation and the importance of ontogenetic niche shifts. Canadian Journal of Fisheries and Aquatic Sciences **45(1):** 17-26.

[3]Huckins, C.J.F., 1997. Functional linkages among morphology, feeding performance, diet, and competitive ability of molluscivorous sunfish. Ecology **78:** 2401-2414.

[4]Werner, E.E. and D.J. Hall, 1977. Competition and habitat shift in two sunfishes (Centrarchidae). Ecology **58 (4):** 869-876.

[5]Werner, E.E., G.G. Mittelbach and D.J. Hall, 1981. The role of foraging profitability and experience in habitat use by the bluegill sunfish. Ecology **62 (1):** 116-125.

[6]Stegemann, E.C., 1990. Department of Environmental Conservation. The Sunfish of New York State. Retrieved from www.dec.state.ny.us/website/dfwmr/fish/fishspecs/sunfitxt.html - 27k on July 12, 2010.

[7]Mittelbach, G.G., 1981. Foraging efficiency and body size: a study of optimal diet and habitat use by bluegills. Ecology **62:** 1370 -1386.

[8]Schramm, L.H. Jr. and K.J. Jirka, 1989. Epiphytic macroinvertebrates as a food resource for bluegills in Florida lakes. Transactions of the American Fisheries Society **118:** 416-426.

[9]Dewey, M.R., W.B. Richardson and S.J. Zigler, 1997. Patterns of foraging and distribution of bluegill sunfish in a Mississippi river backwater: influence of macrophytes and predation. Ecology of Freshwater Fish **6:** 8 -15.

[10]Olson, N.W., C.P. Paukert, D.W. Willis and J.A. Klammer, 2003. Prey selection and diets of bluegill, *Lepomis macrochirus*, with differing population characteristics in two Nebraska natural lakes. Fisheries Management and Ecology **10:** 31-40.

[11]Engel, S., 1988. The role and interactin of submersed macrophytes in a shallow Wisconsin lake. Journal of Freshwater Ecology **4:** 329 – 341.

[12]Casterlin.M.E. and W.W. Reynolds, 1978. Habitat selection by juvenile bluegill sunfish, *Lepomis macrochirus*. Hydrobiologia. **59(1):** 75-79.

[13]ODWC, Oklahoma Department of Wildlife Conservation. Retrieved from www.wildlifedepartment.com/species/bluegillsunfish.htm. on August 10,2010.

[14]Emery, L., 1984. The physiological effects of electrofishing. Cal-Neva Wildlife Transactions 59-72.

[15]Snyder, D., 2004. Invited overview: Conclusions from a review of electrofishing and its harmful effects on fish. Reviews in Fish Biology and Fisheries. **13:** 445-453.

[16]Department of Environmental Conservation 2002. A primer on aquatic plant management in NYS. Retrieved from www.dec.gov/docs/water_pdf/ch6p3.pdf on April 12, 2011.
[17]Department of Environmental Conservation 2004. Annual report: highlights and accomplishments 2003/2004. Retrieved from www.dec.ny.gov/docs/fish_marine_pdf/fshan_rpt04.pdf on May 13, 2011.
[18]Robinson, B.W., D.L. Wilson, and G.O. Shea, 1996. Trade-offs of ecological specialization: an intraspecific comparison of pumpkinseed sunfish phenotypes. Ecology **77(1):** 170-179.
[19]Johnson, J.H. and D.S. Dropkin, 1995. Diel feeding chronology of six fish species in the Juniata River, Pennsylvania. Journal of Freshwater Ecology **10(1):** 11-18.
[20]Zaret, T.M and S.A Rand, 1971. Competition in tropical stream fishes: support for the competitive exclusion principle. Ecology **52:** 336-342.
[21]Cooner, R.W. and D.R Bayne, 1982. Diet overlap in redbreast sunfish and long ear sunfishes from small streams of east-central Alabama Proc. S.E. Association. Fish and Wildlife Agencies **36:** 106-114.

Fungal Growth on Luria-Bertani Agar Plates Plus or Minus Ampicillin Exposed to the Air in a Laboratory

Amber Tucker, Victoria Ruiz, Allen Burdowski and Kathleen A. Nolan
St. Francis College, 180 Remsen St., Brooklyn, NY 11201

Abstract

Through contamination of some Luria-Bertani (LB) agar plates that were prepared for a course, we learned that there was a significant difference in numbers of molds on plates that were enriched with ampicillin (amp) versus those without amp[1]. The experiment was repeated here with added conditions. LB plates with and without amp were exposed to air in a microbiology teaching laboratory under the following conditions: covered or uncovered for three hours at room temperature (21°C), in a refrigerator (10°C) or in a 30° C incubator (this temperature is commonly used to grow molds). All plates were incubated at 30°C after treatments. There were significant differences in numbers of growths or color (molds and/or yeasts) between covered and uncovered, with and without ampicillin, and among temperatures. A signature black mold was noted on the LB plates plus amp that was not noted on the LB plates alone. Mold growth has been known to be a side effect of media that has had ampicillin added to it, and has also been noted as a side effect of ampicillin use in humans. Inexpensive experiments such as these can re-enforce microbiological techniques for students, and inspire them to delve further into the literature to learn about antibiotics, probiotics, side effects, overuse of antibiotics, and positives and negatives of fungal growth.

Introduction

Ampicillin is a class of penicillin that inhibits cell wall synthesis of both gram-negative and gram-positive bacteria. Ampicillin contains a beta lactam ring. Some bacteria contain or develop antibiotic resistance by breaking down the beta β lactam ring in the antibiotic (Fig. 1) with an enzyme, beta lactamase. Scientists have and are continuing to develop beta lactamase inhibitors that can block this bacterial action. Law *et al*.[2] outlined a fascinating experiment in which they designed a plasmid that contained a gene to produce a beta-lactamase inhibitor that was produced in large enough quantities to be effective. To do this, they transformed a methylotrophic yeast (*Pichia pastoris)* that over-expressed the beta-lactamase inhibitor, when induced with methanol. When combined with ampicillin, 100% inhibition of bacterial growth of *Bacillus subtiliis* was produced as compared to controls. These authors point out our continual need to develop new B lactamase inhibitors because of the constant development of novel beta lactamases by bacteria.

In general, the literature abounds with the growth of fungus in the human microbiome after the use of antibiotics. This is sometimes helpful, or, it can be harmful. Grenni *et al*.[3] provide a review on the effects of antibiotics on soil and

water quality. They deliver comprehensive information about the names, structures, uses, and effects of various antibiotics on bacteria. Jiang *et al.*[4] elucidate an example where fungal growth as a result of the use of antibiotics that kill gut bacteria can be "good" for the mouse body. Figure 2 illustrates how commensal fungi can increase in numbers after antibiotic use. They found that the yeasts *Candida albicans* and *Saccharomyces cerevisiae* produced mannans, which are tough cell wall components that help mice resist infection. Yu-Kyong and Young[5] note that ampicillin can activate phosphorylation in yeast, which might be a mechanism for their increase. Tigini *et al.*[6] are exploring the possibilities of using fungi isolated on waste-water agar as possible bioaugmentation agents in breaking down pollutants.

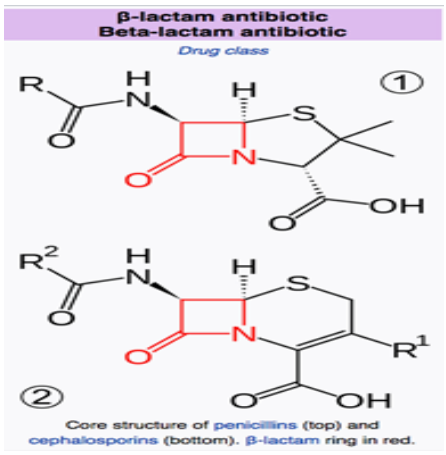


Figure 1. Beta lactam rings, From Wikipedia

Another positive use of fungi has been as probiotics. Kabbani *et al.*[7] studied the effects of the probiotic yeast *Saccharomyces boulardii* on healthy volunteer patients when ingested with and without ampicillin. Ampicillin alone, like other antibiotics caused a "dysbiosis" of the microbiota of the volunteers. The probiotic yeast, in combination with the antibiotic, caused less disruption of the microbiota according to DNA sequencing.

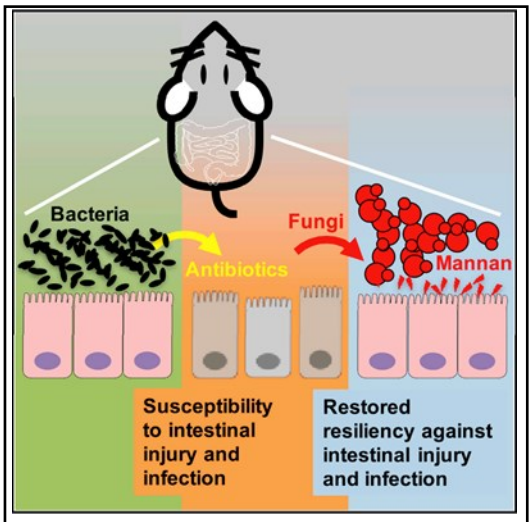


Figure 2. A positive result of a side effect of antibiotics with commensal fungi. Permission to use graphic by Sing sing Way granted 3/4/19, singsing.way@cchmc.org

However, the balance of the microbiota of the body can, conversely, be "upset" by the use of antibiotics. Babies, when treated with antibiotics for ear infections, often develop a diaper rash caused by yeast. Typically, an antifungal such as Nystatin will then be used. As a result of this microbiome "unbalance" additional infections such as those that cause impetigo can set in[8,9]. More serious examples include fungal infections of immunocompromised patients, such as those with HIV or cystic fibrosis. Dollive[10] in order to see how the microbiome changed with the use of antibiotics, delivered a cocktail of four antibiotics; vancomycin, ampicillin, neomycin and metronidazole, to mice. They noted the demise of the bacterial microbiome, and the rise in number of fungi. When the antibiotics were discontinued, the bacterial fauna returned, but some of the bacterial assemblages were altered, as well as persistence of some fungi such as *Candida spp.*

Another effect of the use of antibiotics, which has also been associated with other conditions such as smoking or a dry mouth, has been Black Hairy Tongue (BHT)[11]. The microorganism(s) that cause BHT are not well understood nor characterized. BHT resembles a black mold growing on the tongue. Zimmerman *et al*.[12] note that overprescribing antibiotics may further contribute to side effects of these antibiotics. Wiwin and Rejeki[13] found highly resistant *E. coli* in fecal cultures from hospitalized children (blood diseases) in Indonesia. Another complication[14] pointed out that all organisms have natural defense molecules called cytotoxic peptides, which could help explain the growth of the fungi in people with opportunistic infections, such as tuberculosis, HIV, and cystic fibrosis. Heteroresistance can develop in fungi, which is a differential resistance of fungi to antifungals, which is a growing and alarming problem[15]. There is a need to develop new molecular targets for antifungals[16]. Rojo *et al*.[17] provide a review of the human microbiome and factors that might alter its composition. This study is a good starting point for learning about the complicated interactions of the microbiota in our bodies. However, as DNA and rRNA sequencing becomes more available (less expensive), more human microbiomes are appearing in the literature, and there is much variation in the composition.

In a preliminary experiment in which LB plates were exposed to air and then refrigerated, there was a significantly greater difference in mold growth on LB plates plus ampicillin than on LB plates alone[1]. Ten white and 87 reddish brown colonies were found on the LB control plates, whereas 29 white and 112 reddish brown colonies were found on the LB + ampicillin plates. ($p < 0.01$ with a Chi-squared analysis.) The white colony size in mm average was slightly larger in LB control plates versus LB + amp plates (18 and 12 respectively), but the reddish brown colony size average was approximately 7 mm in both. It was surmised that this experiment could represents a simulation of what can occur in the body as a result of antibiotic use

For this paper, we repeated the experiment above, but added additional parameters. LB plates with and without amp were left open for three hours at room temperature (RT), in a refrigerator (10^{o}C), and then in a 30^{o}C incubator, and then covered and covered and incubated at 30^{o}C, the optimal temperature for mold growth. Covered controls were also provided. This experiment was conducted in an attempt to learn: a. what molds are present (as spores) in the laboratory air? b. does ampicillin select for different molds? and c. what temperatures provide the optimum growth for this mold? This experiment was an examination of the selective effects of an antibiotic, and does not require any other cultures or animals—just the air.

Materials and Methods

Bottles of Luria Bertani media (water, tryptone, NaCl, yeast extract and agar at pH 7 were melted in a microwave and poured into sterile petri plates. Ampicillin was added at a concentration of 100ul/ml to one-half of the agar after it had cooled to 50^{o}C. Ten plates each were used for the treatment and control plates at room temperature (21^{o}C) and at 10^{o}C, and five plates were used each for the treatment in the 30^{o}C incubator. LB with and without amp was streaked with *E. coli* to test the efficacy of the ampicillin.

Results

There was little or no growth on the closed LB alone plates. (We only used open plates in the 30°C incubator.) The greatest amount of growth occurred on the open LB alone and open LB + amp plates at RT. A Chi-squared analysis revealed a significant difference in distribution of colonies by color under the two treatments. The number of molds/ yeasts by color were: 25 black, 3 brown, 18 orange, 2 tan, 32 white, and 45 yellow for LB amp alone open at RT. The numbers were: 39 black, 6 brown, 7 orange, 7 tan, 20 white, and 3 yellow on LB= amp plates open at RT. ($X2 = 88$; $p<0.01$ for 5 degrees of freedom). There was a significant difference between the number of growths on the LB alone plates (125) versus 81 for the LB + amp at RT. These results vary significantly from what was obtained in the preliminary experiment[1]. There was a greater variety of colors and sizes of molds in our experiment.

The second highest amount of fungal growth occurred on open plates at 30°C. There were twice as many colonies on the LB + amp plates. Growth was the same on both types of plates in the refrigerator at 10°C (16 and 20 colonies respectively). Interestingly, there were approximately the same numbers of growths overall for the entire experiment for both LB alone versus LB + amp plates: 167 and 173. (Figs. 3, 4; Table 1.)

Discussion

This experiment was an attempt to extend a previous experiment in which we showed that there was an increase in fungal growth on plates supplemented with ampicillin versus those that were not. The literature indicates that a side effect of the use of some antibiotics is increased fungal growth. We repeated this experiment under different treatments, including covered and uncovered plates at 10°C in a refrigerator, and uncovered in an incubator at 30°C, which is considered the optimal temperature for fungal growth. We did not find the dramatic differences in growth between LB alone and LB + amp plates that was revealed from a previous experiment[17], but we did find significant differences, using a Chi-squared analysis, between open plates at RT between LB plates alone and LB + amp in this experiment in categories of molds based on color. Future work includes: a) specific chemical tests for fungi b) microscopic examination and photography of molds and yeasts and c) DNA barcoding of specific molds and yeasts that we have refrigerated. If we are able to identify species of fungi, we can then delve into analysis of this environmental microbiome. We might then produce rank-abundance curves, as well as analyze species richness and evenness. We would then be able to calculate species diversity on the various media for comparative purposes, such as Simpson's or Shannon-Weiner Biodiversity indices. Smithee *et al.*[17] added creatinine to their agar (Sabaraud's) to inhibit bacterial growth. We have used Sabaraud's for this project but did obtain too much bacterial growth. Adding creatinine might make an interesting project for isolating bacteria from fungi. Smyth[19] has used experiments such as these to teach her students about the microbiome of urban spaces and the built environment. She and her students design experiments and test hypotheses. The model of using various media alone or supplemented with various antibiotics can teach students about how antibiotics work (or

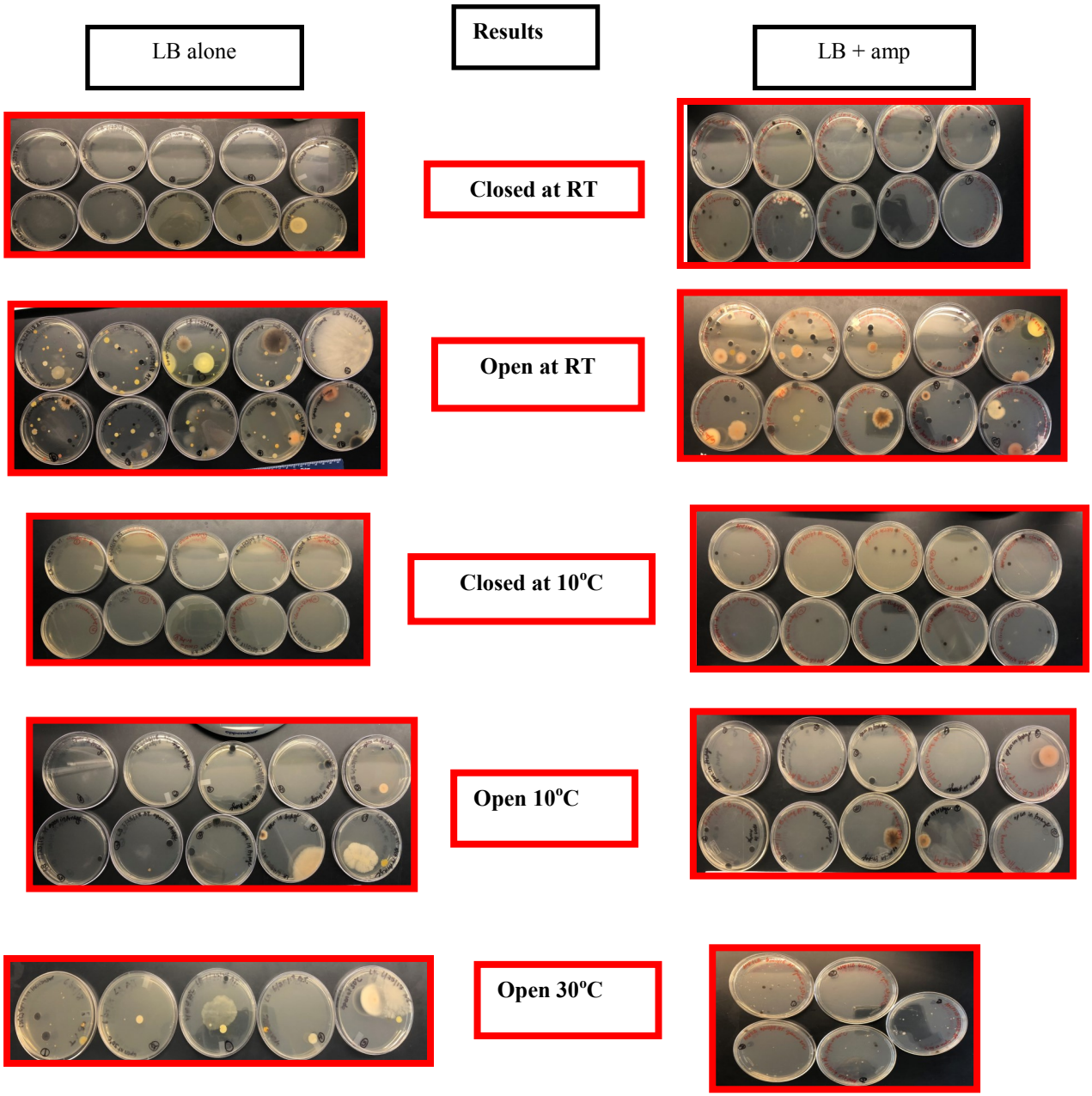
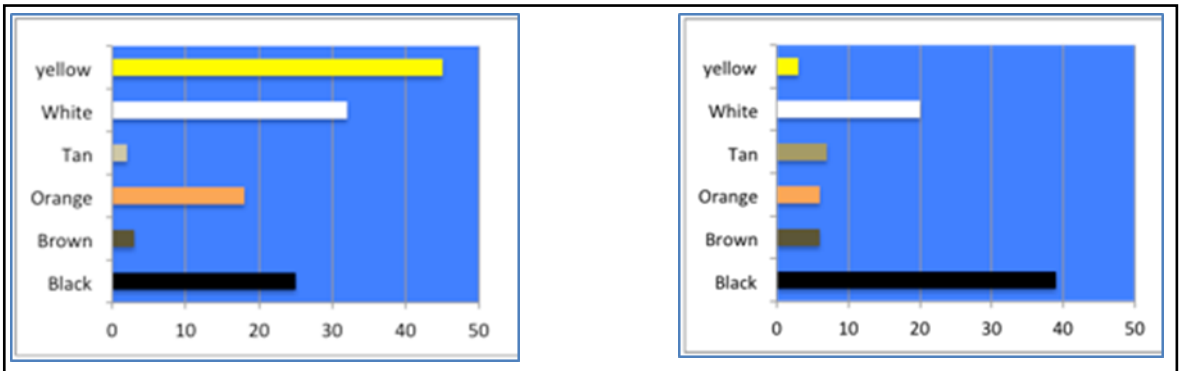


Figure 3. LB plates with and without amp covered or uncovered at RT, 10°C and 30°C.



LB alone open at RT

LB + amp open at RT

Figure 4. A pictorial display of the two different distributions of mold by color.

Table 1. Summary of numbers of molds of various colors on LB plates with and without amp at RT (21°C)

	LB alone (RT)	LB + amp (RT)
Black	25	39
Brown	3	6
Orange	18	6
Tan	2	7
White	32	20
yellow	45	3
TOTALS	125	81

do not work) against microorganisms that are found naturally in the environment around us. The experiments will re-enforce microbiological technique, as well as forge a deeper understanding about microbiology, antibiotics, and our environment.

References

[1]Nolan, K.A., V. Ruiz, A.J. Burdowski, K. Casares and O. Brown, 2019. A simple experiment that reveals overgrowth of fungi as a "side-effect" of antibiotic use. In: McMahon K, editor. Tested studies for laboratory teaching. Proceedings of the 39th Conference of the Association for Biology Laboratory Education (ABLE). **40:** 69: 1-6.

[2]Law K.H., M.W. Tsang, Y.K. Wong, M.S. Tsang, P.Y. Lau, K.Y. Wong, K.P. Ho and Y.C. Leung, 2018. Efficient production of secretory *Streptomyces clavuligerus* β-lactamase inhibitory protein (BLIP) in *Pichia pastoris*. AMB Express. **8(1):** 64-75.

[3]Grenni, P., V. Ancona and A.B. Caracciolo, 2018. Ecological effects of antibiotics on natural ecosystems: a review. Microchemical Journal **136:** 25-39.

[4]Jiang,T.T., T. Shao, W.X.G. Ang, J.K. Kinder, L.H. Turner, G. Pham, J. Whitt, T. Alenghat and S.S. Way, 2017. Commensal fungi recapitulate the protective benefits of intestinal bacteria Cell Host & Microbe **22:** 809-816.

[5]Yu-Kyong, S. and K. Ki-Young, 2017. Ampicillin activates Mpk1 phosphorylation in *Saccharomyces cerevisiae* and ERK1/2 phosphorylation in HepG2 cells. Turkish Journal of Biology **41(4):** 600-607.

[6]Tigini, V., F. Bevione, V. Prigione, A. Poli, L. Ranieri, F. Spennati, G. Munz and G.C. Varese, 2019. Wastewater-Agar as a selection environment: A first step towards a fungal in-situ bioaugmentation strategy. Ecotoxicology and Environmental Safety **171:** 443-450.

[7]Kabbani, T.A., K. Pallav, S.E. Dowd, J. Villafuerte-Galvez, R.R. Vanga, N.E. Castillo, J. Hansen, M. Dennis, D,A. Leffler and C.P. Kelly, 2016. Prospective randomized controlled study on the effects of *Saccharomyces boulardii* CNCM I-745 and amoxicillin-clavulanate or the combination on the gut microbiota of healthy volunteers. Gut microbes **8(1):** 17-32.

[8]Voychuk, S.I., E.N. Gromozova and M.G. Sadovskiy, 2010. The model of fungal population dynamics affected by nystatin. International Journal of Quantum Chemistry, **110(1):** 242-251.

[9]Kathleen Nolan Personal Communications.

[10]Dollive, S., Y.Y Chen, S. Grunberg, K. Bittinger, C. Hoffmann, L. Vandivier, and F.D. Bushman, 2013. Fungi of the murine gut: episodic variation and proliferation during antibiotic treatment. PLoS ONE, 8**(8):** 1-12.

[11]Thompson, D.F. and T.L. Kessler, 2010. Drug-Induced Black Hairy Tongue. Pharmacotherapy **30(6):** 585-593.

[12]Zimmerman, S., C.M. Mitchell, A. Song Beeber, C. Kistler, D. Reed, L. Chisholm and P.D. Sloane, 2015. Strategies to reduce potentially inappropriate antibiotic prescribing in assisted living and nursing homes and inform other quality improvement efforts. American Journal of Medical Research **2(1):** 41-52.

[13]Wiwin, J. and I.P.S. Rejeki, 2015. Microbial Pattern and Antibiotic Sensitivity Test of Hospitalized Children. Folia Medica Indonesiana **51(3):** 190-192.

[14]Anghel, R., D. Jitaru, L. Bãdescu, I. Bararu, M. Ciocoiu, and M. Bãdescu, 2013. Magains and Cecropins, Cytotoxic Peptides with Multiple Biological Effects. Romanian Journal of Functional and Clinical, Macro-Microscopical Anatomy of Anthropology; Revista Româna de Anatomie Functionala Si Clinica, Macro Si Microscopica Si de Antropologie **12 (4):** 292-303.

[15]Ferreira, G.F. and D.A. Santos, 2017. Heteroresistance and fungi. Mycoses **60 (9):** 562-568.

[16]Sangamwar, A.T., U.D. Deshpande and S.S. Pekamwar, 2008. Antifungals: need to search for a new molecular target. Indian Journal of Pharmaceutical Sciences **70(4):** 423-430.

[17]Rojo, D., C. Méndez-García, R.A. Raczkowska, R. Bargiela, A, Moya, M. Ferrara and C. Barbas, 2017. Exploring the human microbiome from multiple perspectives: factors altering its composition and function. FEMS Microbiology Reviews **41(4):** 453-478.
[18]Smithee, S., S. Tracy, K. Drescher, L.A. Pitz and T. McDonald, 2014. A novel, broadly applicable approach to isolation of fungi in diverse growth media. Journal of Microbiological Methods **105:** 155-161.
[19]Smyth, D., 2017. An authentic course based research experience in antibiotic resistance and microbial genomics. Science Education and Civic Engagement: An International Journal **8(2):** 59-65.

The Metropolitan Association of College and University Biologists thanks the following Affiliate Members for their support

AD Instruments
Anatomy in Clay Systems
BioPac Systems
Cengage Learning
Heyden McNeil Publishing
I. Miller Microscopes
John Wiley & Sons
McGraw Hill Publishing
Micro-Optics Precision Instruments
Pasco Scientific
Pearson Education
W. H. Freeman and Company

Please make every effort to support these affiliate members.
Their participation help us keep registration fees at a reasonable price.

Dr. Edward J. Catapane
Department of Biology
Medgar Evers College
1638 Bedford Ave
Brooklyn, New York 11225