IDENTIFICATION AND DISTRIBUTION OF BACTERIA ASSOCIATED WITH LABORATORY CULTURES OF *HYDRA PSEUDOLIGACTIS*

by

James Earl Deramo

Submitted in Partial Fulfillment of the Requirements

for the Degree of

Master of Science

in the

Biology

Program

Paul VanGundy 2/23/73

Adviser Date

Karl E. Kill February 28, 1973

Dean of the Graduate School Date

YOUNGSTOWN STATE UNIVERSITY

March, 1973

ABSTRACT

IDENTIFICATION AND DISTRIBUTION OF BACTERIA ASSOCIATED WITH LABORATORY CULTURES OF *HYDRA PSEUDOLIGACTIS*

James Earl Deramo

Master of Science

Youngstown State University, 1973

Six genera of bacteria, *Flavobacterium*, *Brevibacterium*, *Escherichia*, *Salmonella*, *Arizona*, and *Staphylococcus* were isolated from a laboratory population of *Hydra pseudoligactis* fed *Artemia nauplii*. The population of total bacteria and the per centages of each kind remain constant with time. A significant difference exists between the types of bacteria most closely associated with the inside and outer surface of the hydra itself and with the culture media. The genera *Salmonella* and *Arizona* are the most prevalent bacteria in the media: 50 ± 2%. *Flavobacterium* (orange) are the most prevalent on the hydra's outer surface: 78 ± 1%. *Flavobacterium* (yellow) are the most common on the inside of the hydra: 78 ± 1%.

ACKNOWLEDGEMENTS

I extend special acknowledgements to Paul D. Van Zandt, Ph.D. and Lauren Schroeder, Ph.D., of the Department of Biology, Youngstown State University, for providing encouragement and direction to my research and for providing the materials of this study. I also extend special acknowledgements to all those who were of assistance to me in this project.

TABLE OF CONTENTS

PAGE

ABSTRACT ii

ACKNOWLEDGEMENTS iii

TABLE OF CONTENTS. iv

LIST OF SYMBOLS. v

LIST OF FIGURES. vi

LIST OF TABLES vii

CHAPTER

I. INTRODUCTION. 1

II. MATERIALS AND METHODS 5

III. RESULTS AND DISCUSSION. 10

Identification of Bacteria. 10

Distribution of Bacteria. 19

In the Hydra Media 24

Associated with the Hydra. 27

IV. CONCLUSIONS 35

APPENDIX A. EXPERIMENTAL DATA (TWENTY-FIVE EXPERIMENTS). 36

APPENDIX B. MAINTENANCE CHART 62

REFERENCES CITED 63

LIST OF SYMBOLS

SYMBOL	DEFINITION
$\bar{X}$	Mean
$\bar{\bar{X}}$	Mean of Means
T	Total Bacteria
T_o	Total Bacteria on the Hydra's Outer Surface
T_i	Total Bacteria inside the Hydra
C	Control
Wash 1-3	1 ml sample of each wash
sup. 1 or 2: A or B	first or second supernatant: A = 1 ml sample; B = 2 ml sample
crushed Hydra	Crushed Hydra Tissue
* (in data tables)	Dilution = 1:1,000 all other dilutions = 1:10,000
* (in F tables)	significant at 0.05 level
** (in F tables)	significant at 0.01 level

LIST OF FIGURES

FIGURE PAGE

1. The Compositions of the Bacterial Communities Associated with the Hydra Media, the Hydra's Outer Surface, and Inside the Hydra, Based on Mean Percentages and Standard Deviations of Each Bacterial Group: White, Orange, Yellow, and Beige 22

LIST OF TABLES

TABLE		PAGE
1.	Bacteria Identification *Flavobacterium species* (orange).	12
2.	Bacteria Identification *Flavobacterium species* (yellow).	13
3.	Bacteria Identification *Brevibacterium species* (beige)	14
4.	Bacteria Identification *Salmonella species* (white)	15
5.	Bacteria Identification *Arizona species* (white)	16
6.	Bacteria Identification *Escherichia species* (white)	17
7.	Bacteria Identification *Staphylococcus species* (white)	18
8.	Mean Percentages and Standard Deviations of Each Bacterial Group: In the Hydra Media; on the Hydra's Outer Surface; and Inside the Hydra	21
9.	Sample Experimental Data; Date: April 27 . . .	23
10.	F Values Based on Analyses of Variance of Bacteria in the Hydra Media: Effect of Sampling date (Time); and the Effect of Temperature. . .	25
11.	Regression Coefficient, Squared Deviation, and t Statistic of the Total and Mean Percentages of Bacteria on the Hydra's Outer Surface and Inside the Hydra: A, B, and C	28
12.	F Values Based on Analyses of Variance of Bacteria Associated with the Hydra: Bacteria on Hydra Outer Surface vs. Inside (Media Washed); Bacteria on Hydra Outer Surface vs. Inside (Disinfectant Washed); Bacteria on Inside the Hydra (Media Washed) vs. Inside (Disinfectant Washed)	29

LIST OF TABLES CONTINUED

TABLE PAGE

13. F values based on Analyses of Variance of Mean Percentages of Bacterial Groups Associated with Media and Hydra: Bacteria in the Media vs. Outer Surface of Hydra; Bacteria in the Media vs. Inside the Hydra 33

14. F Values Based on the Analyses of Variance of Mean Percentages of Each Bacterial Group: In the Hydra Media; On the Outer Surface of the Hydra; Inside the Hydra 34

CHAPTER I

INTRODUCTION

Hydra have served in the investigation of biological phenomena from the outset of their discovery by Antony van Leeuwenhoek (1632-1723) in 1702 (Dobell, 1960). Abraham Trembley (1710-1784) published in 1744, the first controlled experiments on hydra, including regeneration, the first successful animal grafting experiments, the first investigations of phototaxis in lower invertebrates, the first vital staining of tissues, and thorough proof of asexual reproduction by budding (Lenhoff and Loomis, 1961). As a result of cutting the polyps lengthwise, into sections, and not completely through, Trembley produced a polyp having seven "heads" and as many bodies, but only one "tail", which he compared to the many-headed mythical monster, Hydre of Lernea. This was the first use of the word 'hydra', by which the animal is now known. Later Linnaeus used the word as a generic name (Lentz, 1966).

Hydra are small, 3 mm to 5 cm long, tubular shaped common freshwater coelenterates with a mouth, surrounded by six to ten tentacles, which opens into a saclike gastrovascular cavity (Lentz, 1966). The hydra, having six basic cell types, is a bicellular leaflet of ectoderm and endoderm separated by a non-cellular mesoglea, which supports the body

and tentacles (Lenhoff and Loomis, 1961).

Usually small crustaceans such as daphnia and cyclops or small annelids serve as food for hydra. Food brought into the digestive cavity is partially broken down by proteolytic enzymes (Beutler, 1924) and ingested by digestive cells. Protein digestion is completed intracellularly (Lenhoff, 1961a). Undigested remnants such as chitinous exoskeletons are ejected, as a cohesive bolus, through the mouth five to six hours after feeding.

Growing hydra in the laboratory in solutions of known composition according to the methods of Loomis (1954) is a development that has enabled investigators to experiment with hydra under controlled and rigorous conditions. There are at least three innate qualities of hydra that make them ideal for certain biological studies: (1) the genotype of the animal is constant when animals descending from a single individual by budding are used; (2) the small size of hydra and lack of skeleton lend them to many quantitative techniques (Lenhoff, 1961b; Lenhoff and Loomis, 1957); and (3) their lack of a definite self-regulated internal extracellular fluid. The culture solution, in place of this fluid, can be regulated and controlled by the experimenter.

Much of what is known today about organization, induction, gradients, polarity, and other growth and regeneration phenomena resulted from research on hydra. Hydra have served as experimental organisms for studies on feeding

response (Loomis, 1955; Lenhoff, 1961a; Forrest, 1962; Burnett, 1963), aging (Strehler, 1961), regeneration (Eakin, 1961), growth factors (Spangenberg, 1961), sexual and cellular differentiation (Loomis, 1961; Slautterback and Fawcett, 1959), epithelial cell interactions (Wood, 1961), pain and sting factors (Welsh, 1961), and ecology (Schroeder, 1969; Forrest, 1963; Slobodkin, 1964; Stiven, 1962).

Hydra release into the media a significant portion of ingested energy in the form of dissolved and particulate organic material (Poddar, 1972). A slime consisting of sloughed cells, egested boli, and other wastes forms on the bottom and sides of the culture dish. These substances then become a source of potential energy within the hydra culture community. The appearance of "cloudy" culture media, due to the excess accumulation of these organic materials, increases the bacterial population and effects a decline in the general health of the hydra population. It is conceivable that bacteria present in the hydra culture may influence many or all of the hydra's physiological activities including growth, respiration, regeneration, aging, and sexual differentiation, which is controlled externally by the media (Loomis, 1961). However, bacteria present in the culture media, are not a source of significant error in experiments measuring absorption and excretion of dissolved organic and inorganic materials (Poddar, 1972).

Although the ecological importance of bacterial

communities is recognized (Odum, 1959), there is to date no accumulation of data concerning the identification and distribution of the bacterial community associated with the hydra and its laboratory environment. This environment may differ between the inside and outer surface of the hydra and cause a difference in the size and structure of the bacterial community associated with the inside and outside of the hydra. Non-motile bacteria are most likely to predominate on the surfaces of the hydra and the culture dish.

Thus, this study is descriptive in nature and two-fold in objective: first, to isolate and identify the bacteria associated with hydra and hydra cultures, and second, to determine relative distribution of the bacterial community associated with hydra and the culture media.

CHAPTER II

MATERIALS AND METHODS

A stock culture of Hydra pseudoligactis was maintained in laboratory culture at 16°C ± 1°C according to the methods of Loomis (1953) and Lenhoff and Brown (1970). A corresponding maintenance and temperature chart was kept (Appendix B). Bacteria were collected from the culture media[1] by means of the membrane filter technique (Standard Methods, 1971). Prior to feeding, two 1 ml samples were taken from the center of the culture dish at a depth approximately halfway between the surface of the media and the bottom of the culture dish. These 1 ml samples of media were serially diluted to a concentration of 1:10,000 using a sterile system of four 16 mm capped test tubes containing 9 ml sterile hydra media. All dilutions were made with sterile 1 ml pipettes. A 1, 2, and 5 ml sample of each 1:10,000 dilution was transferred respectively into three sterile 15 ml Millipore apparatus funnels, and a 3 ml sample of each 1:1,000 dilution was transferred to a fourth sterile Millipore funnel. Each funnel was previously fitted with a 24 mm Millipore membrane having a 0.45 u pore size (Millipore, 1969). Membranes were aseptically transferred,

---

[1] 10^{-3}N $CaCl_2$, 10^{-4}N KCl, 10^{-4}N $NaHCO_3$ in distilled, deionized H_2O.

using alcohol dipped forceps, to 20 ml Brain Liver Heart agar plates (Difco, 1953). Four replicates were incubated at 16°C, 48 hrs. and four replicates at 30°C, 24 hrs. In experiments in which the presence of the genera Escherichia or Staphylococcus were to be determined, the membranes were incubated on Eosin Methylene Blue (EMB) agar and Staphylococcus 110 Medium respectively. Also, a 1 ml sample of a 1:10,000 media control was filtered and incubated according to the procedure given above. After incubation all plates were examined under a binocular scope at 10x and total colony counts were made for each replicate. The number of each type of colony was also counted on the basis of color: white, orange, yellow, beige. This experiment was performed twenty-five times on different days (Appendix A).

Bacteria associated with the outer surface of the hydra were washed from single unbudded hydra. Each hydra was transferred, using sterile forceps, from the stock culture, prior to feeding, to a capped sterile 16 mm test tube containing 10 ml of sterile hydra media. The hydra was agitated gently for 15 seconds using a Vortex Junior mixing apparatus. Each hydra was washed a total of three times, being transferred from one test tube to the next using sterile forceps. 1 ml of each 10 ml wash and a media control, without dilution, was filtered, plated, incubated, and counted as described above.

Bacteria inside the hydra were collected by crushing the "washed" hydra, from the previous procedure, in a sterile

15 ml pyrex glass tissue crushing apparatus containing ½ ml sterile hydra media. The hydra was maneuvered so that it rested on the side of the tubular mortar rather than at the bottom. The crushing pestal was turned three times, the last time with a quick upward motion. This sample, containing the ground hydra tissue, was rinsed with 9½ ml of sterile hydra media into a sterile 12 ml conical centrifuge tube, resuspended and centrifuged on a standard seriological table-top laboratory centrifuge (International Clinical Centrifuge, Model CL), at half speed for three minutes. A 1 and 2 ml sample of the resulting supernatant were filtered. The remaining supernatant was decanted. The tissue was resuspended in 10 ml sterile media, centrifuged for three minutes at half speed, and a 1 ml sample of the second supernatant was filtered. The second supernatant was then decanted. The remaining crushed hydra tissue was removed, with a minimum amount of sterile media, from the centrifuge tube using a sterile 1 ml pipette and filtered. All samples, including a media control, were plated, incubated at 30°C, and counted as described above.

To evaluate the effectiveness of the washing and crushing technique, to determine the bacteria on the outer surface and inside of a hydra, a second unbudded hydra was removed, using sterile forceps, from the stock culture, prior to feeding, to a capped sterile 16 mm test tube containing 10 ml of Zephiran Chloride (1:750) disinfectant. The hydra was agitated gently for 10 seconds using a Vortex Junior mixing apparatus. The hydra was transferred, using

sterile forceps, to a capped sterile 16 mm test tube containing 9 ml sterile hydra media. It was washed a total of three times and crushed, and samples were filtered, incubated, and counted according to the procedure described above. A control of ½ ml sterile hydra media was used in all hydra crush experiments. These experiments were done twenty-five times on different days, coinciding with the media sampling experiments (Appendix A).

The streak plate method was also employed to determine bacteria associated with the slime layer covering the surfaces of the hydra culture dish. A flamed wire inoculating loop was streaked across the bottom of the stock culture dish several times. Two Brain Liver Heart agar plates were streaked with this inoculum. One plate was incubated at 16°C, 48 hrs., the other at 30°C, 24 hrs. On the basis of colonial appearance, colonies were isolated, and pure cultures were made for identification. This procedure was performed six times on six different days.

Basic microbiological procedures for isolation, culturing, staining, and biochemical testing were employed in the identification of bacteria (Bradshaw, 1963). The bacteria were identified to genus by verification of test results according to Bergey's Manual of Determinative Bacteriology (1957).

Morphology of each type of bacteria was noted according to its colonial appearance: size, color, edge, consistency, and odor. Also, spore, capsule, acid fast, and Gram stains were made. The physiology of each type

of bacteria was tested by the fermentation of the following sugars: adonitol, arabinose, cellobiose, dextrin, dextrose, dulcitol, fructose, galactose, inositol, inulin, lactose, maltose, mannitol, melibiose, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, and xylose. The following miscellaneous tests were performed: catalase, citrate, coagulase, gelatin, H_2S, indol, litmus milk, motility, methyl red, Voges-Proskauer, nitrate, oxidase, oxygen, starch, temperature, urea, O.N.P.G. (o-nitrophenyl-B-D-galactopyranoside), P.D. (phenylalanine deaminase), L.D. (lysine decarboxylase), and C.O. (cytochrome oxidase). The following special media was employed: Mannitol Salt Agar, MacConkey's Agar, EMB (Eosin Methylene Blue) Agar, Bismuth Sulfite Agar, S-S Agar, Staphylococcus 110 Medium, Hektoen Agar, Blood Agar, and the TSI (Triple Sugar Iron) Slant. All tests were performed six times on each organism on six different days.

Analysis of variance, Student's t test, and linear regression were used where applicable to analyze all numerical data.

CHAPTER III

RESULTS AND DISCUSSION

The results of this study are divided into two sections. First, the identification of each kind of bacteria isolated from the hydra and the hydra culture. Second, the distribution of the bacterial community in the hydra media and on the hydra. This distribution is defined by the results of the statistical analysis applied to the total bacteria and the percentages of each bacterial group (colored: white, orange, yellow, and beige) associated with the hydra and the media.

IDENTIFICATION OF BACTERIA

The results of all microbiological and biochemical tests performed on each kind of bacteria isolated are presented in Tables 1-7. The bacteria identified belong to the genera: Flavobacterium, Brevibacterium, Salmonella, Arizona, Escherichia, and Staphylococcus. On the basis of consistent morphological characteristics, i.e., colonial appearance, four groups were observed. These four groups are comprised of bacteria whose colonies are colored white, orange, yellow, or beige when grown on Brain Liver Heart agar plates. The bacteria were isolated from the hydra media, the hydra, and the film sediment associated with the bottom of the hydra culture dish. Each bacterial group was tested six different times, and all times

identical results were obtained for each respective group. The results of all tests were compared to standard tabulated test results (Breed, 1957). Thus, it was determined that the orange and yellow colonies were a species of Flavobacterium, the beige colonies were a species of Brevibacterium, and among the whitish-gray colored colonies were species of the genera: Salmonella, Arizona, Escherichia, and Staphylococcus. All six genera were isolated from the film sediment of the culture dish by the streak plate method. However, no members of the genera Escherichia or Staphylococcus were isolated from the media or the hydra's outside or inside. This was determined by growing samples on EMB and Staphylococcus 110 Medium respectively. All the genera of bacteria isolated from the hydra and its culture are common to freshwater and hence, to environmental conditions suitable to hydra.

Flavobacterium occurs in freshwater and characteristically produces orange, yellow, red, or yellow-brown pigmentation. Many freshwater Flavobacterium have been isolated from the slime of fishes, and these bacteria, like those identified in this study, are non-motile (Breed, 1957). Thus, the association of Flavobacterium with the surfaces of hydra is not unlikely. Brevibacterium, found in freshwater, is generally non-motile, and produces a red, orange, or brown pigmentation. The species isolated in this study produces a beige, flesh-colored surface colony typical of Brevibacterium brunneum, originally isolated

Table 1

BACTERIA IDENTIFICATION

Name of Organism: Flavobacterium species (orange)

MORPHOLOGY:

Colonial Appearance:	Brain Liver Heart Agar	Staining:
size	2-3 mm	spore -
color	dirty orange	capsule -
edge	entire	acid fast -
consistency	clay-like	Gram Stain: gram negative, coccobacillus (24 hr. culture)

PHYSIOLOGY:

Fermentation:		Miscellaneous:		Special Media:
adonitol	-			Mannitol Salt Agar -
arabinose	+	catalase	+	NA
cellobiose	+	citrate	-	
dextrin	-	coagulase	NA*	MacConkey's Agar -
dextrose	+	gelatin	-	no growth
dulcitol	-	H_2S	-	
fructose	+	indol	-	EMB Agar -
galactose	+	litmus milk	+	NA
inositol	-	motility	-	
inulin	-	MR	-	Bismuth Sulfite Agar -
lactose	-	VP	-	NA
maltose	+	Nitrate	-	
mannitol	-	oxidase	NA	S-S Agar-
melibiose	-	oxygen	+	NA
raffinose	+	starch	-	
rhamnose	-	temperature	30°C	Staph. 110 Medium -
salicin	-	urea	-	NA
sorbitol	-	O.N.P.G.	NA	
sucrose	+	P.D.	"	Hektoen Agar -
trehalose	V	L.D.	"	NA
xylose	+	C.O.	"	

Blood Agar - good growth, no hemolysis

TSI Slant - orange slant and butt, no H_2S produced, no gas produced

NOTE: Surface colonies on BLH agar were always large (2-3mm) and dirty orange in color.

*NA: not applicable

Table 2

BACTERIA IDENTIFICATION

Name of Organism: Flavobacterium species (yellow)

MORPHOLOGY:

Colonial Appearance:	Brain Liver Heart Agar	Staining:
size	1-3 mm	spore -
color	yellow	capsule -
edge	entire	acid fast -
consistency	creamy	Gram stain: gram negative, small thin rods (24 hr. culture)
odor	repugnant	

PHYSIOLOGY:

Fermentation:		Miscellaneous:		Special Media:
adonitol	-			Mannitol Salt Agar - NA
arabinose	+	catalase	+	
cellobiose	+	citrate	-	
dextrin	-	coagulase	NA*	MacConkey's Agar - no growth
dextrose	+	gelatin	-	
dulcitol	-	H_2S	-	
fructose	+	indol	-	EMB Agar - NA
galactose	+	litmus milk	+	
inositol	-	motility	-	
inulin	-	MR	-	Bismuth Sulfite Agar - NA
lactose	-	VP	-	
maltose	+	nitrate	-	
mannitol	-	oxidase	NA	S-S Agar - NA
melibiose	-	oxygen	+	
raffinose	-	starch	-	
rhamnose	-	temperature	30°C	Staph. 110 Medium - NA
salicin	-	urea	-	
sorbitol	-	O.N.P.G.	NA	
sucrose	+	P.D.	"	Hektoen Agar - NA
trehalose	V	L.D.	"	
xylose	+	C.O.	"	Blood Agar - growth, no hemolysis
				TSI Slant - orange slant and butt, no H_2S produced, no gas produced

NOTE: Surface colonies on BLH agar were always small (1-2mm) and yellow.

*NA: not applicable

Table 3

BACTERIA IDENTIFICATION

Name of Organism: Brevibacterium species (beige)

MORPHOLOGY:

Colonial Appearance: Brain Liver Heart Agar

size	1-2 mm
color	beige-flesh
edge	entire
consistency	creamy
odor	cheesy

Staining:

spore	-
capsule	-
acid fast	-

Gram stain: gram positive, very short thin rods (24 hr. culture)

PHYSIOLOGY:

Fermentation:

adonitol	-
arabinose	-
cellobiose	-
dextrin	-
dextrose	-
dulcitol	-
fructose	-
galactose	-
inositol	-
inulin	-
lactose	-
maltose	-
mannitol	-
melibiose	-
raffinose	-
rhamnose	-
salicin	-
sorbitol	-
sucrose	-
trehalose	-
xylose	-

Miscellaneous:

catalase	+
citrate	-
coagulase	NA*
gelatin	+
H_2S	-
indol	-
litmus milk	-
motility	-
MR	-
VP	-
nitrate	-
oxidase	NA
oxygen	+
starch	-
temperature	30°C
urea	-
O.N.P.G.	NA
P.D.	"
L.D.	"
C.O.	"

Special Media:

Mannitol Salt Agar - NA

MacConkey's Agar - NA

EMB Agar- NA

Bismuth Sulfite Agar - NA

S-S Agar - NA

Staph. 110 Medium - NA

Hektoen Agar - NA

Blood Agar - no growth

TSI Slant - no growth

NOTE: Surface colonies on BLH agar were always small (1-2 mm) and beige-flesh colored.

*NA: not applicable

Table 4

BACTERIA IDENTIFICATION

Name of Organism: Salmonella species (white)

MORPHOLOGY:

Colonial Appearance: Brain Liver Heart Agar

size	1-3 mm
color	whitish-gray
edge	entire
consistency	creamy
odor	fecal

Staining:

spore	-
capsule	-
acid fast	-

Gram stain: gram negative, rods (24 hr. culture)

PHYSIOLOGY:

Fermentation:

adonitol	-
arabinose	+
cellobiose	+
dextrin	-
dextrose	+ gas
dulcitol	+
fructose	-
galactose	-
inositol	+
inulin	-
lactose	-
maltose	+
mannitol	+
melibiose	-
raffinose	-
rhamnose	+
salicin	-
sorbitol	+
sucrose	-
trehalose	+
xylose	+

Miscellaneous:

catalase	+
citrate	+
coagulase	NA*
gelatin	-
H_2S	+
indol	-
litmus milk	+
motility	+
MR	+
VP	-
nitrate	+
oxidase	-
oxygen	+
starch	+
temperature	30°C
urea	-
O.N.P.G.	-
P.D.	-
L.D.	+
C.O.	NA

Special Media:

Mannitol Salt Agar - NA

MacConkey's Agar - slight beige colored growth

EMB Agar - pink colonies

Bismuth Sulfite Agar - large black colonies

S-S Agar - good growth, pink colonies, yellow discoloration

Staph. 110 Medium - NA

Hektoen Agar - NA

Blood Agar - no hemolysis

TSI Slant - alkaline slant, black butt with gas, H_2S produced

NOTE: Surface colonies on BLH agar were medium (1-3 mm) and whitish-gray in color, but not easily distinguished from other white colonies.

*NA: not applicable

Table 5

BACTERIA IDENTIFICATION

Name of Organism: Arizona species (white)

MORPHOLOGY:

Colonial Appearance:	Brain Liver Heart Agar	Staining:
size	2-3 mm	spore -
color	whitish-gray	capsule -
edge	entire	acid fast -
consistency	creamy	Gram stain: - gram negative, short rods (24 hr. culture)
odor	repugnant	

PHYSIOLOGY:

Fermentation:		Miscellaneous:		Special Media:
adonitol	-			Mannitol Salt Agar - NA
arabinose	+	catalase	+	
cellobiose	+	citrate	+	
dextrin	-	coagulase	NA*	MacConkey's Agar - large brick red colonies
dextrose	+ gas	gelatin	+	
dulcitol	-	H_2S	+	EMB Agar - pink colonies
fructose	-	indol	-	
galactose	-	litmus milk	+	
inositol	-	motility	+	
inulin	-	MR	+	Bismuth Sulfite Agar - large black colonies
lactose	+	VP	-	
maltose	+	nitrate	+	
mannitol	+	oxidase	-	S-S Agar - pink colonies, yellow discoloration
melibiose	-	oxygen	+	
raffinose	-	starch	-	
rhamnose	+	temperature	30°C	Staph. 110 Medium - NA
salicin	-	urea	-	
sorbitol	+	O.N.P.G.	+	
sucrose	-	P.D.	-	Hektoen Agar - NA
trehalose	+	L.D.	+	
xylose	+	C.O.	NA	
				Blood Agar - no hemolysis
				TSI Slant - yellow slant, black butt with gas, H_2S produced

NOTE: Surface colonies on BLH agar were large (2-3 mm) and whitish-gray in color, but not easily distinguished from other white colonies.

*NA: not applicable

Table 6

BACTERIA IDENTIFICATION

Name of Organism: Escherichia species (white)

MORPHOLOGY:

Colonial Appearance:	Brain Liver Heart Agar	Staining:
size	1-3 mm	spore -
color	whitish-gray	capsule -
edge	entire	acid fast -
consistency	creamy	Gram stain: gram negative, short rods (24 hr. culture)
odor	fecal	

PHYSIOLOGY:

Fermentation:		Miscellaneous:		Special Media:
adonitol	-			Mannitol Salt Agar - NA
arabinose	+	catalase	+	
cellobiose	-	citrate	+	
dextrin	+	coagulase	NA*	MacConkey's Agar - brick red colonies
dextrose	+	gelatin	-	
dulcitol	+	H_2S	-	
fructose	+	indol	-	EMB Agar - colonies with green metallic sheen
galactose	+	litmus milk	-	
inositol	-	motility	-	
inulin	-	MR	+	Bismuth Sulfite Agar - NA
lactose	+	VP	-	
maltose	+	nitrate	+	
mannitol	+	oxidase	NA	S-S Agar - NA
melibiose	+	oxygen	+	
raffinose	-	starch		
rhamnose	+	temperature	30°C	Staph. 110 Medium - NA
salicin	-	urea	-	
sorbitol	+	O.N.P.G.	NA	
sucrose	-	P.D.	"	Hektoen Agar - orange-yellow colonies
trehalose	+	L.D.	"	
xylose	+	C.O.	"	Blood Agar - no hemolysis
				TSI Slant - yellow slant and butt, no H_2S produced

NOTE: Surface colonies on BLH agar were always medium (1-3 mm) and not easily distinguished from other white colonies.

*NA: not applicable

Table 7

BACTERIA IDENTIFICATION

Name of Organism: Staphylococcus species (white)

MORPHOLOGY:

Colonial Appearance:	Brain Liver Heart Agar	Staining:
size	1-2 mm	spore -
color	white	capsule -
edge	entire	acid fast -
consistency	creamy	Gram stain: gram positive, cocci in grape-like clusters (24 hr. culture)
odor	none	

PHYSIOLOGY:

Fermentation:		Miscellaneous:		Special Media:
adonitol	-			Mannitol Salt Agar - good growth, small colonies, no discoloration
arabinose	-	catalase	+	
cellobiose	-	citrate	+	
dextrin	V	coagulase	-	MacConkey's Agar - NA
dextrose	+	gelatin	-	
dulcitol	V	H_2S	-	
fructose	+	indol	NA*	EMB Agar - NA
galactose	+	litmus milk	+	
inositol	V	motility	-	
inulin	-	MR	NA	Bismuth SulfiteAgar - NA
lactose	+	VP	NA	
maltose	+	nitrate	+	
mannitol	-	oxidase	NA	S-S Agar - NA
melibiose	V	oxygen	+	
raffinose	-	starch	-	
rhamnose	-	temperature	30°C	Staph. 110 Medium - very good growth, large white colonies
salicin	-	urea	NA	
sorbitol	-	O.N.P.G.	NA	Hektoen Agar - NA
sucrose	+	P.D.	"	
trehalose	+	L.D.	"	
xylose	-	C.O.	"	Blood Agar - no hemolysis
				TSI Slant - yellow slant and butt, no H_2S produced

NOTE: Surface colonies on BLH agar were always small (1-2 mm) and not easily distinguished from other white colonies.

*NA: not applicable

from Pittsburgh, Pennsylvania tap water (Breed, 1957). Salmonella is found in water, food, and the bodies of cold and warm blooded animals. The bacteria isolated exhibits the basic characteristics of the genus Salmonella and represents one of the species compatible to freshwater and a temperature range of 16°C to 30°C, at which experimental bacterial cultures were incubated (Breed, 1957). The genus Arizona is widely distributed in surface water, soil, and in the bodies of cold and warm blooded animals. It is motile and bears close resemblance to the genus Salmonella. Escherichia is also widely distributed in nature. Its agar colonies are generally whitish-gray and it is either motile or non-motile. The coliform isolated from the film sediment of the hydra culture closely resembles Escherichia intermedia, a non-fecal, freshwater coliform with a growth range of 10°C to 47°C. Escherichia would be well-suited to the temperature of the hydra culture, 16°C ± 1°C (Breed, 1957). Staphylococcus is tolerant of media containing high levels of salt, and is widely distributed. It is often found on body surfaces, skin, or mucous membranes of man and other animals. The Staphylococcus isolated here was found only in the film sediment of the culture dish and may have been present as a contaminant (Breed, 1957).

DISTRIBUTION OF BACTERIA

The composition of the bacterial community associated with the hydra media and the hydra outside and inside

is presented in Table 8 and in Figure 1. The white colored bacterial group is the most common (50 ± 2%) in the hydra media. This group represents the motile genera, Salmonella and Arizona, which are least commonly associated with the hydra's outer surface and inside. This distribution is understandable since these bacteria are motile. The orange species of Flavobacterium is by far the most numerous (78 ± 1%) of the bacteria associated with the outer surface of the hydra. It is also the major Flavobacterium present in the hydra media (27 ± 1%). The yellow species of Flavobacterium is the predominant bacterial group associated with the inside of the hydra. It is not as common as the orange Flavobacterium in the hydra medium or on the hydra's outer surface. Thus, these non-motile species of Flavobacterium are associated chiefly with the hydra. This is also reasonable since these bacteria are often isolated from such sources as the slime of freshwater fishes (Breed, 1957). Also, since these Flavobacterium are non-motile, a close association with sessile surfaces is expected. The Brevibacterium is more closely associated with the inside than the outer surface of the hydra, but the highest percentage of Brevibacterium exists in the media.

In Table 9 is presented a sample set of data used for statistical analysis in this study. This data is, in form, representative of the data of all twenty-five daily experiments. The sample presented is the actual data of April 27, the date of the first experiment performed. All

TABLE 8

MEAN PERCENTAGES AND STANDARD DEVIATIONS OF EACH BACTERIAL GROUP: IN THE HYDRA MEDIA; ON THE HYDRA'S OUTER SURFACE; AND INSIDE THE HYDRA

	MEDIA	HYDRA OUTSIDE	HYDRA INSIDE
% White	50 ± 2	1 ± 0.5	2 ± 1
% Orange	27 ± 1	78 ± 1	18 ± 1
% Yellow	17 ± 1	18 ± 1	78 ± 1
% Beige	8 ± 1	2 ± 1	3 ± 1

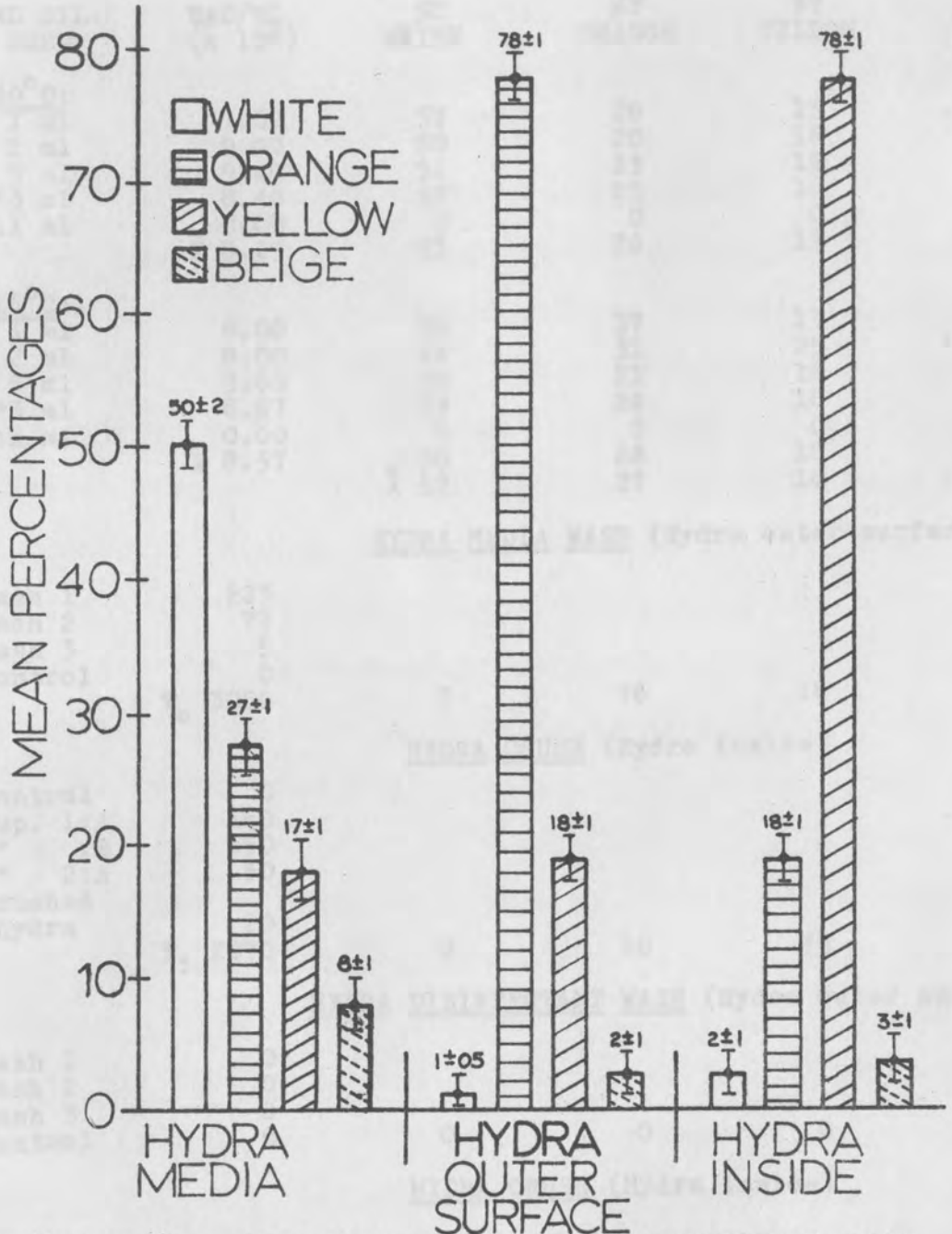

figure1: THE COMPOSITION OF THE BACTERIAL COMMUNITIES ASSOCIATED WITH THE HYDRA MEDIA, THE HYDRA'S OUTER SURFACE, AND INSIDE THE HYDRA, BASED ON MEAN PERCENTAGES AND STANDARD DEVIATIONS OF EACH BACTERIAL GROUP: WHITE, ORANGE, YELLOW, & BEIGE

data used for statistical analysis and all raw data, for all twenty-five experiments, is found in Appendix A. Analysis of variance, Student's t test, and linear regression were employed to determine if all twenty-five experiments were replicates.

DISTRIBUTION OF BACTERIA IN THE HYDRA MEDIA

In each of the twenty-five experiments performed on the hydra media, there were eight replicates: four incubated at 30°C and four at 16°C. Incubation at two temperatures was employed in order to detect variations in the types of bacteria sampled. Thus, species of bacteria would not be eliminated from observation by a non-permissive temperature. All twenty-five experiments for determining total bacteria per ml and the percentages of bacterial groups per ml in the media (Table 9) were shown to be replicates by the analysis of variance. All replicate values of total bacteria per ml, both at 30°C and 16°C, for all twenty-five experiments, were submitted to analysis of variance; the F value was insignificant (Table 10). Therefore, there is no significant difference in the total bacteria per ml for the replicates of all twenty-five experiments whether incubated at 30°C or 16°C, or sampled on different days. Also, all replicate values for percentages of each bacterial group per ml were submitted to analysis of variance; these F values were insignificant. Thus, the twenty-five experiments performed were treated as replicates.

TABLE 10

F VALUES BASED ON ANALYSES OF VARIANCE OF BACTERIA IN THE HYDRA MEDIA: EFFECT OF SAMPLING DATE (TIME): AND THE EFFECT OF TEMPERATURE

	Time Sampling Date	Temperature 30°C vs 16°C
Total bacteria/ml	1.30	0.09
% White/ml	0.80	0.52
% Orange/ml	0.80	0.46
% Yellow/ml	0.88	0.03
% Beige/ml	1.04	0.35
Tabulated F (0.05)	1.39	3.84
Degrees of freedom	49-150	1-198

* Denotes significance at the 0.05 level

** Denotes significance at the 0.01 level

Since there was no difference due to the date of sampling, all data of total bacteria per ml incubated at 30°C were combined and compared, by analysis of variance, to all combined data of total bacteria per ml incubated at 16°C. The results confirm that no significant difference exists between replicates incubated at either temperature (Table 10). Also, all replicate values for the percentages of each bacterial group per ml were combined and analyzed in the same manner; the same results were obtained (Table 10).

Since the bacteria present in the hydra culture are equally viable at 30°C and 16°C, incubation temperature is not a source of variation in colony count data. Also, since the total bacteria per ml and the percentages of each bacterial group per ml do not change significantly with time, a relatively stable bacterial community is present in the hydra culture media. This suggests a fairly stable environment in which conditions are similar day to day (Appendix B). Culturing hydra according to the methods of Loomis (1953) and Lenhoff and Brown (1970) provides uniform environmental conditions. These culturing methods are highly suitable for growing hydra, and possibly the bacterial community present under such conditions may essentially be considered no source of deleterious interference to hydra.

DISTRIBUTION OF BACTERIA ASSOCIATED WITH THE HYDRA

One replicate was performed per experiment to determine the composition and distribution of the bacterial community on the hydra's outer surface and inside. The results of linear regression and the Student's t test indicate that the total bacteria and the percentages of each bacterial group on the outer surface of a hydra, and on the inside of a hydra first washed with media, and on the inside of a hydra first washed with disinfectant do not change significantly with time since the slopes of these regressed lines are not significantly different from zero (Table 11). This indicates that the bacterial community associated with the outer surface and inside of the hydra is constant under similar daily conditions. The results of the twenty-five washing and crushing experiments (Table 9 and Appendix A) do not change significantly with time. Hence, these experiments may be treated as replicates.

By the analysis of variance it was determined that there is a significant difference in the total bacteria and in the percentages of each bacterial group between the outer surface and inside of a hydra washed first with media and then crushed (Table 12). There is also a significant difference in the total bacteria and in the percentages of each bacterial group between the outer surface of a hydra and the inside of a hydra first washed in disinfectant and then crushed (Table 12). The composition of

TABLE 11

REGRESSION COEFFICIENT, SQUARED DEVIATION, AND t STATISTIC OF THE TOTAL AND MEAN PERCENTAGES OF BACTERIA ON THE HYDRA'S OUTER SURFACE AND INSIDE THE HYDRA: A, B, AND C

A. Bacteria on Hydra's Outer Surface.

B. Bacteria on Hydra Inside (media washed).

C. Bacteria on Hydra Inside (disinfectant washed).

	Regression Coefficient	Squared Deviation	T Statistic (0.01 = 2.807)
Total Bacteria:			
A.	0.154	17.728	0.300
B.	-0.142	14.519	0.340
C.	0.559	14.144	1.371
% White:			
A.	0.007	0.461	0.491
B.	0.007	0.671	0.345
C.	-0.005	0.600	0.266
% Orange:			
A.	0.087	1.076	2.800
B.	0.028	0.770	1.280
C.	0.074	0.929	2.751
% Yellow:			
A.	0.052	0.919	1.970
B.	0.081	1.382	2.023
C.	0.013	1.386	0.327
% Beige:			
A.	-0.088	0.733	4.136**
B.	-0.105	0.828	4.370**
C.	0.092	0.969	3.294**

** Denotes significance at the 0.01 level

TABLE 12

F VALUES BASED ON ANALYSES OF VARIANCE OF BACTERIA ASSOCIATED WITH THE HYDRA: BACTERIA ON HYDRA OUTER SURFACE VS. INSIDE (MEDIA WASHED); BACTERIA ON HYDRA OUTER SURFACE VS. INSIDE (DISINFECTANT WASHED); BACTERIA INSIDE THE HYDRA (MEDIA WASHED) VS. INSIDE (DISINFECTANT WASHED)

	Outer Surface vs. Inside (media washed)	Outer Surface vs. Inside (disnf. washed)	Inside (media washed) vs. Inside (disnf. washed)
Total bacteria	43001.41**	42121.86**	0.14
% White	4.66*	10.75**	0.27
% Orange	39640.36**	32667.36**	0.34
% Yellow	26211.64**	29089.08**	0.00
% Beige	0.28	3.24	1.46
Tabulated F (0.05)	4.04	4.04	4.04
Degrees of freedom	1-48	1-48	1-48

* Denotes significance at the 0.05 level

** Denotes significance at the 0.01 level

the bacterial community associated with the outer surface and inside of a hydra is significantly different, and remains relatively constant under the similar daily conditions maintained in this study (Appendix B).

There is, however, no significant difference in the total bacteria and in the percentages of each bacterial group between the inside of a hydra washed with media first and then crushed and the inside of a hydra washed with disinfectant first, media next, and then crushed (Table 12). This suggests that both techniques of washing and crushing hydra are effective in determining the bacteria associated with its outer surface and inside. Since successive washing of the hydra in sterile media removed nearly all bacteria present on its outer surface, the bacteria recovered on crushing the hydra should be associated with the inside (Table 9 and Appendix A). To check this technique, all bacteria on the outer surface of the hydra were killed by washing in Zephiran Chloride (1:750). There were no bacteria isolated from the washings of a hydra which had been washed in disinfectant first (Table 9 and Appendix A). It was assumed bacteria on the inside of the hydra would not be effected because the mouth of the hydra, open only during feeding, would be closed while being washed. There is no significant difference in the composition of the bacterial community between the inside of a hydra washed in media only and a hydra washed in disinfectant first, and media next. Therefore, the bacteria isolated from the

hydra by crushing are probably associated with the inside of the hydra. This is substantiated further because there is a significant difference in the composition of the bacterial community washed from the outer surface of the hydra and the community associated with the inside of the hydra, whether washed in media only, or in disinfectant and media. The total bacteria present on the outer surface of the hydra is significantly larger than inside the hydra.

The percentages of each bacterial group in the media is significantly different from these percentages on the hydra's outer surface and inside the hydra (Table 13). Therefore, the composition of the bacterial community associated with the media is different in structure from that associated with the hydra itself. Also, within the media itself, there is a significant difference between the percentages of each bacterial group (Table 14). The same is true for percentages of each bacterial group on the hydra's outer surface and inside the hydra (Table 14). The analysis of variance for Tables 13 and 14 was based on the mean of all mean percentages of each bacterial group associated with the media, the percentage of each group associated with the hydra's outer surface, and the mean percentages of the groups associated with the inside of the hydra, washed with media or disinfectant (Table 9 and Appendix A). Thus, the compositions of the bacterial communities in the media, and on the hydra's outer surface and inside are significantly different. Also, the

percentages of the four bacterial groups in the media, and on the hydra's outer surface, and inside are significant.

TABLE 13

F VALUES BASED ON ANALYSES OF VARIANCE OF MEAN PERCENTAGES OF BACTERIAL GROUPS ASSOCIATED WITH MEDIA AND HYDRA: BACTERIA IN THE MEDIA VS. OUTER SURFACE OF HYDRA; BACTERIA IN THE MEDIA VS. INSIDE THE HYDRA

	Media vs. Hydra Outer Surface	Media vs. Inside The Hydra
% White	13931.74**	13462.00**
% Orange	21493.88**	872.19**
% Yellow	16.41**	30363.72**
% Beige	205.30**	163.56**
Tabulated F (0.05)	4.04	4.04
Degrees of freedom	1-48	1-48

TABLE 14

F VALUES BASED ON THE ANALYSES OF VARIANCE OF MEAN PERCENTAGES OF EACH BACTERIAL GROUP: IN THE HYDRA MEDIA; ON THE OUTER SURFACE OF THE HYDRA; INSIDE THE HYDRA

	In The Media	On The Hydra's Outer Surface	Inside The Hydra
$\bar{X}$% White vs. $\bar{X}$% Orange	2443.90**	80542.50**	7360.09**
$\bar{X}$% White vs. $\bar{X}$% Yellow	4591.57**	5716.13**	104621.88**
$\bar{X}$% White vs. $\bar{X}$% Beige	7525.09**	44.89**	50.23**
$\bar{X}$% Orange vs. $\bar{X}$% Yellow	730.94**	43082.79**	50820.77**
$\bar{X}$% Orange vs. $\bar{X}$% Beige	2712.50**	54909.95**	3649.06**
$\bar{X}$% Yellow vs. $\bar{X}$% Beige	540.15**	2974.83**	69886.81**
Tabulated F (0.05)	4.04	4.04	4.04
Degrees of freedom	1-48	1-48	1-48

CHAPTER IV

CONCLUSIONS

1. The bacteria associated with the hydra and its culture are members of the genera: Flavobacterium, Brevibacterium, Salmonella, Arizona, Escherichia, and Staphylococcus.

2. The total bacteria and the percentages of each bacterial group do not change significantly with time in the hydra media, or on the hydra's outer surface or inside. Under similar daily environmental conditions there is a stable bacterial community.

3. The compositions of the bacterial communities in the media, and on the hydra's outer surface and inside are significantly different. Also, the percentages of the four bacterial groups in the media and on the hydra's outer surface and inside the hydra are significant.

4. The bacterial group displaying white colonies, determined to be the motile Salmonella and Arizona, is predominant in the media.

5. The bacterial group displaying orange-colored colonies, a non-motile Flavobacterium, is predominant on the outer surface of the hydra.

6. The bacterial group displaying yellow colonies, a non-motile Flavobacterium, is predominant inside the hydra.

APPENDIX A

EXPERIMENTAL DATA: BACTERIAL COLONY COUNTS FOR TWENTY-FIVE EXPERIMENTS

LIST OF SYMBOLS

SYMBOL	DEFINITION
$\bar{X}$	Mean
$\bar{\bar{X}}$	Mean of Means
T_o	Total bacteria outside hydra
T_i	Total bacteria inside hydra
C	Control
Wash 1-3	1 ml sample of each wash
sup. 1 or 2: A or B	first or second supernatant: A = 1 ml sample; B = 2 ml sample
crushed hydra	crushed hydra tissue
*	Dilution = 1:1,000 all other dilutions = 1:10,000

APPENDIX A: EXPERIMENTAL DATA: DATE: April, 27

MEDIA

ML DIL. USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	7	7.00	4	57	2	28	1	15	0	0
2 ml	18	9.00	9	50	5	28	3	16	1	6
5 ml	47	9.40	24	51	11	23	9	19	3	6
*3 ml	252	8.40	131	52	57	23	40	16	24	9
C:1 ml	0	0.00	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.45		53		26		17		6
16°C:										
1 ml	8	8.00	4	50	3	37	1	13	0	0
2 ml	6	8.00	7	44	5	31	4	25	0	0
5 ml	48	9.60	26	54	10	21	8	16	4	8
*3 ml	260	8.67	139	53	61	24	43	16	17	7
C:1 ml	0	0.00	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.57		=50		28		18		4
				$\bar{X}$52		27		18		5
			HYDRA MEDIA WASH							
Wash 1	223	223	4		171		39		9	
Wash 2	77	77	2		56		16		3	
Wash 3	5	5	0		4		1		0	
control	0	0	0		0		0		0	
		T_o 3050	6	2	231	76	56	18	12	4
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	160	160	3		29		121		7	
" :B	300	150	1		27		117		5	
		$\bar{X}$ 155	2		28		119		6	
sup. 2:A	50	50	1		12		35		2	
crushed hydra	20	--	0		3		16		1	
		T_i 2070	30	2	403	19	1556	75	81	4
			HYDRA DISINFECTANT WASH							
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	173	173	5		31		127		10	
" :B	296	148	2		20		122		4	
		$\bar{X}$ 160.5	3.5		25.5		124.5		7	
sup. 2:A	41	41	1		6		31		3	
crushed hydra	28	--	1		5		20		2	
		T_i 2043	46	2	20	16	1575	77	102	7
				$\bar{X}$ 2		18		76		6

APPENDIX A: EXPERIMENTAL DATA; DATE: April 28

MEDIA

ML DIL. USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	8	8.00	4	50	2	25	1	13	0	0
2 ml	15	7.50	8	53	4	26	2	13	1	7
5 ml	43	8.60	21	49	13	30	7	16	3	7
*3 ml	261	8.70	140	54	62	24	45	17	17	7
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.20		52		26		15		5
16°C:										
1 ml	8	8.00	4	50	3	38	0	0	0	0
2 ml	19	9.50	10	52	5	26	3	16	1	5
5 ml	34	6.80	18	53	10	29	4	12	2	6
*3 ml	245	8.17	126	51	48	20	50	20	21	9
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.12		$\bar{\bar{X}}$ 52		28		12		5
				$\bar{\bar{X}}$ 52		27		14		5
			HYDRA MEDIA WASH							
Wash 1	234	234	3		187		37		7	
Wash 2	67	67	1		52		11		3	
Wash 3	3	3	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 3040	4	1	241	79	49	16	10	3
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	156	156	1		28		123		4	
" :B	298	149	2		23		119		5	
		$\bar{X}$ 152.5	1.5		25.5		121		4.5	
sup. 2:A	52	52	1		9		40		2	
crushed hydra	21	--	0		3				2	
		T_i 2066	25	1	348	17	1626	79	67	3
			HYDRA DISINFECTANT WASH							
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	149	149	3		20		119		7	
" :B	301	150.5	3		27		115		6	
		$\bar{X}$ 149.8	3		23.5		117		6.5	
sup. 2:A	53	53	1		9		40		3	
crushed hydra	19	--	0		3		15		1	
		T_i 2047	40	$\bar{\bar{X}}$ 2	328	16	1585	77	96	5
				$\bar{\bar{X}}$ 2		17		78		4

APPENDIX A: EXPERIMENTAL DATA; DATE: April, 29

MEDIA

ML DIL. USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	8	8.00	3	38	2	25	2	25	1	13
2 ml	17	8.50	7	41	5	29	3	18	2	12
5 ml	40	8.00	18	45	13	32	6	15	3	7
*3 ml	247	8.23	128	52	54	22	44	18	21	8
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.18		44		27		19		10
16°C:										
1 ml	9	9.00	4	44	3	33	2	22	0	0
2 ml	17	8.50	8	47	6	35	2	12	1	6
5 ml	40	8.00	20	50	9	22	7	18	4	10
*3 ml	244	8.13	117	48	63	26	45	18	19	8
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.41		$\bar{X}$ 47		29		17		6
				$\bar{X}$ 46		28		18		8
HYDRA MEDIA WASH										
Wash 1	199	199	3		155		34		7	
Wash 2	99	102	1		81		17		2	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 3010	4	1	236	78	52	17	9	3
HYDRA CRUSH										
control	0	0	0		0		0		0	
sup. 1:A	150	150	3		22		118		7	
" :B	299	$\bar{X}$ 149.5	1		28		115		6	
		$\bar{X}$ 149.8	2		25		116.5		6.5	
sup. 2:A	52	52	1		12		37		2	
crushed hydra	24	--	0		4		19		1	
		T_i 2042	30	2	374	18	1554	76	86	4
HYDRA DISINFECTANT WASH										
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
HYDRA CRUSH										
control	0	0	0		0		0		0	
sup. 1:A	146	146	1		25		116		4	
" :B	295	$\bar{X}$ 147.5	1		28		113		6	
		$\bar{X}$ 146.8	2		26.5		114.5		5	
sup. 2:A	56	56	1		11		42		2	
crushed hydra	22	--	0		5		16		1	
		T_i 2050	30	$\bar{X}$ 2	380	18	1581	77	71	4
				$\bar{X}$ 2		18		77		4

APPENDIX A: EXPERIMENTAL DATA; DATE: May, 4

MEDIA

ML DIL. USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	8	8.00	3	38	2	25	2	25	1	13
2 ml	19	9.50	9	47	6	32	3	16	1	5
5 ml	50	10.00	27	54	16	32	4	8	3	6
*3 ml	203	6.77	106	52	53	26	30	15	14	7
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.57		48		29		16		8
16°C:										
1 ml	7	7.00	3	43	2	29	2	29	0	0
2 ml	18	9.00	8	44	5	28	3	17	2	11
5 ml	46	9.20	20	43	11	24	9	19	6	13
*3 ml	212	7.07	103	49	56	26	35	16	18	8
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.07		45		27		20		8
				$\bar{\bar{X}}$ 47		28		18		8
			HYDRA MEDIA WASH							
Wash 1	234	234	2		184		37		11	
Wash 2	65	65	1		50		12		2	
Wash 3	1	1	0		1		0		0	
control	0	0	0		0		0		0	
		T_o 3000	0	1	235	78	49	16	13	4
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	171	171	3		29		129		10	
" :B	302	151	1		27		116		7	
		$\bar{X}$ 161	2		28		122.5		8.5	
sup, 2:A	46	46	1		8		35		2	
crushed hydra	19	--	0		4		14		1	
		T_i 2089	30	1	364	17	1589	76	106	5
			HYDRA DISINFECTANT WASH							
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	177	177	1		30		141		5	
" :B	291	145.5	2		26		113		5	
		$\bar{X}$ 161.3	1.5		28		127		5	
sup. 2:A	42	42	1		7		32		2	
crushed hydra	23	--	0		5		17		1	
		T_i 2056	25	1	355	17	1607	78	71	3
				$\bar{\bar{X}}$ 1		17		77		4

APPENDIX A: EXPERIMENTAL DATA; DATE: May 5

MEDIA

ML DIL. USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	8	8.00	3	38	3	38	2	25	0	0
2 ml	16	8.00	7	44	5	31	3	19	1	6
5 ml	42	8.40	19	45	16	38	4	9	3	7
*3 ml	253	8.43	131	52	57	22	43	17	22	9
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.21		45		32		18		5
16°C:										
1 ml	7	7.00	3	43	2	29	2	29	0	0
2 ml	16	8.00	9	56	3	19	3	19	1	6
5 ml	41	8.20	25	61	8	19	5	12	3	7
*3 ml	260	8.67	139	53	65	25	38	15	18	7
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 7.97		53		23		19		5
				$\bar{\bar{X}}$ 49		28		19		5

HYDRA MEDIA WASH

Wash 1	230	230	3		179		40		8	
Wash 2	76	76	1		56		16		3	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 3060	4	1	235	77	56	18	11	4

HYDRA CRUSH

control	0	0	0		0		0		0	
sup. 1:A	166	166	3		28		127		8	
" :B	289	144.5	4		26		110		5	
		$\bar{X}$ 155.3	3.5		27		118.5		6.5	
sup. 2:A	51	51	1		9		38		3	
crushed hydra	18	--	0		4		13		1	
		T_i 2081	45	2	364	18	1578	76	96	5

HYDRA DISINFECTANT WASH

Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0

HYDRA CRUSH

control	0	0	0		0		0		0	
sup. 1:A	181	181	3		30		144		4	
" :B	293	146.5	1		24		119		3	
		$\bar{X}$ 163.8	2		27		131.5		3.5	
sup. 2:A	39	39	1		6		30		2	
crushed hydra	27	--	1		6		18		2	
		T_i 2055	40	2	336	16	1633	80	57	3
				$\bar{X}$ 2		17		78		4

APPENDIX A: EXPERIMENTAL DATA; DATE: May 6

MEDIA

ML DIL USED	COLONY COUNTS	BAC/ML ($\times 10^4$)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	8	8.00	3	38	2	25	2	25	1	13
2 ml	15	7.50	7	47	4	27	3	20	1	7
5 ml	42	8.40	26	62	9	21	5	12	2	5
*3 ml	249	8.30	129	52	61	25	40	16	19	8
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.05		50		24		18		8
16°C:										
1 ml	8	8.00	4	50	2	25	1	13	1	13
2 ml	16	8.00	9	56	4	25	2	13	1	6
5 ml	41	8.20	25	61	8	19	5	12	3	9
*3 ml	251	8.37	130	52	66	26	39	15	16	6
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.14		$\bar{X}$ 55		24		13		9
				$\bar{X}$ 53		24		16		9
			HYDRA MEDIA WASH							
Wash 1	197	197	3		150		37		7	
Wash 2	98	98	1		78		16		3	
Wash 3	7	7	2		3		1		1	
control	0	0	0		0		0		0	
		T_o 3020	6	2	231	77	54	18	11	4
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	171	171	1		30		136		3	
" :B	288	144	2		25		113		4	
		$\bar{X}$ 157.5	1.5		27.5		124.5		3.5	
sup. 2:A	44	44	1		8		33		2	
crushed hydra	34	--	1		5		25		3	
		T_i 2049	26	1	360	17	1600	78	58	3
			HYDRA DISINFECTANT WASH							
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	184	180	1		32		146		2	
" :B	284	142	3		25		107		7	
		$\bar{X}$ 161	2		28.5		126.5		4.5	
sup. 2:A	44	44	1		6		34		3	
crushed hydra	29	--	0		5		23		1	
		T_i 2082	30	1	350	17	1628	78	76	4
				$\bar{X}$ 1		17		78		4

APPENDIX A: EXPERIMENTAL DATA; DATE: May 7

MEDIA

ML DIL. USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	7	7.00	3	43	2	29	1	14	1	14
2 ml	17	8.50	8	47	4	23	3	18	2	12
5 ml	43	8.60	26	60	10	23	4	9	3	7
*3 ml	248	8.27	129	52	64	26	40	16	15	6
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.09		50		25		14		10
16°C:										
1 ml	8	8.00	4	50	2	25	2	25	0	0
2 ml	18	9.00	10	56	4	22	3	17	1	6
5 ml	42	8.40	17	40	12	29	9	21	4	9
*3 ml	256	8.53	131	51	69	27	37	14	19	7
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.48		49		26		19		6
				$\bar{X}$ 50		26		17		8
			HYDRA MEDIA WASH							
Wash 1	232	232	2		177		44		9	
Wash 2	68	68	1		53		12		2	
Wash 3	1	1	0		1		0		0	
control	0	0	0		0		0		0	
		T_o 3010	3	1	231	76	56	19	11	4
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	155	155	2		29		119		5	
" :B	294	147	1		26		116		4	
		$\bar{X}$ 151	1.5		27.5		117.5		4.5	
sup. 2:A	54	54	1		9		41		3	
crushed hydra	21	--	1		3		16		1	
		T_i 2071	26	1	368	18	1601	77	76	4
			HYDRA DISINFECTANT WASH							
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	172	172	1		29		138		4	
" :B	299	149.5	1		27		119		3	
		$\bar{X}$ 160.3	1		28		128.5		3.5	
sup. 2:A	46	46	1		8		35		2	
crushed hydra	19	--	0		4		14		1	
		T_i 2087	20	1	364	17	1649	79	56	3
				$\bar{X}$ 1		18		78		4

APPENDIX A: EXPERIMENTAL DATA; DATE: May 11

MEDIA

ML DIL USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	8	8.00	3	38	3	38	2	25	0	0
2 ml	16	8.00	9	56	4	25	2	13	1	6
5 ml	46	9.20	29	63	8	17	6	13	3	6
*3 ml	241	8.03	132	55	59	24	33	14	17	7
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.31		53		26		16		5
16°C:										
1 ml	9	9.00	4	44	2	22	2	22	1	11
2 ml	17	8.50	7	41	6	35	3	18	1	6
5 ml	40	8.00	23	58	9	22	5	13	3	8
*3 ml	261	8.70	138	53	68	26	37	14	18	7
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.55		49		26		17		8
				$\bar{X}$ 51		26		17		7

HYDRA MEDIA WASH

Wash 1	220	220	3		168		42		7	
Wash 2	79	83	2		66		13		2	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 3030	5	1	234	77	55	18	9	3

HYDRA CRUSH

control	0	0	0		0		0		0	
sup. 1:A	150	150	2		28		119		6	
" :B	321	160.5	2		30		124		5	
		$\bar{X}$ 155.3	2		29		119		5.5	
sup. 2:A	48	48	1		8		36		3	
crushed hydra	26	--	1		4		19		2	
		T_i 2059	31	2	374		1569	76	87	4

HYDRA DISINFECTANT WASH

Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0

HYDRA CRUSH

control	0	0	0		0		0		0	
sup. 1:A	160	160	2		30		123		5	
" :B	300	150	2		29		115		4	
		$\bar{X}$ 155	2		29.5		119		4.5	
sup. 2:A	50	50	1		9		38		2	
crushed hydra	25	--	1		4		18		2	
		T_i 2075	31	2	389	19	1588	77	67	3
				$\bar{X}$ 2		19		77		4

APPENDIX A: EXPERIMENTAL DATA; DATE: May 12

MEDIA

ML DIL. USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	9	9.00	4	44	3	33	2	22	0	0
2 ml	16	8.00	8	50	4	25	3	19	1	6
5 ml	40	8.00	18	45	13	32	6	15	3	8
*3 ml	281	9.37	145	52	71	25	44	16	21	7
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.59		48		29		18		5
16°C:										
1 ml	8	8.00	4	50	2	25	1	13	1	13
2 ml	17	8.50	8	47	5	29	3	18	1	6
5 ml	48	9.60	29	60	9	19	7	14	3	6
*3 ml	255	8.50	132	52	67	26	37	14	19	7
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.65		52		25		15		8
				$\bar{\bar{X}}$ 50		27		17		7
			HYDRA MEDIA WASH							
Wash 1	232	232	4		176		44		8	
Wash 2	65	65	1		51		11		2	
Wash 3	5	5	0		3		2		0	
control	0	0	0		0		0		0	
		T_o 3020	5	2	230	76	57	19	10	3
			HYDRA CRUSH							
control	0	0	C		0		0		0	
sup. 1:A	152	152	1		27		121		2	
" :B	307	153.5	2		29		118		5	
		$\bar{X}$ 152.3	1.5		28		119.5		3.5	
sup. 2:A	52	52	1		10		38		3	
crushed hydra	29	--	1		4		21		3	
		T_i 2072	26	1	384	18	1596	77	68	3
			HYDRA DISINFECTANT WASH							
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	168	168	2		32		131		3	
" :B	294	147	2		24		118		3	
		$\bar{X}$ 157.5	2		38		124.5		3	
sup. 2:A	43	43	1		9		30		3	
crushed hydra	36	--	1		6		27		2	
		T_i 2041	31	2	376	18	1572	77	62	3
				$\bar{X}$ 2		18		77		3

APPENDIX A: EXPERIMENTAL DATA; DATE: May 13

ML DIL USED	COLONY COUNTS	BAC/ML (x 10^4)	MEDIA WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	8	8.00	3	38	2	25	2	25	1	13
2 ml	19	9.50	10	53	4	21	3	16	2	10
5 ml	39	7.80	21	54	10	26	5	13	3	7
*3 ml	249	8.30	129	52	57	23	42	17	21	8
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.40		49		24		18		10
16°C:										
1 ml	7	7.00	3	43	2	29	1	14	1	14
2 ml	19	9.50	8	42	6	32	3	16	2	10
5 ml	46	9.20	31	67	8	17	4	9	3	6
*3 ml	250	8.33	129	56	63	75	39	16	19	8
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.51		= 52		26		14		10
				$\bar{X}$ 51		25		16		10
			HYDRA MEDIA WASH							
Wash 1	241	241	2		192		40		7	
Wash 2	65	65	1		51		12		1	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 3060	3	1	243	79	52	17	8	3
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	163	163	2		27		130		4	
" :B	297	148.5	3		28		113		5	
		$\bar{X}$ 155.8	2.5		27.5		121.5		4.5	
sup. 2:A	47	47	1		8		36		2	
crushed hydra	24	--	1		4		17		2	
		T_i 2052	36	2	359	18	1592	78	67	3
			HYDRA DISINFECTANT WASH							
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	168	168	4		31		126		7	
" :B	289	144.5	2		27		112		4	
		$\bar{X}$ 156.3	3		29		119		5.5	
sup. 2:A	48	48	1		8		37		2	
crushed hydra	31	--	1		6		23		1	
		T_i 2074	41	= 2	376	18	1583	76	76	4
				$\bar{X}$ 2		18		77		4

APPENDIX A: EXPERIMENTAL DATA; DATE: May 14

MEDIA

ML DIL. USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	8	8.00	3	38	2	25	2	25	1	13
2 ml	20	10.00	9	45	6	30	3	15	2	10
5 ml	39	7.80	21	54	11	28	4	10	3	8
*3 ml	251	8.37	132	53	58	23	41	16	20	8
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.54		47		26		17		10
16°C:										
1 ml	8	8.00	4	50	3	38	1	13	0	0
2 ml	15	7.50	7	47	5	33	2	13	1	7
5 ml	49	9.80	28	57	11	22	6	12	4	8
*3 ml	239	7.97	125	52	53	22	41	17	20	1
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.32		= 52		29		14		6
				$\bar{X}$ 50		28		16		8
			HYDRA MEDIA WASH							
Wash 1	241	241	2		192		40		7	
Wash 2	59	59	1		46		11		1	
Wash 3	3	3	0		3		0		0	
control	0	0	0		0		0		0	
		T_o 3030	3	1	241	80	51	17	8	3
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	152	152	3		28		117		4	
" :B	307	153.5	1		26		125		2	
		$\bar{X}$ 152.8	2		27		121		3	
sup. 2:A	53	53	1		11		40		1	
crushed hydra	22	--	0		5		16		1	
		T_i 2080	30	1	385	19	1626	78	41	2
			HYDRA DISINFECTANT WASH							
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	179	179	1		32		143		3	
" :B	285	142.5	2		24		115		2	
		$\bar{X}$ 160.8	1.5		28		129		2.5	
sup. 2:A	43	43	1		7		33		3	
crushed hydra	25	--	1		4		18		2	
		T_i 2063	26	$\bar{X}$ 1	354	17	1638	79	57	3
				$\bar{X}$ 1		18		79		3

APPENDIX A: EXPERIMENTAL DATA; DATE: May 15

MEDIA

ML DIL USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	8	8.00	3	38	2	25	2	25	1	13
2 ml	18	9.00	9	50	5	28	3	17	1	6
5 ml	47	9.40	28	59	10	21	6	13	3	6
*3 ml	248	8.27	133	53	52	21	42	17	21	8
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.67		50		24		18		8
16°C:										
1 ml	8	8.00	4	50	2	25	1	13	1	13
2 ml	19	9.50	8	42	6	32	3	16	2	11
5 ml	41	8.20	19	46	13	32	5	12	4	10
*3 ml	244	8.13	128	52	53	32	38	16	25	10
C:1 ml	0	0	0	0	0	27	0	0	0	0
		$\bar{X}$ 8.46		48		28		14		11
				$\bar{\bar{X}}$ 49		26		16		10
			HYDRA MEDIA WASH							
Wash 1	224	224	3		172		42		7	
Wash 2	75	75	1		58		14		2	
Wash 3	3	3	0		2		1		0	
control	0	0	0		0		0		0	
		T_o 3020	4	1	232	77	57	19	9	3
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	154	154	2		30		117		5	
" :B	308	154	3		26		121		4	
		$\bar{X}$ 154	2.5		28		119		4.5	
sup. 2:A	49	49	1		7		39		2	
crushed hydra	23	--	0		3		19		1	
		T_i 2053	35	2	353	17	1599	78	66	3
			HYDRA DISINFECTANT WASH							
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	00		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	169	169	3		32		128		6	
" :B	297	148.5	3		31		111		4	
		$\bar{X}$ 158.8	3		31.5		119.5		5	
sup. 2:A	46	46	1		9		34		2	
crushed hydra	32	--	1		6		23		2	
		T_i 2080	41	2	411	20	1558	75	72	3
				$\bar{X}$ 2		19		77		3

APPENDIX A: EXPERIMENTAL DATA; DATE: May 18

MEDIA

ML DIL. USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	9	9.00	4	44	3	33	2	22	0	0
2 ml	17	8.50	7	41	6	35	3	18	1	6
5 ml	43	8.60	26	60	8	19	5	12	4	9
*3 ml	250	8.33	130	52	57	23	42	16	21	8
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.61		49		28		17		6
16°C:										
1 ml	7	7.00	3	43	2	29	1	14	1	14
2 ml	18	9.00	10	56	5	28	2	11	1	6
5 ml	50	10.00	31	62	9	18	7	14	3	6
*3 ml	230	7.67	119	52	61	26	33	14	17	7
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.42		53		25		13		8
				$\bar{\bar{X}}$ 51		27		15		7
			HYDRA MEDIA WASH							
Wash 1	230	230	2		181		44		3	
Wash 2	74	74	0		59		13		2	
Wash 3	1	1	0		1		0		0	
control	0	0	0		0		0		0	
		T_o 3050	2	1	241	79	57	19	5	2
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	158	158	3		28		122		5	
" :B	288	144	0		30		113		1	
		$\bar{X}$ 151	1.5		29		117.5		3	
sup. 2:A	51	51	1		11		36		3	
crushed hydra	19	--	0		4		15		0	
		T_i 2039	25	1	404	20	1550	76	60	3
			HYDRA DISINFECTANT WASH							
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	172	172	3		32		133		4	
" :B	294	147	2		27		115		3	
		$\bar{X}$ 159.5	2.5		29.5		124		3.5	
sup. 2:A	42	42	1		6		31		4	
crushed hydra	25	--	0		6		19		0	
		T_i 2040	35	2	361	18	1569	77	75	4
				$\bar{X}$ 2		19		77		4

APPENDIX A: EXPERIMENTAL DATA; DATE: May 19

MEDIA

ML DIL USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
$30^{o}C$:										
1 ml	8	8.00	3	38	2	25	2	25	1	13
2 ml	17	8.50	9	53	5	29	2	12	1	6
5 ml	41	8.20	23	56	9	22	5	12	4	10
*3 ml	238	7.93	121	51	62	26	37	16	18	8
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.16		50		26		16		9
$16^{o}C$:										
1 ml	8	8.00	3	38	2	25	2	25	1	13
2 ml	18	9.00	8	44	6	33	3	16	1	6
5 ml	40	8.00	18	45	11	28	7	18	4	10
*3 ml	240	8.00	119	50	65	27	40	17	16	7
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.25		44		28		19		9
				$\bar{\bar{X}}$ 47		27		18		9
			HYDRA MEDIA WASH							
Wash 1	212	212	1		170		39		2	
Wash 2	86	86	2		65		15		4	
Wash 3	5	5	0		3		1		1	
control	0	0	0		0		0		0	
		T_o 3030	3	1	238	79	55	18	7	2
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	155	155	2		28		124		1	
" :B	305	152.5	4		26		119		4	
		$\bar{X}$ 153.3	3		27		1215		2.5	
sup. 2:A	50	50	2		8		38		2	
crushed hydra	27	--	0		3		22		2	
		T_i 2060	50	3	353	17	1617	78	47	2
			HYDRA DISINFECTANT WASH							
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	185	185	3		35		140		7	
" :B	311	155.5	2		29		179		6	
		$\bar{X}$ 170.3	2.5		32		129.5		6.5	
sup. 2:A	33	33	1		6		25		1	
crushed hydra	19	--	0		3		15		1	
		T_i 2052	35	$\bar{X}$ 2	383	19	1560	76	76	4
				$\bar{X}$ 3		18		77		3

APPENDIX A: EXPERIMENTAL DATA; DATE: May 20

MEDIA

ML DIL. USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
$30^{\circ}C$:										
1 ml	7	7.00	4	57	2	29	1	14	0	0
2 ml	17	8.50	8	47	6	35	3	18	0	0
5 ml	52	10.40	24	46	15	29	7	14	6	12
*3 ml	261	8.70	135	52	63	24	42	16	21	8
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.65		51		29		16		5
$16^{\circ}C$:										
1 ml	8	8.00	4	50	2	25	1	13	1	13
2 ml	19	9.50	10	53	4	21	3	16	2	11
5 ml	37	7.40	23	62	8	22	4	11	2	5
*3 ml	216	7.20	105	49	57	26	36	17	18	8
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.03		$\bar{\bar{X}}$ 54		24		14		9
				$\bar{\bar{X}}$ 53		27		15		7
			HYDRA MEDIA WASH							
Wash 1	230	230	2		183		42		3	
Wash 2	66	66	1		51		13		1	
Wash 3	4	4	0		3		1		0	
control	0	0	0		0		0		0	
		T_o 3000	3	1	237	79	56	19	4	1
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	152	152	3		25		119		5	
" :B	309	154.5	1		30		122		2	
		$\bar{X}$ 153.25	2		27.5		120.5		3.5	
sup. 2:A	49	49	2		8		37		2	
crushed hydra	29	--	1		4		23		1	
		T_i 2052	41	2	359	18	1598	78	56	3
			HYDRA DISINFECTANT WASH							
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	181	181	1		33		146		1	
" :B	286	143	3		26		109		5	
		$\bar{X}$ 162	2		29.5		127.5		3	
sup. 2:A	41	41	1		7		31		2	
crushed hydra	32	--	0		6		25		1	
		T_i 2062	30	2	371	18	1610	78	51	3
				$\bar{X}$ 2		18		78		3

APPENDIX A: EXPERIMENTAL DATA; DATE: May 21

MEDIA

ML DIL USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	8	8.00	3	38	2	25	2	25	1	13
2 ml	17	8.50	9	53	4	24	2	12	2	12
5 ml	48	9.60	25	52	13	27	6	13	4	8
*3 ml	223	7.43	116	52	54	24	37	17	16	7
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.38		49		25		17		10
16°C:										
1 ml	8	8.00	4	50	2	25	2	25	0	0
2 ml	17	8.50	8	47	6	35	2	12	1	6
5 ml	42	8.40	22	52	10	24	6	14	4	10
*3 ml	238	7.93	121	51	63	27	36	15	18	8
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.21		50		28		17		6
				$\bar{\bar{X}}$ 50		27		17		8
			HYDRA MEDIA WASH							
Wash 1	231	231	2		183		44		2	
Wash 2	70	70	4		51		12		3	
Wash 3	3	3	0		2		1		0	
control	0	0	0		0		0		0	
		T_o 3040	6	2	236	77	57	19	5	2
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	175	175	2		35		136		2	
" :B	288	144	1		27		114		2	
		$\bar{X}$ 159.5	1.5		31		125		2	
sup. 2:A	45	45	1		6		37		1	
crushed hydra	19	--	2		2		14		1	
		T_i 2064	27	1	372	18	1634	79	31	2
			HYDRA DISINFECTANT WASH							
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	170	170	6		34		121		9	
" :B	301	150.5	1		30		119		1	
		$\bar{X}$ 160.25	3.5		32		120		5	
sup. 2:A	42	42	2		7		30		3	
crushed hydra	36	--	3		7		24		2	
		T_i 2059	58	3	397	19	1524	74	82	4
				$\bar{X}$ 2		19		77		3

APPENDIX A: EXPERIMENTAL DATA; DATE: May 25

MEDIA

ML DIL. USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	9	9.00	5	56	2	22	2	22	0	0
2 ml	16	8.00	7	44	4	25	3	19	2	13
5 ml	39	7.80	20	51	9	23	7	18	3	8
*3 ml	241	8.03	125	52	67	28	33	14	16	7
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.21		51		25		18		7
16°C:										
1 ml	8	8.00	3	38	3	38	1	13	1	13
2 ml	19	9.50	9	47	5	26	3	16	2	11
5 ml	39	7.80	22	56	9	23	5	13	3	8
*3 ml	211	7.03	110	52	52	25	32	13	17	8
C:1 ml	0	0	0	0	0	0	0	15	0	0
		$\bar{X}$ 8.08		$\bar{X}$ 48		28		14		10
				$\bar{X}$ 50		27		16		9
			HYDRA MEDIA WASH							
Wash 1	219	219	1		171		43		4	
Wash 2	81	81	3		60		14		4	
Wash 3	4	4	0		2		2		0	
control	0	0	0		0		0		0	
		T_o 3040	4	1	233	77	59	19	8	3
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	163	163	1		31		129		2	
" :B	302	151	2		26		121		2	
		$\bar{X}$ 157	1.5		28.5		125		2	
sup. 2:A	45	45	3		8		34		0	
crushed hydra	22	--	1		4		15		2	
		T_i 2042	46	2	369	18	1605	79	22	1
			HYDRA DISINFECTANT WASH							
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	161	161	0		30		131		0	
" :B	291	145.5	2		28		114		2	
		$\bar{X}$ 153.3	1		29		122.5		1	
sup. 2:A	52	52	3		9		37		3	
crushed hydra	30	--	0		6		22		2	
		T_i 2083	40	2	386	19	1617	78	42	2
				$\bar{X}$ 2		19		79		2

APPENDIX A: EXPERIMENTAL DATA; DATE: May 26

MEDIA

ML DIL USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	8	8.00	3	38	2	25	1	13	1	13
2 ml	16	8.00	8	50	5	31	3	19	1	6
5 ml	41	8.20	19	46	11	27	8	20	3	7
*3 ml	270	9.00	139	52	70	26	38	14	23	9
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.30		47		27		17		9
16°C:										
1 ml	8	8.00	3	38	2	25	2	25	1	13
2 ml	18	9.00	10	56	5	28	2	11	1	6
5 ml	43	8.60	26	60	9	21	5	12	3	7
*3 ml	255	8.50	132	52	66	26	40	16	17	7
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.53		$\bar{X}$ 52		25		16		8
				$\bar{X}$ 50		26		17		9
			HYDRA MEDIA WASH							
Wash 1	238	238	0		191		45		2	
Wash 2	64	64	1		49		11		3	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 3020	1	.3	240	80	56	19	5	2
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	158	158	0		30		126		2	
sup. :B	303	151.5	1		2		121		1	
		$\bar{X}$ 154.75	.5		29.5		123.5		1.5	
sup. 2:A	52	52	1		9		40		2	
crushed hydra	19	--	0		2		16		1	
		T_i 2087	16	1	387	19	1651	79	36	2
			HYDRA DISINFECTANT WASH							
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	171	171	2		34		135		0	
" :B	295	147.5	2		25		118		3	
		$\bar{X}$ 159.25	2		29.5		126.5		1.5	
sup. 2:A	46	46	0		9		35		1	
crushed hydra	24	--	1		5		17		1	
		T_i 2077	21	$\bar{X}$ 1	390	19	1632	79	26	1
				$\bar{X}$ 1		19		79		2

APPENDIX A: EXPERIMENTAL DATA; DATE: May 27

MEDIA

ML DIL USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30^{o}C:										
1 ml	9	9.00	4	44	3	33	2	22	0	0
2 ml	18	9.00	10	56	4	22	2	11	2	11
5 ml	42	8.40	19	45	16	38	4	9	3	7
*3 ml	280	9.33	133	48	78	27	46	16	24	8
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.93		48		30		14		6
16^{o}C:										
1 ml	9	9.00	3	33	3	33	2	22	1	11
2 ml	17	8.50	7	41	5	29	4	23	1	6
5 ml	49	9.80	29	59	11	22	6	12	3	6
*3 ml	280	9.33	141	50	69	25	44	16	26	9
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 9.16		46		27		18		8
				$\bar{\bar{X}}$ 47		29		16		7
			HYDRA MEDIA WASH							
Wash 1	224	224	3		172		43		6	
Wash 2	75	75	2		61		10		2	
Wash 3	3	3	0		2		1		0	
control	0	0	0		0		0		0	
		T_o 3020	5	2	235	78	54	18	8	3
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	156	156	0		31		124		1	
" :B	310	155	2		27		120		6	
		$\bar{X}$ 155.5	1		29		122		3.5	
sup. 2:A	48	48	2		8		36		2	
crushed hydra	17	--	0		3		13		1	
		T_i 2052	30	2	373	18	1593	78	56	3
			HYDRA DISINFECTANT WASH							
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	169	169	2		33		131		3	
" :B	301	150.5	1		26		121		3	
		$\bar{X}$ 159.75	1.5		29.5		126		3	
sup. 2:A	45	45	2		6		36		1	
crushed hydra	20	--	0		3		15		2	
		T_i 2068	35	2	358	17	1635	79	42	2
				$\bar{X}$ 2		18		79		3

APPENDIX A: EXPERIMENTAL DATA; DATE: May 28

MEDIA

ML DIL USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	9	9.00	5	56	3	33	1	11	0	0
2 ml	19	9.50	8	42	5	26	4	21	2	10
5 ml	52	10.40	29	56	16	31	4	8	3	6
*3 ml	280	9.33	138	49	69	25	49	17	24	9
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 9.33		50		29		14		6
16°C:										
1 ml	9	9.00	4	44	3	33	2	22	0	0
2 ml	18	9.00	9	50	4	22	4	22	1	6
5 ml	58	11.60	32	55	13	22	9	15	4	7
*3 ml	284	9.47	147	52	65	23	45	16	29	9
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 9.77		50		25		19		6
				$\bar{\bar{X}}$ 50		27		17		6
			HYDRA MEDIA WASH							
Wash 1	239	239	1		189		46		3	
Wash 2	60	60	2		44		13		1	
Wash 3	2	2	0		2		0		0	
control	0	0	0		0		0		0	
		T_o 3010	3	1	235	78	59	20	4	1
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	153	153	0		30		122		1	
" :B	307	153.5	3		28		120		3	
		$\bar{X}$ 153.25	1.5		29		121		2	
sup. 2:A	52	52	0		10		41		1	
crushed hydra	17	--	1		3		12		1	
		T_i 2070	16	1	393	19	1632	79	31	2
			HYDRA DISINFECTANT WASH							
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	180	180	3		34		141		2	
" :B	282	141	1		26		111		3	
		$\bar{X}$ 160.5	2		30		126		2.5	
sup. 2:A	43	43	0		7		34		2	
crushed hydra	31	--	2		3		26		0	
		T_i 2066	22	1	373	18	1626	79	45	2
				$\bar{X}$ 1		19		79		2

APPENDIX A: EXPERIMENTAL DATA; DATE: June 2

MEDIA

ML DIL USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	10	10.00	6	60	2	20	2	20	0	0
2 ml	27	8.50	15	56	7	26	3	11	2	7
5 ml	51	10.20	26	51	13	25	8	16	4	8
*3 ml	248	8.27	122	49	61	25	43	17	22	9
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 9.24		54		24		16		6
16°C:										
1 ml	10	10.00	5	50	2	20	2	20	2	10
2 ml	17	8.50	10	59	4	23	4	23	2	6
5 ml	44	8.80	19	43	12	27	12	27	8	1
*3 ml	250	8.33	128	51	59	24	59	24	44	8
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.91		51		24		24		9
				$\bar{\bar{X}}$ 53		24		20		8
			HYDRA MEDIA WASH							
Wash 1	211	211	2		166		41		2	
Wash 2	82	92	1		72		17		2	
Wash 3	3	3	0		3		0		0	
control	0	0	0		0		0		0	
		T_o 3060	3	1	241	79	58	19	4	1
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	171	171	4		31		135		1	
" :B	293	146.5	0		29		118		0	
		$\bar{X}$ 158.75	2		30		126.5		.5	
sup. 2:A	47	47	3		8		34		2	
crushed hydra	29	--	0		5		22		2	
		T_i 2087	50	2	385	18	1627	78	27	1
			HYDRA DISINFECTANT WASH							
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	174	174	1		32		138		3	
" :B	293	146.5	2		29		114		2	
		$\bar{X}$ 160.25	1.5		30.5		126		2.5	
sup. 2:A	41	41	0		7		33		1	
crushed hydra	33	--	2		4		24		3	
		T_i 2046	17	1	379	19	1614	79	38	2
				$\bar{X}$ 2		19		79		x

APPENDIX A: EXPERIMENTAL DATA; DATE: June 3

ML DIL USED	COLONY COUNTS	BAC/ML (x 10^4)	MEDIA WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	9	9.00	4	44	3	33	2	11	0	0
2 ml	22	11.00	10	46	7	32	3	14	2	9
5 ml	49	9.80	25	51	11	22	9	18	4	8
*3 ml	257	8.57	133	52	60	22	39	15	25	10
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 9.59		48		27		15		7
16°C:										
1 ml	9	9.00	4	44	2	22	2	22	1	11
2 ml	21	10.50	12	57	4	19	2	10	1	5
5 ml	50	10.00	27	54	15	30	5	10	3	6
*3 ml	277	9.23	143	52	67	24	45	16	22	8
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 9.68		52		24		15		8
				$\bar{\bar{X}}$ 50		26		15		8
			HYDRA MEDIA WASH							
Wash 1	222	222	1		178		41		2	
Wash 2	83	83	4		64		12		3	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 3050	5	2	242	79	53	18	5	2
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	162	162	1		29		131		1	
" :B	298	149	0		29		120		0	
		$\bar{X}$ 155.5	.5		29		125.5		15	
sup. 2:A	48	48	0		6		40		2	
crushed hydra	21	--	2		3		16		0	
		T_i 2056	7	.3	353	17	1671	81	25	1
			HYDRA DISINFECTANT WASH							
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	184	184	3		33		142		6	
" :B	288	144	2		22		117		3	
		$\bar{X}$ 164	2.5		27.5		129.5		4.5	
sup. 2:A	42	42	4		8		31		1	
crushed hydra	18	--	0		3		14		1	
		T_i 2078	65	3	358	17	1619	78	56	3
				$\bar{X}$ 2		17		80		2

APPENDIX A: EXPERIMENTAL DATA; DATE: June 4

MEDIA

ML DIL USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
$30^{\circ}C$:										
1 ml	9	9.00	4	44	3	33	1	11	1	1
2 ml	16	8.00	7	44	4	25	3	18	2	12
5 ml	50	10.00	29	58	11	22	7	14	3	6
*3 ml	251	8.37	127	51	61	24	42	17	21	8
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.84		49		26		15		7
$16^{\circ}C$:										
1 ml	8	8.00	4	50	3	37	1	13	0	0
2 ml	19	9.50	10	53	4	21	3	16	2	11
5 ml	39	7.80	21	54	10	26	5	13	3	8
*3 ml	263	8.77	136	52	71	27	37	14	19	7
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.52		$\bar{X}$ 52		28		14		7
				$\bar{X}$ 51		27		15		7

HYDRA MEDIA WASH

Wash 1	220	220	2		176		40		2	
Wash 2	81	81	0		65		14		2	
Wash 3	2	2	0		2		0		0	
control	0	0	0		0		0		0	
		T_o 3030	2	1	243	80	54	18	4	1

HYDRA CRUSH

control	0	0	0		0		0		0	
sup. 1:A	173	173	9		32		121		11	
" :B	285	142.5	3		29		109		2	
		$\bar{X}$ 157.75	6		30.5		115		6.5	
sup. 2:A	46	46	1		8		35		2	
crushed hydra	23	--	0		3		19		1	
		T_i 2061	70	3	388	19	1519	74	86	4

HYDRA DISINFECTANT WASH

Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0

HYDRA CRUSH

control	0	0	0		0		0		0	
sup. 1:A	178	178	0		33		141		4	
" :B	299	149.5	1		29		118		2	
		$\bar{X}$ 163.75	.5		31		129.5		3	
sup. 2:A	42	42	2		7		31		2	
crushed hydra	22	--	0		4		18		0	
		T_i 2080	25	1	384	18	1623	78	50	2
				$\bar{X}$ 2		19		76		3

APPENDIX A: EXPERIMENTAL DATA; DATE: June 6

MEDIA

ML DIL USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	8	8.00	4	50	2	25	2	25	0	0
2 ml	16	8.00	7	44	5	31	3	18	1	6
5 ml	39	7.80	20	51	11	28	5	13	3	8
*3 ml	253	8.43	132	52	65	26	38	15	18	7
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.06		49		28		18		5
16°C:										
1 ml	9	9.00	4	44	3	33	1	11	1	11
2 ml	17	8.50	8	47	5	29	3	18	1	6
5 ml	39	7.80	18	47	9	23	8	21	4	10
*3 ml	210	7.00	102	49	55	26	36	17	17	8
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.08		47		28		17		9
				$\bar{X}$ 48		28		18		7

HYDRA MEDIA WASH

Wash 1	221	221	2		173		39		7	
Wash 2	79	79	0		62		16		1	
Wash 3	4	4	0		4		0		0	
control	0	0	0		0		0		0	
		T_o 3040	2	1	239	79	55	18	8	3

HYDRA CRUSH

control	0	0	0		0		0		0	
sup. 1:A	161	161	2		33		122		4	
" :B	294	147	1		27		116		3	
		$\bar{X}$ 154	1.5		30		119		3.5	
sup. 2:A	52	52	1		8		42		1	
crushed hydra	16	--	0		3		12		1	
		T_i 2076	25	1	383	19	1622	78	46	2

HYDRA DISINFECTANT WASH

Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0

HYDRA CRUSH

control	0	0	0		0		0		0	
sup. 1:A	172	172	2		35		129		6	
" :B	300	150	3		27		116		4	
		$\bar{X}$ 161	2.5		31		122.5		5	
sup. 2:A	44	44	1		8		33		2	
crushed hydra	29	--	1		7		19		2	
		T_i 2079	36	2	397	19	1574	76	72	4
				$\bar{X}$ 2		19		77		3

APPENDIX A: EXPERIMENTAL DATA; DATE: June 7

MEDIA

ML DIL USED	COLONY COUNTS	BAC/ML (x 10^4)	WHITE COUNT	%T	ORANGE COUNT	%T	YELLOW COUNT	%T	BEIGE COUNT	%T
30°C:										
1 ml	8	8.00	4	50	3	38	1	13	0	0
2 ml	16	8.00	7	44	5	31	3	19	1	6
5 ml	41	8.20	19	46	12	29	7	17	3	7
*3 ml	259	8.63	134	52	67	26	42	16	16	6
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.21		48		24		16		5
16°C:										
1 ml	8	8.00	3	38	2	25	2	25	1	13
2 ml	17	8.50	8	47	5	29	3	18	1	6
5 ml	38	7.60	18	47	11	29	5	13	4	11
*3 ml	244	8.13	123	50	63	26	39	16	19	8
C:1 ml	0	0	0	0	0	0	0	0	0	0
		$\bar{X}$ 8.06		45		27		18		9
				$\bar{\bar{X}}$ 47		26		17		7
			HYDRA MEDIA WASH							
Wash 1	223	223	3		176		37		7	
Wash 2	79	79	1		63		13		2	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 3020	4	1	239	79	50	17	9	3
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	162	162	4		29		124		5	
" :B	297	148.5	1		28		118		2	
		$\bar{X}$ 155.25	2.5		28.5		121		3.5	
sup. 2:A	47	47	1		8		37		1	
crushed hydra	24	--	1		5		16		2	
		T_i 2047	36	2	370	18	1596	78	42	2
			HYDRA DISINFECTANT WASH							
Wash 1	0	0	0		0		0		0	
Wash 2	0	0	0		0		0		0	
Wash 3	0	0	0		0		0		0	
control	0	0	0		0		0		0	
		T_o 0	0	0	0	0	0	0	0	0
			HYDRA CRUSH							
control	0	0	0		0		0		0	
sup. 1:A	170	170	1		29		136		4	
" :B	291	145.5	2		28		113		3	
		$\bar{X}$ 157.75	1.5		28.5		124.5		3.5	
sup. 2:A	44	44	1		7		34		2	
crushed hydra	31	--	0		8		22		1	
		T_i 2049	25	1	363	17	1607	78	56	3
				$\bar{X}$ 1		17		78		3

APPENDIX B

MAINTENANCE CHART

DATE	FED (AM)	CHANGE I (AM)	CHANGE II(PM)	TEMPERATURE °C
4/27	9:00	10:00	17:00	16.0
4/28	9:00	10:00	16:30	16.0
4/29	10:00	11:00	18:00	15.8
5/1	9:45	10:45	18:00	15.8
5/2	10:00	11:00	17:00	15.8
5/3	10:00	11:00	22:00	16.0
5/4	9:30	10:30	18:00	16.0
5/5	10:00	11:00	18:00	16.0
5/6	10:00	11:00	17:00	15.8
5/8	9:00	10:00	16:00	16.0
5/9	10:00	11:00	16:30	16.0
5/10	10:00	11:00	17:00	16.0
5/11	10:00	11:30	17:00	16.0
5/12	9:30	10:30	17:00	15.6
5/15	10:00	11:00	16:30	15.8
5/16	10:00	11:00	17:00	15.8
5/17	10:00	11:00	17:00	16.0
5/18	10:00	12:00	17:00	16.0
5/19	10:00	11:00	17:00	16.0
5/20	10:00	11:00	17:00	16.0
5/22	9:00	10:00	16:00	16.0
5/23	9:30	10:30	16:00	16.0
5/24	9:30	10:30	16:00	16.0
5/25	10:00	11:00	17:00	16.0
5/26	10:00	11:00	16:30	15.8
5/27	10:00	11:30	18:00	16.0
5/30	9:00	10:00	16:00	15.6
5/31	9:30	10:30	16:30	15.8
6/1	9:30	10:30	16:30	15.8
6/2	10:00	11:00	17:00	16.0
6/3	9:00	10:00	16:00	16.0
6/5	10:00	11:00	17:30	16.0
6/6	10:00	11:30	16:30	16.0

REFERENCES CITED

Breed, Robert. Murray, E.G.D. and Smith, Nathan. 1957. Bergey's Manual of Determinative Bacteriology. The Williams and Wilkins Company. Baltimore. pp. 309, 335, 373, 368, 464, 490.

Beutler, R. 1924. Experimentelle Untersuchungen uber die Verdauung bei Hydra. Z. Vergl. Physiol. 1:1-56.

Bradshaw, L.J. 1963. Laboratory Microbiology. W.B. Saunders Co. Philadelphia, pp. 117-130

Burnett, A. L. Davidson, R. and Wiernik, P. 1963. On the Presence of a Feeding Hormone in the Nematocyst of Hydra pirardi. Biol. Bull. 125: 226-233.

Difco Laboratories. 1953. Difco Manual. Detroit. p. 139.

Dobell, Clifford. 1960. Antony van Leeuwenhoek and his "Little Animals", Letter 149, December 25, 1702. Dover Publs. New York. pp. 280-281.

Eakin, Robert E. 1961. "Studies on Chemical Inhibition of Regeneration in Hydra". The Biology of Hydra and of some other Coelenterates: 1961. Edited by Lenhoff, H.M. and Loomis, W.F. University of Miami Press. Coral Gables, Florida. pp. 399-412.

Forrest, H. 1962. Lack of Dependence of the Feeding Reaction in Hydra on Reduced Glutathione. Biol. Bull. 122: 343-363.

_____. 1963. Immortality and Pearl's survivorship curve for hydra. Ecology 44:609-610.

Hess, Arthur. 1961. "The Fine Structure of Cells in Hydra". The Biology of Hydra and of some other Coelenterates: 1961. Edited by Lenhoff, H.M. and Loomis, W.F. pp. 1-50.

Lenhoff, Howard M. 1961a. Activation of the Feeding Reflex in Hydra littoralis. I. Role Played by Reduced Glutathione and Quantitative Assay of Feeding Refles. J. Gen. Physiol 45: 331-344.

_____. 1961b. Digestion of Protein in Hydra as Studied Using Radiography and Fractionation by Differential Solubilities. Exp. Cell. Res. 23: 335.

_____ and Brown, Ray Dubois. 1970. Mass Culture of Hydra: An Improved Method and its Application to other Aquatic Invertebrates. Lab. Anim. 4(1): 139-154.

_____ and Loomis, W. Farnsworth. 1957. Environmental Factors Controlling Respiration in Hydra. J. Exp. Zool. 134: 171-182.

_____(eds.). The Biology of Hydra and of some other Coelenterates: 1961. University of Miami Press. Coral Gables, Florida. pp. v-vii.

Lentz, Thomas L. 1966. The Cell Biology of Hydra. John Wiley and Sons, Inc. New York pp. 1-18.

Loomis, W. Farnsworth. 1953. The Cultivation of Hydra under Controlled Conditions. Science 117: 565-566.

_____. 1955. Control of Sexual Differentiation in Hydra by pCO_2. Ann. N.Y. Acad. Sci. 77: 73-86.

_____. 1961. "Feedback Factors Affecting Sexual Differentiation in Hydra littoralis." The Biology of Hydra and of some other Coelenterates: 1961. Edited by Lenhoff, H.M. and Loomis, W.F. University of Miami Press. Coral Gables, Florida. pp. 337-362.

Millipore Corporation. 1969. Microbiological Analysis of Water. Catalog No. LARO 81/u U.S.A. pp. 1-25.

Odum, Eugene P. 1959. Fundamentals of Ecology. W. B. Saunders Co. Philadelphia. pp. 46-56.

Poddar, Saradell. 1972. Master's Thesis: Loss of Soluble and Particulate Material from Hydra Populations. Youngstown State University. Youngstown, Ohio. pp. 11, 26.

Schroeder, Lauren. 1969. Population Growth Efficiencies of Laboratory Hydra pseudoligactis Hyman populations. Ecology 50(1): 81-86.

Slautterback, E.L. and Fawcett. D.W. 1959. The Development of the Cnidoblasts of Hydra. An Electron Microscope Study of Cell Differentiation. J. Biophysic. and Biochem. Cytol. 5: 441-452.

Slobodkin, L.B. 1964. Experimental Populations of Hydridae. J. Animal Ecol. 53: 131-147.

Spangenberg, Dorothy B. 1961. "A Study of Normal and Abnormal Regeneration of Hydra". The Biology of Hydra and of some other Coelenterates: 1961. Edited by Lenhoff, H.M. and Loomis, W.F. University of Miami Press. Coral Gables, Florida. pp. 413-424.

Standard Methods for the Examination of Water and Waste Water. 13th Edition. Am. Pub. Health Assoc., Washington D.C. 1971.

Stiven, Alan E. 1962. The Effect of Temperature and Feeding on the Intrinsic Rate of Increase of Three Species of Hydra. Ecology 43: 325-328.

Strehler, Bernard L. 1961. "Aging in Coelenterates". The Biology of Hydra and of some other Coelenterates: 1961. Edited by Lenhoff, H.M. and Loomis, W.F. University of Miami Press. Coral Gables, Florida. pp. 373-398.

Welsh, John H. 1961. "Compounds of Pharmacological Interest in Coelenterates". The Biology of Hydra and of some other Coelenterates: 1961. Edited by Lenhoff, H.M. and Loomis, W.F. University of Miami Press. Coral Gables, Florida. pp. 179-186.

Wood, Richard L. 1961. "The Fine Structure of Cells in Hydra". The Biology of Hydra and of some other Coelenterates: 1961. Edited by Lenhoff, H.M. and Loomis, W.F. University of Miami Press. Coral Gables, Florida. pp. 51-68.