Regional Association for Biology Laboratory
Educators Conference
University of California - Irvine
February 21, 2015

Workshop 1

RABLE 2015

RABLE 2015
Museum ecology: using fine art to reinforce ecological concepts

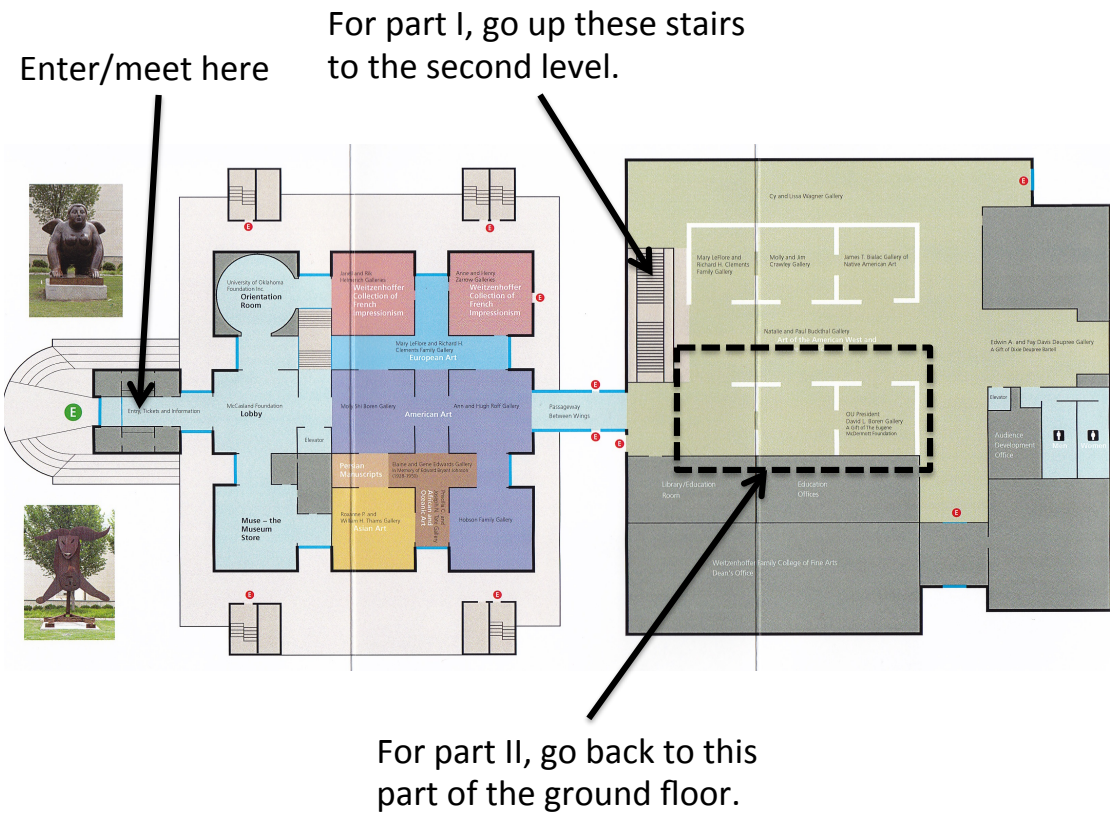
Mariëlle Hoefnagels
University of Oklahoma

RABLE participants: *This is a slightly modified version of a worksheet I use for a lab in my nonmajors biology class. I have left in the rules, gallery maps, point values, and so forth so that you can see the handout as my students see it. If you have an art museum at your own campus, you would of course modify the worksheet to include your own instructions and maps. It should also be possible to modify the activity for an online collection of paintings.*

A few rules while you are here:

- No pens are allowed in the museum galleries – pencils only.
- Do not touch any painting, ever. Do not even look as if you are going to touch any painting, ever – the security guards and other museum personnel cringe whenever you come too close.
- Do not lean on the walls, touch the walls, or use them as a "desk" for filling out your worksheets.
- Museum galleries are typically places of quiet reflection. We don't expect you to be silent while you are here, but please talk quietly and try not to shout to your friends across the gallery.

Ground floor



Part I – General Ecology Questions (7 points)

Instructions: For part 1 of this lab, you will work with your group (no more than three students per group, please) to answer a series of ecology-related questions about paintings on the second floor of the museum. **You must pick a different painting for each of the questions.**

POPULATION ECOLOGY

1. a. What is a population?

b. Find a painting that depicts a **population** of any species (plant or animal, including humans).

- Title:
- Artist:
- General description of subject:

c. Choose any species you can see in the painting. What is it? _____

- For the species you chose, describe the population's density (high? low? somewhere in between?) and pattern of dispersion (random? clumped? a combination?)
- How are members of the species interacting with one another? (competition? mating? something else?)
- What is an example of a **density-dependent control** on the growth of this population? (It can be either evident in the painting or implied)
- What is an example of a **density-independent control** on the growth of this population? (It can be either evident in the painting or implied)

2. In lecture, you learned about the factors that determine whether a population grows or shrinks.

a. What are the events that cause a population's size to increase?

b. What are the events that cause a population's size to decrease?

c. Find a painting that depicts or implies one or more of the events you listed above.

- Title:
- Artist:
- General description of subject:
- Describe the event(s) that are affecting the population's size.

COMMUNITY/ECOSYSTEM ECOLOGY

3. What is the difference between a community and an ecosystem?

4. Find any painting that shows a community with a **large number of different species** (that is, high species diversity).

- Title:
- Artist:
- General description of subject:
- Describe any of the autotrophs you can see in the painting:
- Describe any of the heterotrophs you can see in the painting:
- Which are more prominent in the painting, the heterotrophs or the autotrophs?
- Give an example of a type of organism that MUST be in the scene (because you know how ecosystems work) **but that isn't actually depicted**.

5. Find any painting that shows a community with **low species diversity**.

- Title:
- Artist:
- General description of subject:
- Do you think the species diversity is "naturally" low, or is it low because of human alteration of the environment? How can you tell?

6. a. In class, you learned about three types of **community interactions**. Define each term:

- interspecific competition:
- symbiosis:
- predation:

b. Find a painting that shows (or implies) a **community interaction** of any type.

- Title:
- Artist:
- General description of subject:
- Describe the community interaction.

7. Find a painting that you think shows ***major*** **human alteration** of the environment.

- Title:
- Artist:
- General description of subject:
- How does this painting show human alteration of the environment?

8. Find a painting that you think shows ***minimal*** **human alteration** of the environment.

   - Title:
   - Artist:
   - General description of subject:
   - What clues in the painting suggest that there are few human impacts on the environment?

9. What was your favorite piece in the gallery, and what did you like about it?

Part II – Group Presentation (3 points)

For this part of the exercise, return to the ground floor (see map on first page of this worksheet). Select any painting depicting an outdoor scene. Each group must choose a different painting. *(RABLE note: For museums that do not have multiple collections, the "rules" for Part II can be modified to simply specify that each group must select a different painting and that no group may select a painting they already used in Part I. In fact, those are the rules we're using for the RABLE.)*

1. What are the names of the other people in your group?

2. What is the title of the painting you chose, and who is the artist?

   Title:

   Artist:

3. Give a brief, general description of what is going on in the painting.

4. What can you infer about the weather or climate in the painting? Based on what clues?

5. What types of plants and non-human animals (if any) can you see in the painting? Is the painting realistic enough for you to be able to see any of their adaptations to their climate? If so, what?

6. What does **abiotic** mean? Name three abiotic conditions that are either evident or implied in the painting.

7. What can you infer about the people (if any) in the painting? (Their clothing, activities, posture, etc. may give you clues.)

8. What are three human impacts on the environment that this painting either *depicts* or *implies*? If you can't see three, think about the title and/or the subject and think about some other impacts that are implicit in the subject of the painting.

   a.

   b.

   c.

9. How does each of the three impacts you chose affect the living and/or nonliving environment?

   a.

   b.

   c.

10. Besides what you have already described, choose any other element of the painting that has something to do with biology. How does it relate to something you have learned this semester about biology?

11. Do you see anything in your painting that is *inconsistent* with what you know about biology? If so, what is it?

How the group presentations will work.
When everyone is done with this last set of questions, an instructor will call the class together. He or she will choose someone at random from your group to give a brief presentation about your painting to the rest of the class, explaining your answers to the questions. Each person in your group should be prepared to give the presentation, and everyone in your group will get the same grade based on that person's presentation.

Be sure to turn this entire worksheet in to your TA before you leave!

Museum ecology: using fine art to reinforce ecological concepts

Mariëlle Hoefnagels
University of Oklahoma
Depts. of Biology and
Microbiology/Plant Biology

RABLE – February 2015
10:15 am -12:30 pm

Subscribe to my blog! *nonmajorsbiology.wordpress.com*

About My Class

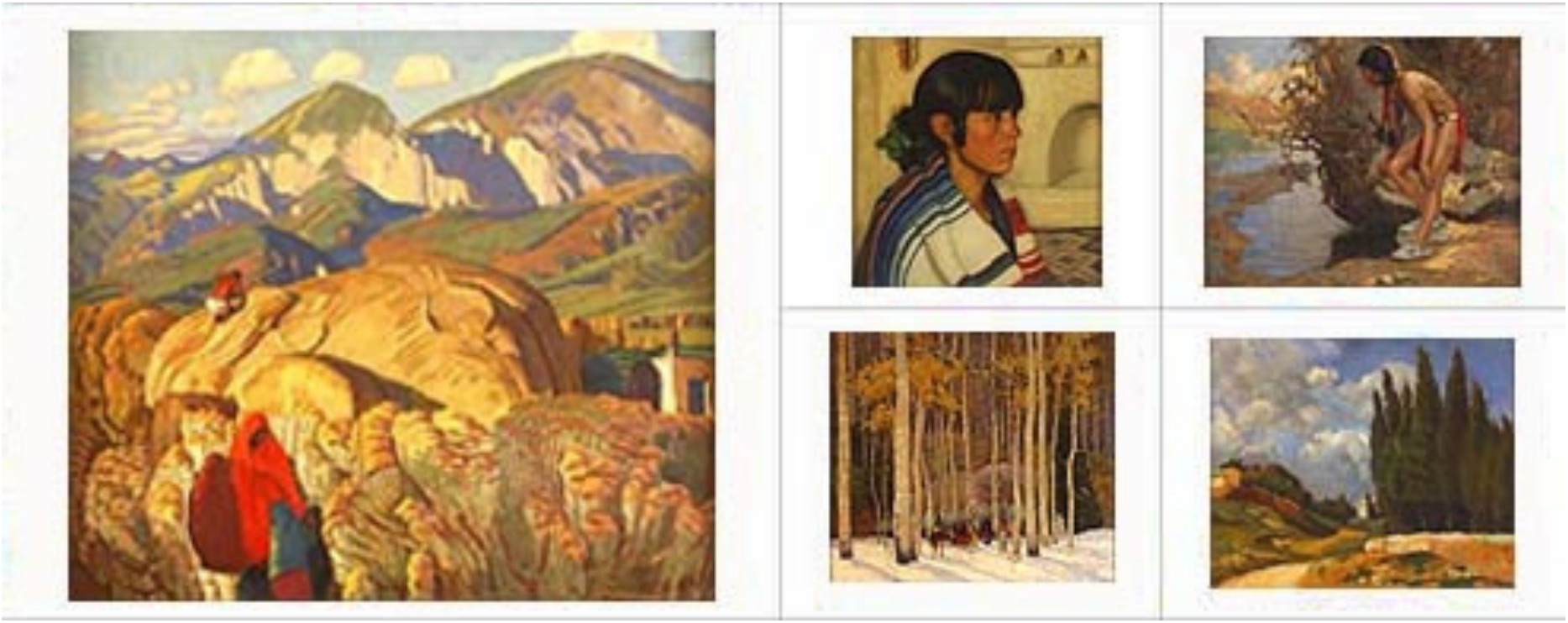
- Non-majors, gen-ed science with a lab; enrolls 70-80
- Each lab section enrolls 30-40
  - This art museum activity takes ~1.5-2 hours
  - Each student signs up for a slot
    - Half come to museum at 1:30
    - The other half come at 2:45

About the Museum

Several Collections Are “Nature-y”

Thams Collection

- Lots of landscapes and images of Native American life, esp. in the Taos (N.M.) area



Tate Collection

More from the Taos Society of Artists

Kenneth Miller Adams
Landscape

Oscar E. Berninghaus
Toas Indians on Mesa

Ernest L. Blumenschein
Yellow Cottonwoods

Eanger Irving Couse
The Medicine Maker

W. Herbert Dunton
Indians of Taos

E. Martin Hennings
Heading for the Pueblo

E. Martin Hennings
Two Women

Victor Higgins
Poplars and Young Fields

Burt Greer Phillips
Indian Fishing

Burt Greer Phillips
Wild Plum Blossoms and Taos Mountain

Joseph Henry Sharp
Crow Reservation - Teepees and Indians (Sweat Lodge)

Walter Ufer
Girls of Isleta

Fleischaker Collection

- Even more from the Taos Society of Artists.

An Art/Biology Partnership

- I got to thinking -- could we use these incredible paintings in a biology course?
- Answer: Yes!

Part I – Ecology Questions

- Please get into pairs.
  - Use the "paintings" *in the hall* (not this room) to fill out **Part I of your worksheets**.
  - Please don't write on the paintings or use the paintings in this room.

Part II – One Painting to Talk About

- Focus on one of the 9 paintings *in this room*
  - Each pair must choose a different one
  - Answer the questions in Part II
  - Again, please don't write on the paintings!
  - When you're done, feel free to take a break; return by 11:30. After the break, we'll have the group presentations and a discussion about the activity.

Part II – Presentations

- I'll show images of the 9 paintings in this room.
  - When your painting comes up, please come to the front and explain your answers to the Part II questions.

Overall student evaluations (**all labs combined**) = **7.2/10**.

Average student evaluation for **this lab** = **8.0/10**.

It's useful for learning ecological concepts …

It's something different …

It's the last lab of the semester …

Oh, and it's short!

Topics for Discussion

- Could you modify this activity for online “art galleries” or for your own campus situation?
- Suggested changes to the questions on the worksheet?
- Additional concepts to ask about? (e.g., relationship between ecology and evolution?)
- Ideas for followup assignments (e.g., write a paragraph for the museum display about the biology behind a painting?)
- Others?

Workshop 2

RABLE 2015

Microbial biodiversity in soil: A research-based introductory biology laboratory course

Regional ABLE Meeting
Irvine CA | February 21, 2015

Stanley M. Lo (smlo@ucsd.edu)
University of California, San Diego

Agenda for today

1. Research-based laboratory courses
2. Microbial biodiversity in soil
3. Data analysis in Excel and sequence alignment

National calls for research-based laboratory courses

Boyer Commission on Educating Undergraduate (1998)

- Restructure undergraduate learning experience
- Focus on inquiry- and research-based learning

President's Council of Advisors for Science and Technology (2012)

- Increase STEM-educated workforce
- Engage students in authentic research in laboratory courses

Research-based courses in introductory biology: Two examples

UC San Diego

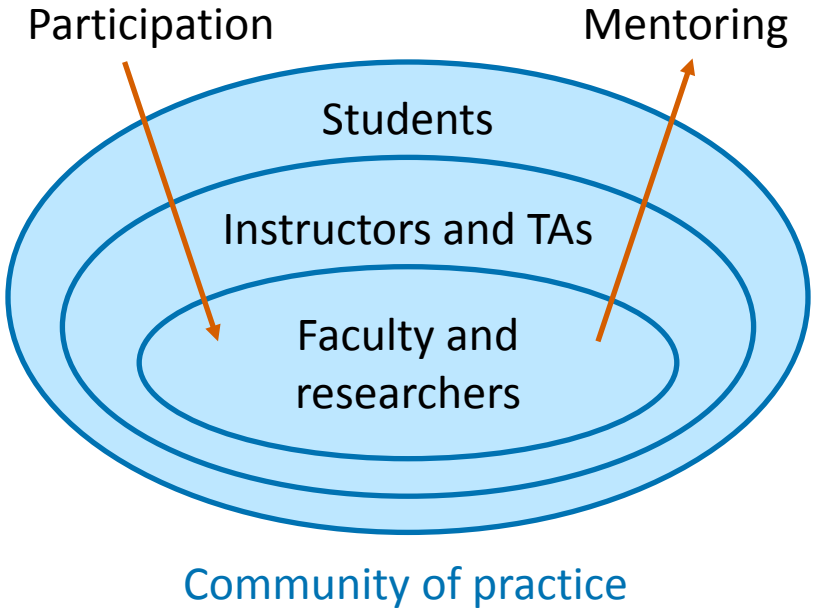
- Stand-alone laboratory course
- Independent on-going research program
- Shared experiments among all students
- Single course in one quarter

NORTHWESTERN UNIVERSITY

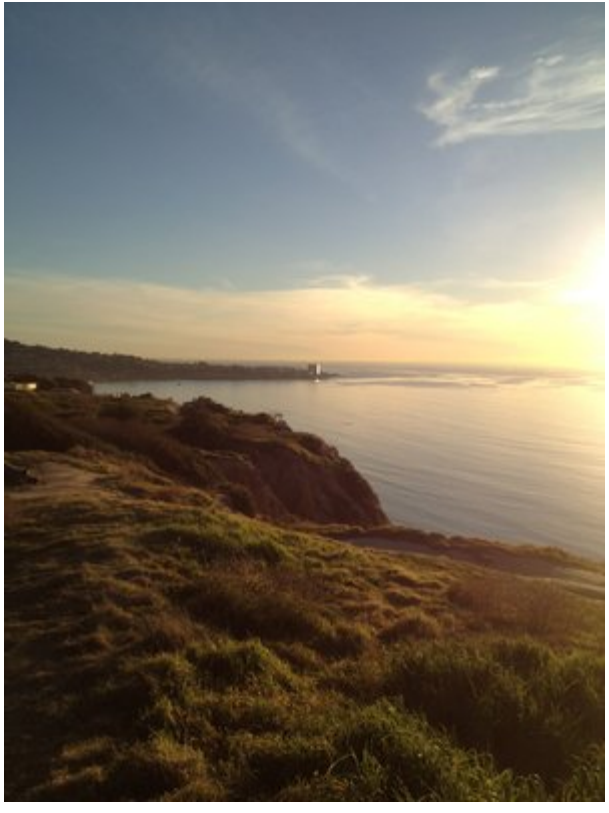
- Stand-alone laboratory courses
- Connected to faculty research programs
- Different experiments among groups
- Sequence of two courses

Design framework: Community of practice

Authentic research experience: Students perform the same tasks as scientists would in the same setting (i.e. legitimate), even though students' level of competence may not be as sophisticated (i.e. peripheral)



UC San Diego: Longitudinal survey on soil microbiomes



Soil properties

- How much moisture does the soil contain?
- How acidic is the soil?

Functional biodiversity

- What carbon sources are metabolized by the microbial community?

Genetic biodiversity

- What microorganisms are present?

Specific research question for each quarter

Native plant species

Invasive plant species

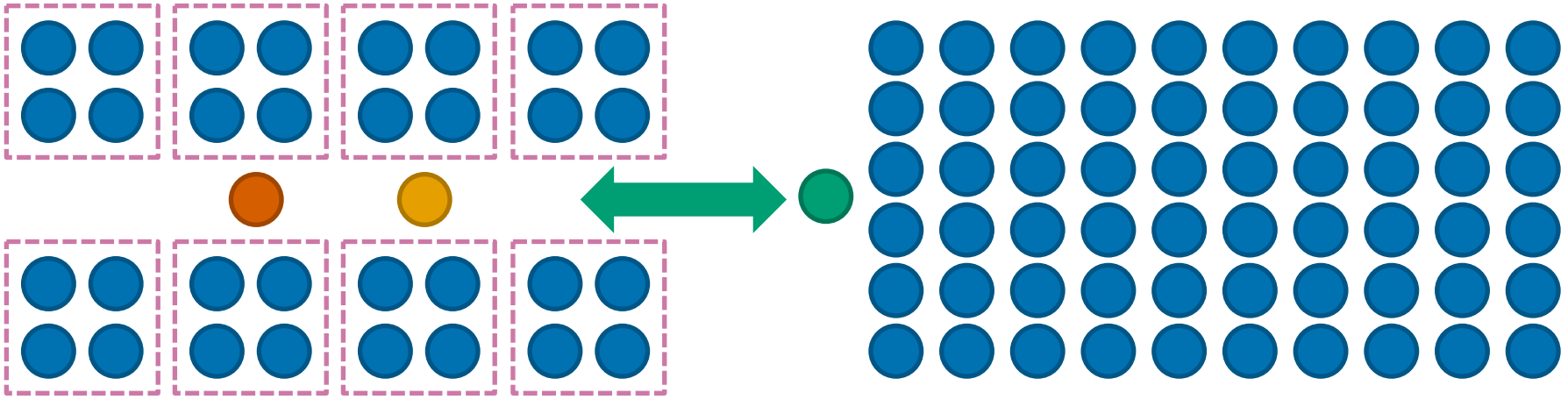
How do soil properties and microbiome biodiversity differ for native and invasive plant species?

Complementary laboratory project and research proposal

Soil microbiome project
Research proposal

- Asking questions
- Making observations
- Generating hypotheses
- Designing and doing experiments
- Collecting and analyzing data
- Drawing conclusions
- Communicating results and ideas

Course structure: Three hours of laboratory per week



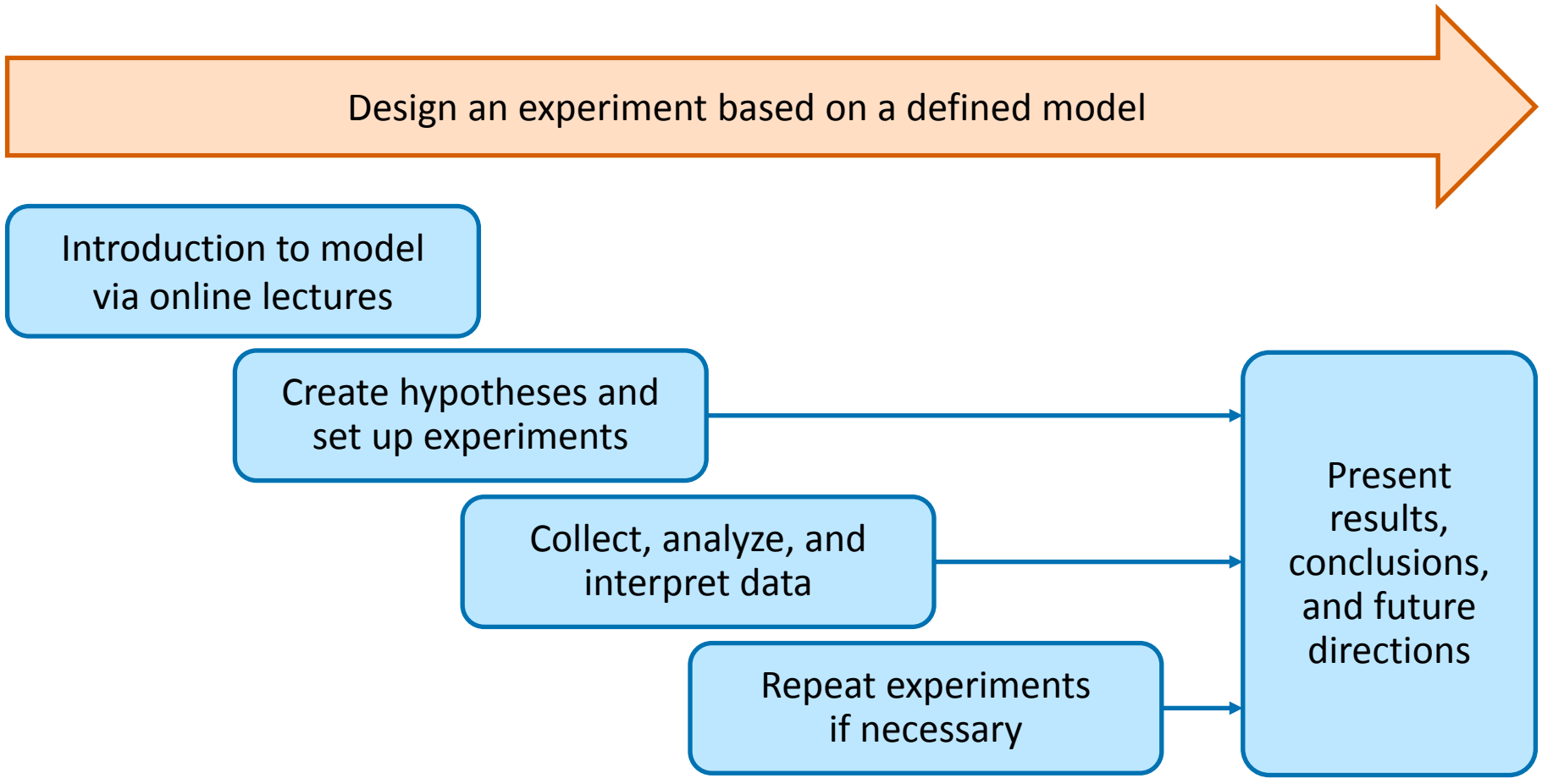
Per laboratory section:

- 32 students in 8 student groups
- 1 undergraduate TA and 1 graduate TA

For all laboratory sections:

- 1 faculty
- Weekly 80-min “lecture”

Northwestern: Focus on experimental design

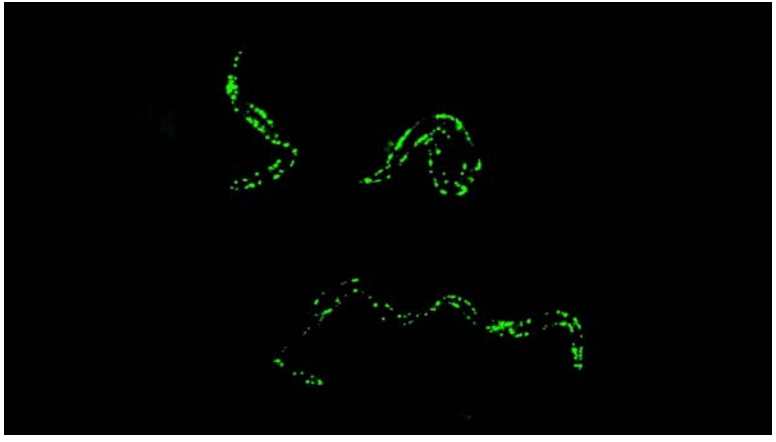


Research project: Protein-folding diseases in model organism

Round worm *C. elegans*

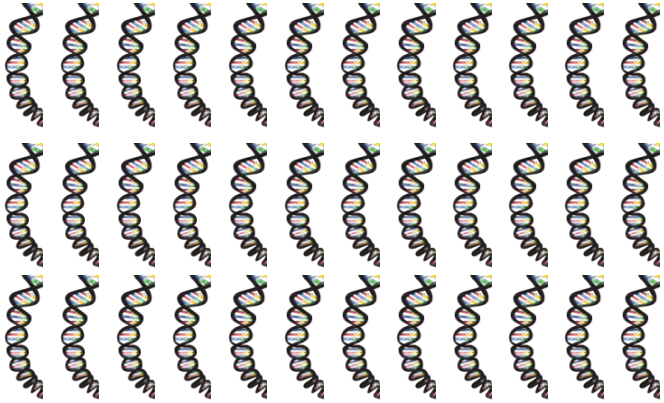
- Visible under standard dissecting scopes
- Grow on agar plates with bacteria
- Store indefinitely in -80°C

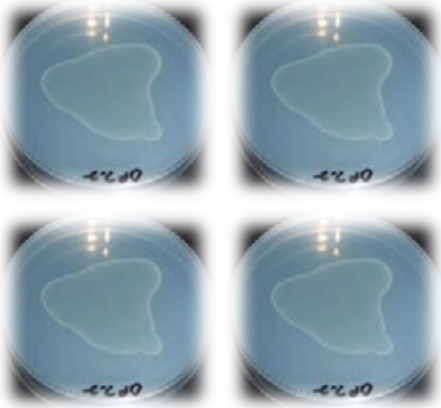
Wild-type worms



Alzheimer's model

Course logistics: Parallel but different experiments





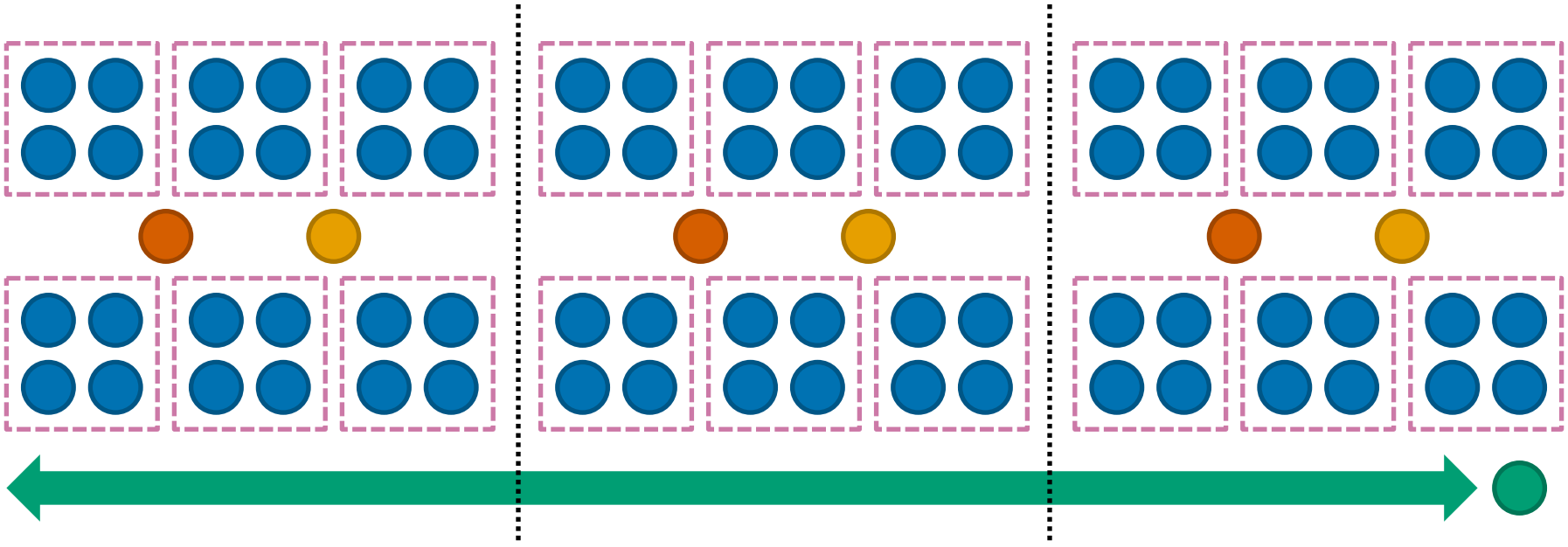
38 candidates genes that may have effects on toxicity:

- Knockdown by RNAi

4 toxicity assays:

- Thrashing, movement, longevity, and egg laying

Course structure: Three hours of laboratory per week



24 students in 6 student groups per section

1 undergraduate TA and 1 graduate TA per section

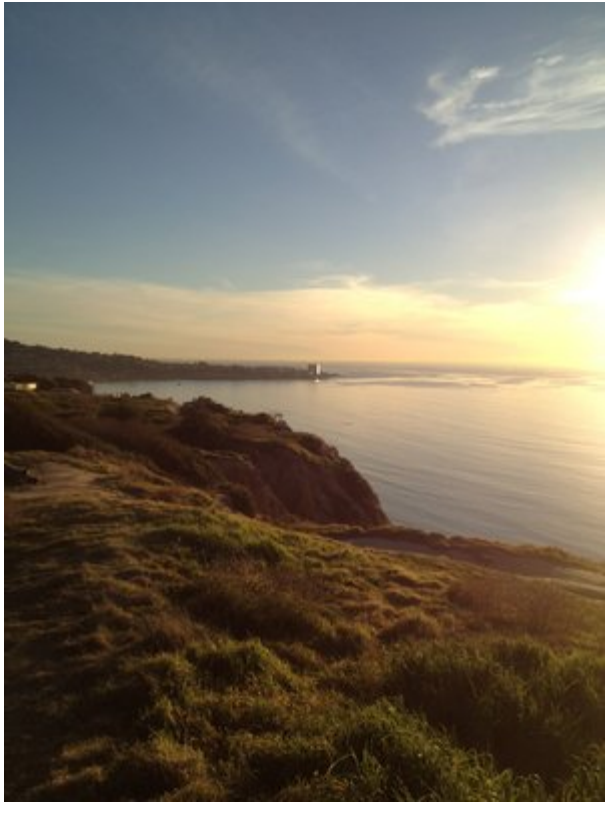
1 faculty per 3 concurrent sections

Brainstorm: Can you do this at your institution?

Write down some ideas on the handout in relation to these questions:

- What resources and support do you need?
- What barriers and challenges do you anticipate?
- What potential solutions can you think of?

Microbial biodiversity in soil



Soil properties

- How much moisture does the soil contain?
- How acidic is the soil?

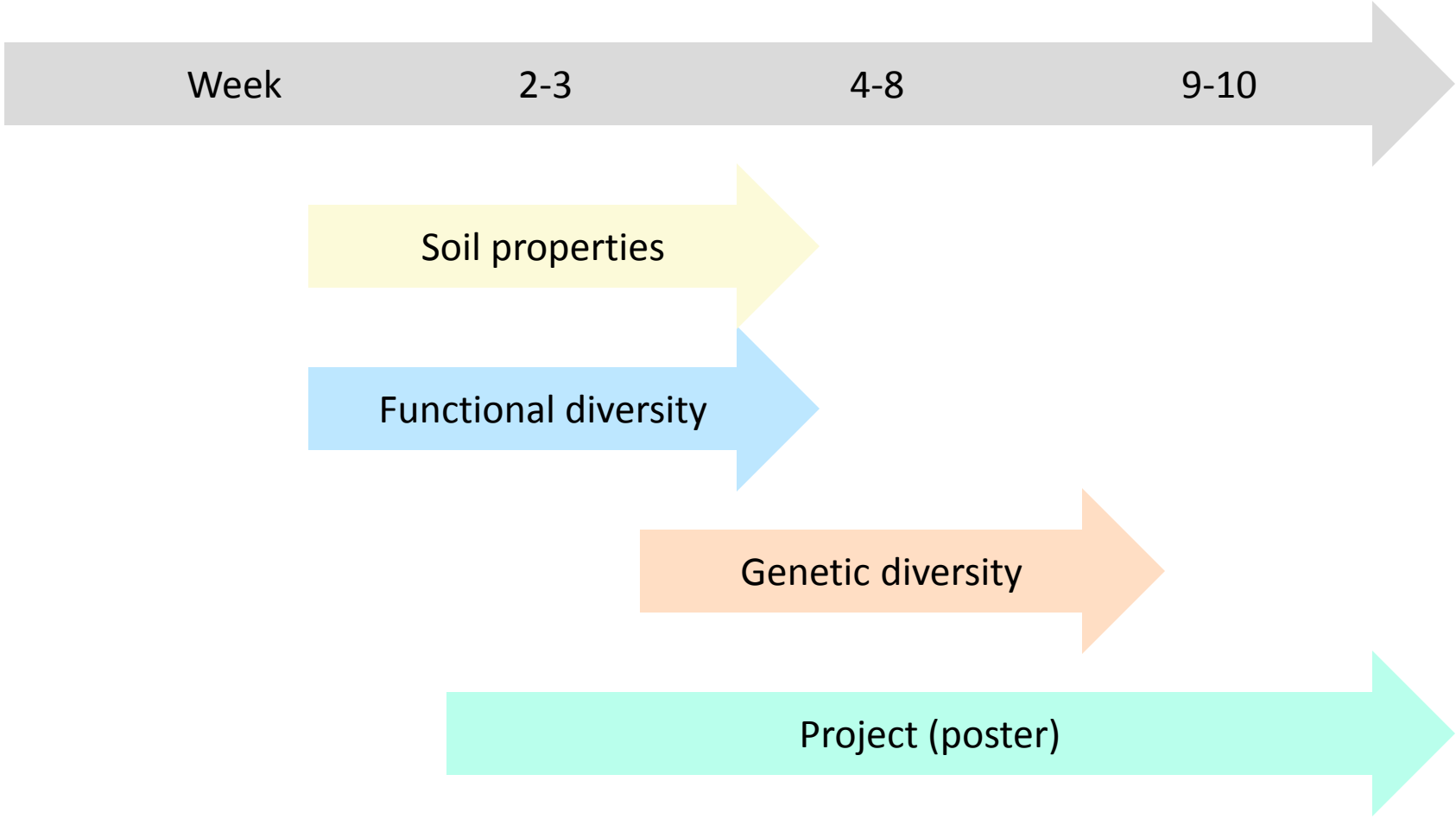
Functional biodiversity

- What carbon sources are metabolized by the microbial community?

Genetic biodiversity

- What microorganisms are present?

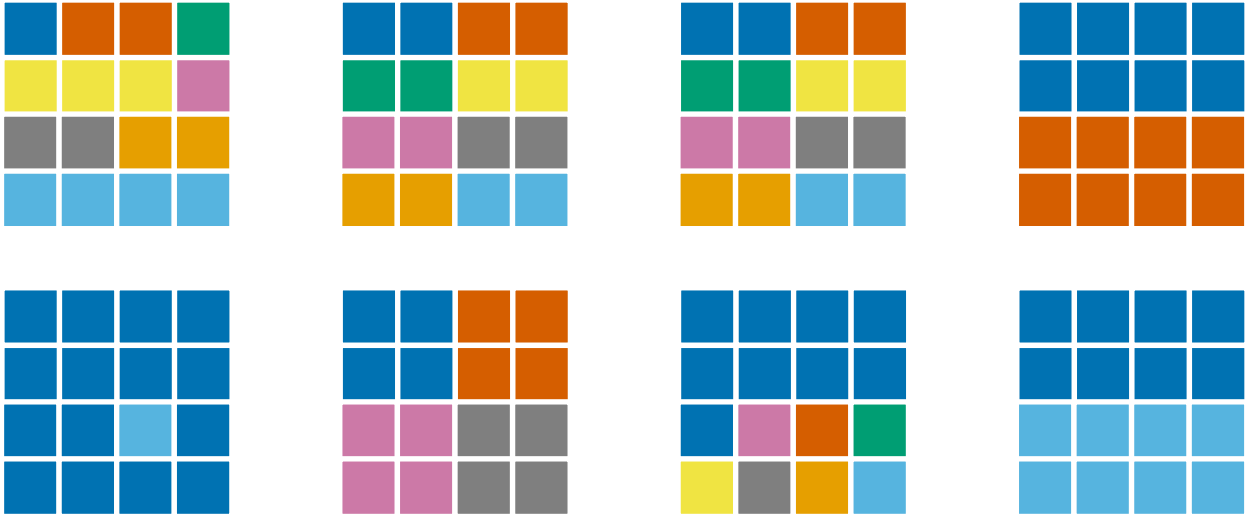
Seven weeks of laboratory experimets



How do we measure biodiversity?

More diverse

Less diverse



Richness Evenness Difference

Shannon diversity index: Richness and evenness

Shannon diversity index
= $H = -\sum p_i \times \ln(p_i)$

Species	Number	p_i	$\ln(p_i)$	$p_i \times \ln(p_i)$
1	40	0.200	-1.609	-0.322
2	40	0.200	-1.609	-0.322
3	40	0.200	-1.609	-0.322
4	40	0.200	-1.609	-0.322
5	40	0.200	-1.609	-0.322
Total	200	1.000		-1.609
				H = 1.609

Shannon diversity index: Richness and evenness

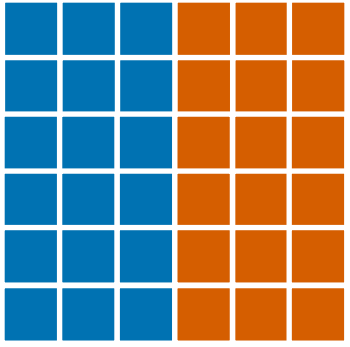
Shannon diversity index
= $H = -\sum p_i \times \ln(p_i)$

Species	Number	p_i	$\ln(p_i)$	$p_i \times \ln(p_i)$
1	1	0.005	-5.298	-0.026
2	1	0.005	-5.298	-0.026
3	196	0.980	-0.020	-0.019
4	1	0.005	-5.298	-0.026
5	1	0.005	-5.298	-0.026
Total	200	1.000		-0.126
				H = 0.126

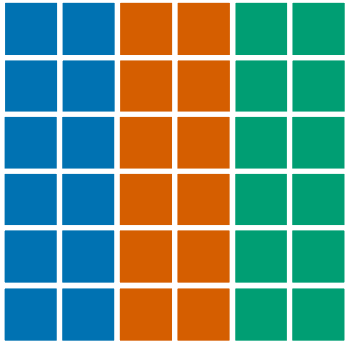
Shannon diversity index (H)

Measures richness

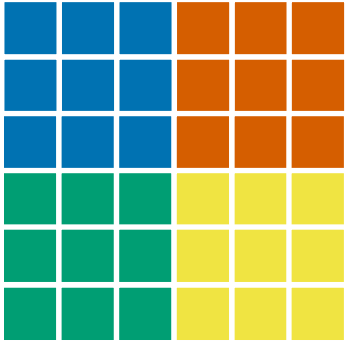
H = 0.69



H = 1.10

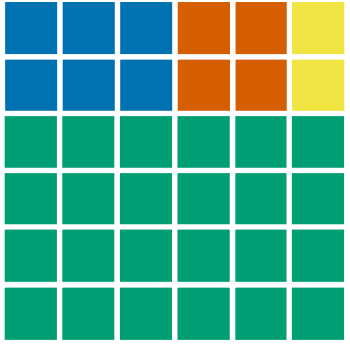


H = 1.39

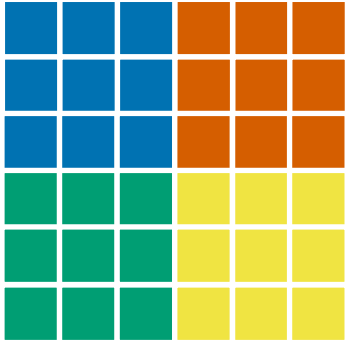


Measures evenness

H = 0.98

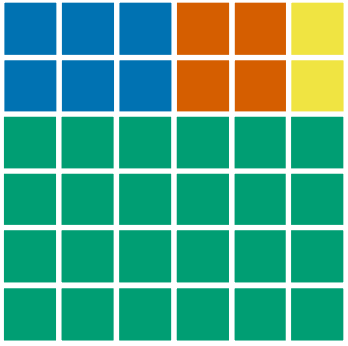


H = 1.39

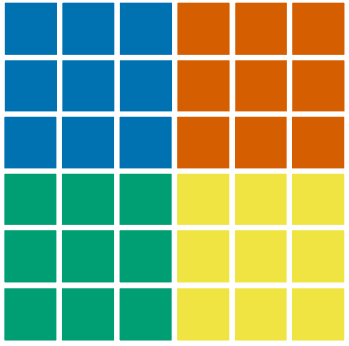


Shannon evenness index

Shannon evenness index
= $E = H / H_{max} = H / \ln (S)$



H = 0.98
H_{max} = 1.39
E = 0.71



H = 1.39
H_{max} = 1.39
E = 1.00

S = richness (number of species)

ln (S) = maximum H given number of species

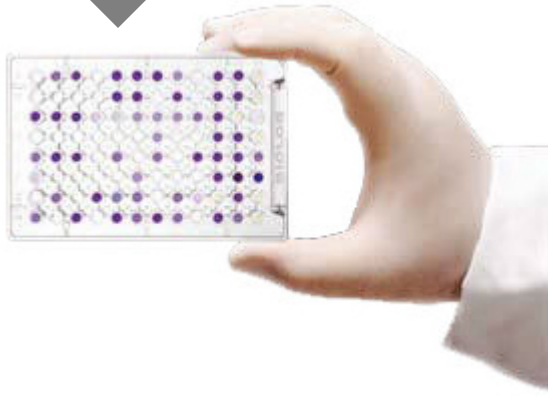
ln (4) = 1.39

Functional biodiversity: Carbon source utilization by Ecoplate

Resuspend microbes in water

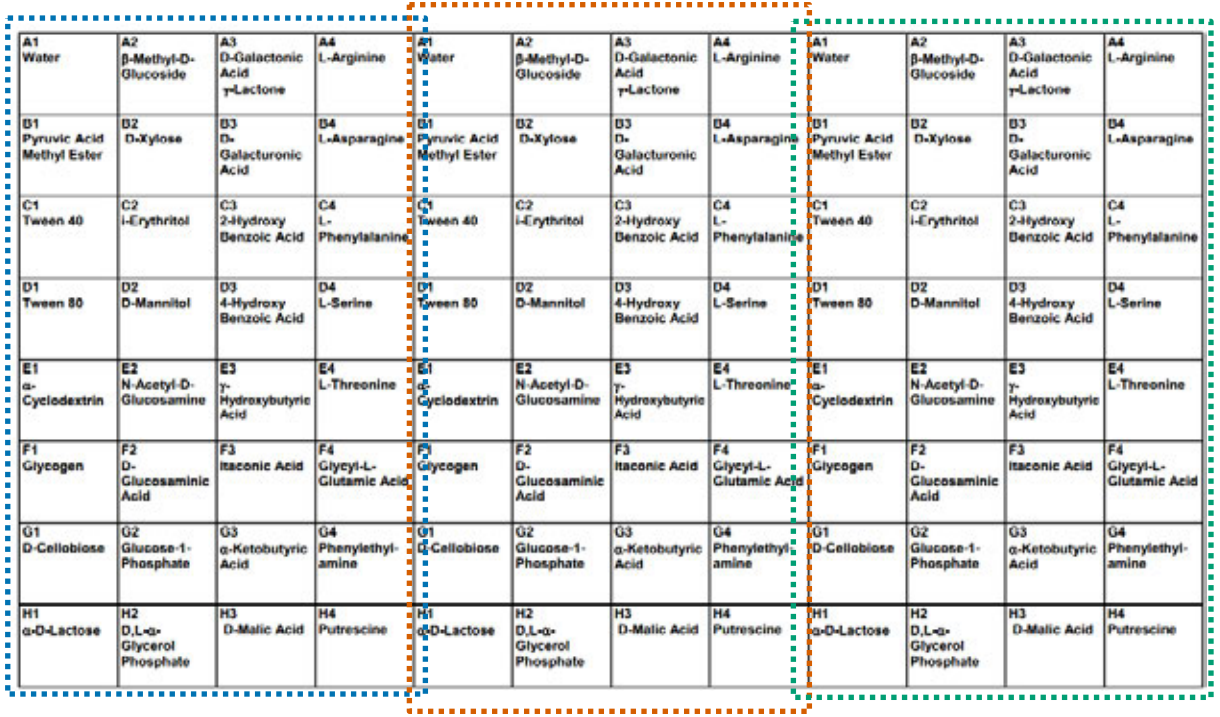
50-mL conical tube

Test carbon source utilization in Ecoplate



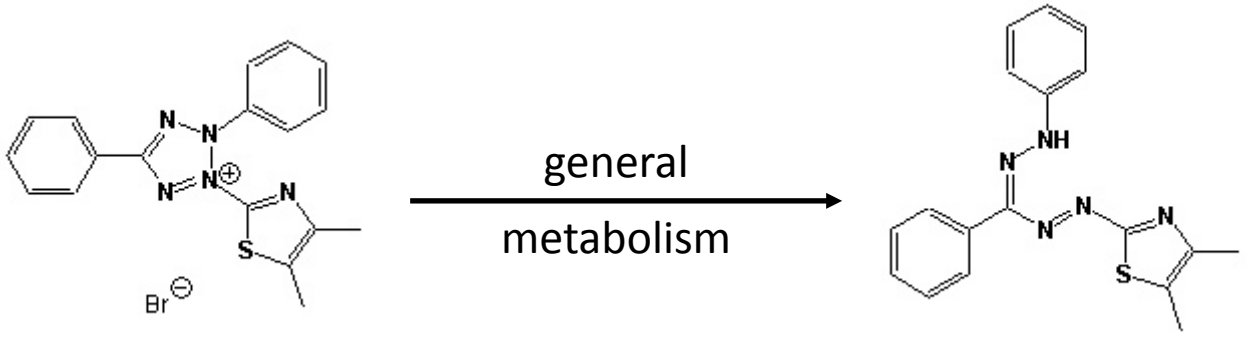
Functional biodiversity: Carbon source utilization by Ecoplate

Triplicate of 31 carbon sources and no carbon source (“water”)



A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine
B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine
C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine
D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine
E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine	E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine	E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine
F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid
G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine	G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine	G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine
H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine

How does Ecoplate work?



Indicator: Colorless

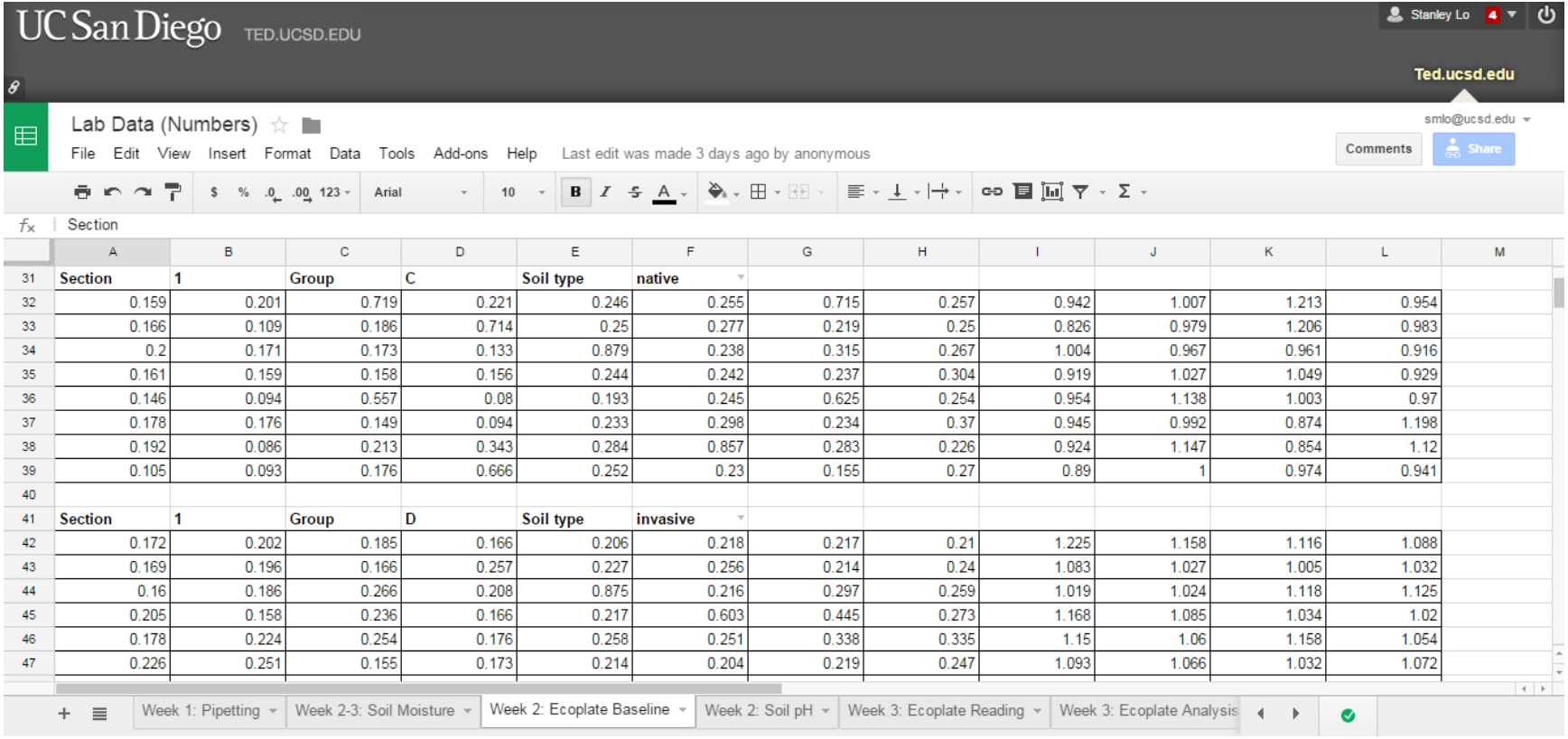
Purple and absorbs light at 590 nm

Soil suspension with controls in Ecoplate

Water | E. coli culture | Soil suspension

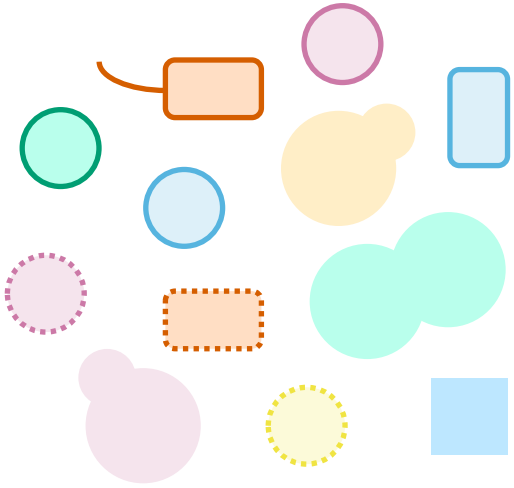
A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine
B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine
C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine
D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine
E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine	E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine	E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine
F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid
G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine	G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine	G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine
H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine

Shared data on Google spreadsheet

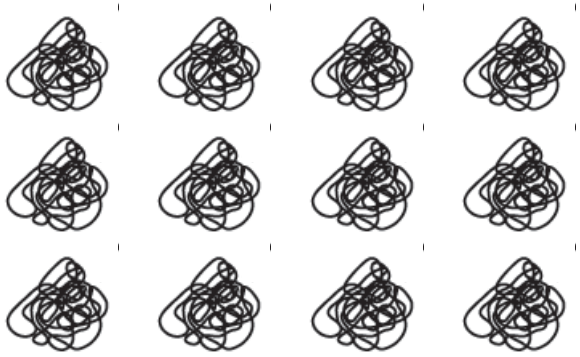


	A	B	C	D	E	F	G	H	I	J	K	L	M
31	**Section**	**1**	**Group**	**C**	**Soil type**	**native**							
32	0.159	0.201	0.719	0.221	0.246	0.255	0.715	0.257	0.942	1.007	1.213	0.954	
33	0.166	0.109	0.186	0.714	0.25	0.277	0.219	0.25	0.826	0.979	1.206	0.983	
34	0.2	0.171	0.173	0.133	0.879	0.238	0.315	0.267	1.004	0.967	0.961	0.916	
35	0.161	0.159	0.158	0.156	0.244	0.242	0.237	0.304	0.919	1.027	1.049	0.929	
36	0.146	0.094	0.557	0.08	0.193	0.245	0.625	0.254	0.954	1.138	1.003	0.97	
37	0.178	0.176	0.149	0.094	0.233	0.298	0.234	0.37	0.945	0.992	0.874	1.198	
38	0.192	0.086	0.213	0.343	0.284	0.857	0.283	0.226	0.924	1.147	0.854	1.12	
39	0.105	0.093	0.176	0.666	0.252	0.23	0.155	0.27	0.89	1	0.974	0.941	
40													
41	**Section**	**1**	**Group**	**D**	**Soil type**	**invasive**							
42	0.172	0.202	0.185	0.166	0.206	0.218	0.217	0.21	1.225	1.158	1.116	1.088	
43	0.169	0.196	0.166	0.257	0.227	0.256	0.214	0.24	1.083	1.027	1.005	1.032	
44	0.16	0.186	0.266	0.208	0.875	0.216	0.297	0.259	1.019	1.024	1.118	1.125	
45	0.205	0.158	0.236	0.166	0.217	0.603	0.445	0.273	1.168	1.085	1.034	1.02	
46	0.178	0.224	0.254	0.176	0.258	0.251	0.338	0.335	1.15	1.06	1.158	1.054	
47	0.226	0.251	0.155	0.173	0.214	0.204	0.219	0.247	1.093	1.066	1.032	1.072	

Genetic biodiversity: 16S rDNA sequencing

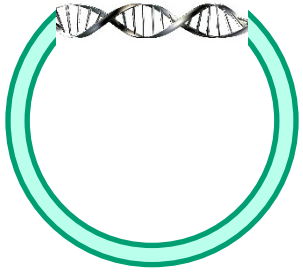


Extract genomic DNA
(Week 4)

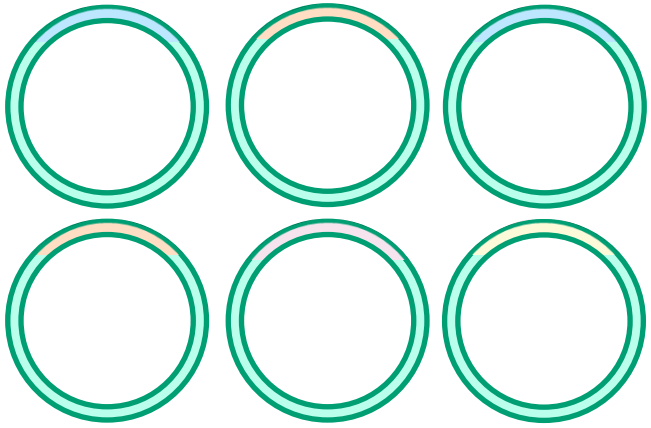


Amplify 16S rDNA
sequences by PCR
(Week 5)

Ligate PRC product into
plasmid (Week 6)

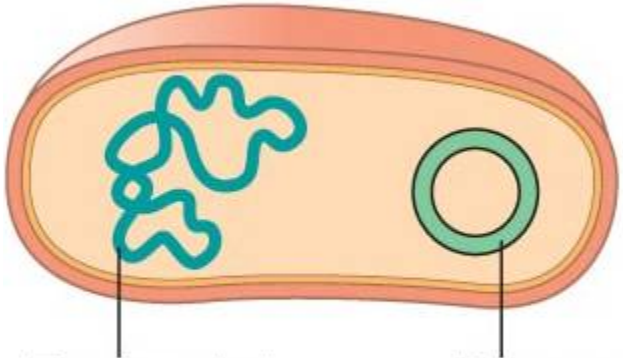


Genetic biodiversity: 16S rDNA sequencing



Transform into E. coli (Week 6)

Bacterium E. coli



Bacterial chromosome

Plasmid

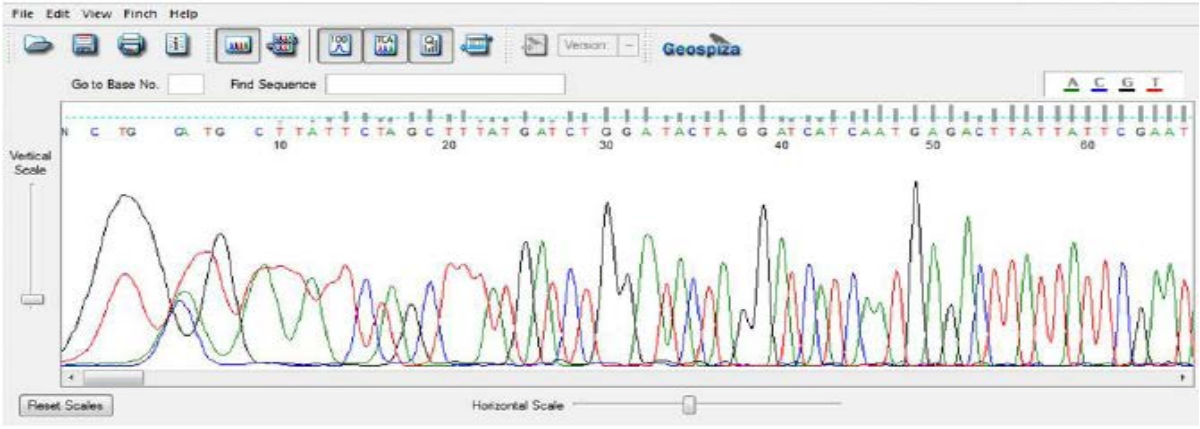
Send bacterial colony to sequence plasmid insert

(Week 7)

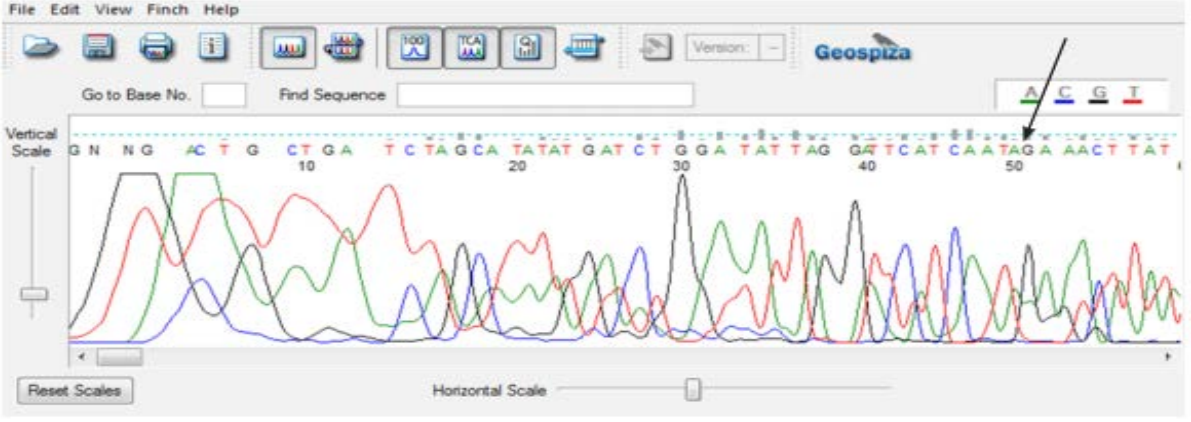
Analyze 16S rDNA sequence data (Week 8)

Quality control in Finch TV

Good sequence

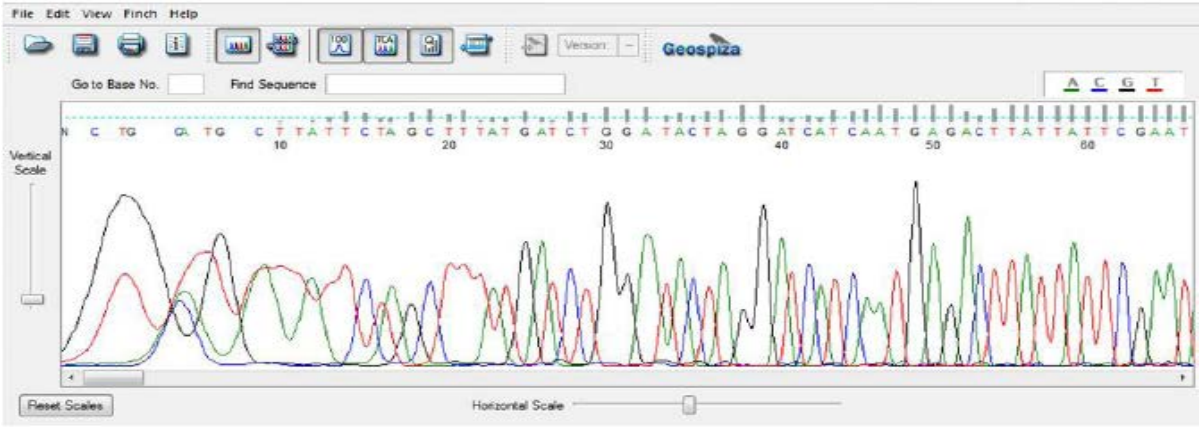


Bad sequence



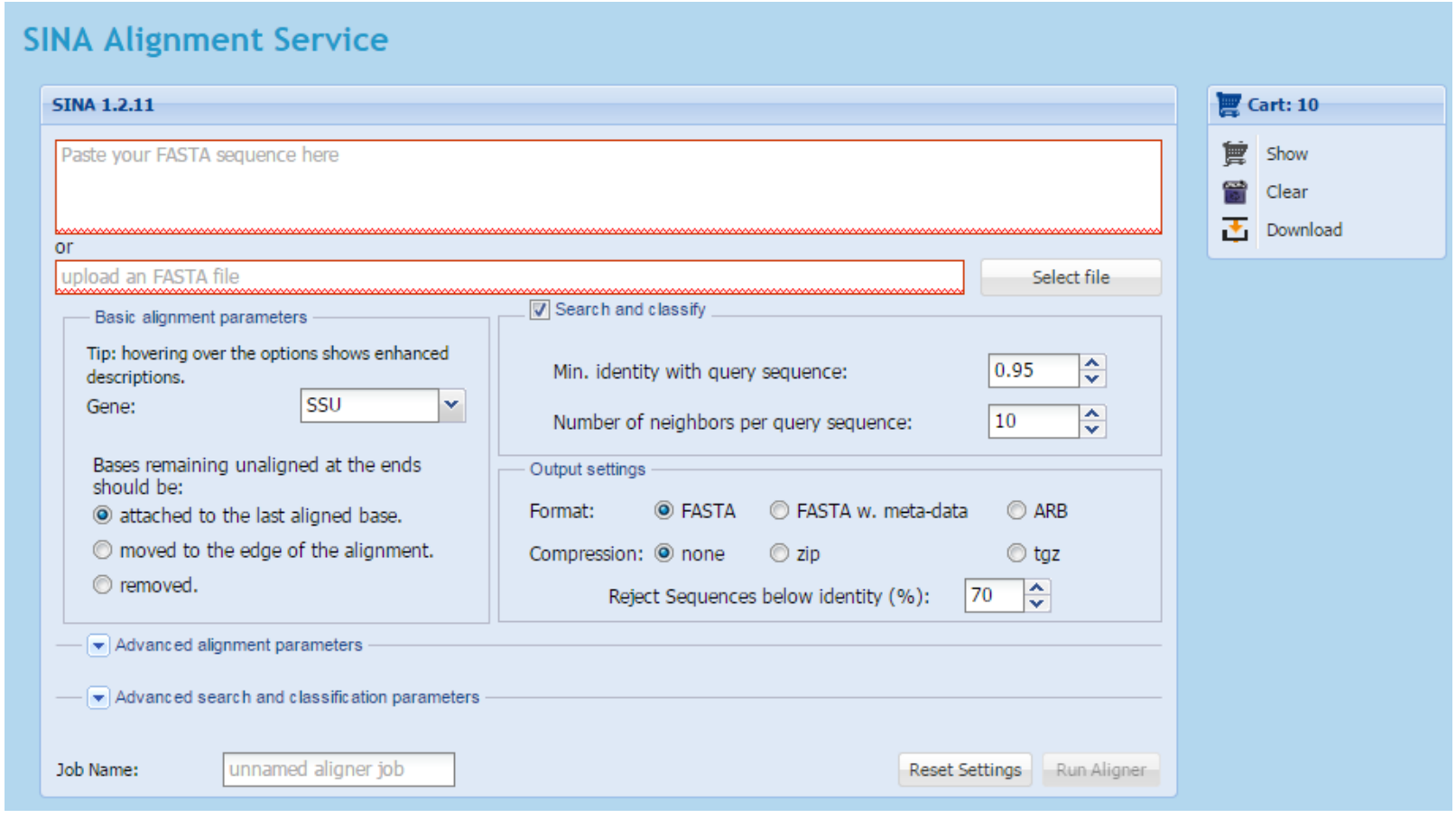
Trimming sequences in Finch TV

Good sequence



1. Remove low quality bases at beginning
2. Remove low quality bases at the end
3. Export sequence in FASTA format (readable by alignment algorithms)
4. Combine all sequences into one FASTA file

Classifying sequences into operational taxonomic units (OTUs)



SINA alignment and classification

1. Take input 16S sequence
2. Identify 10 closest matches in database

accession number	organism name	sequence length	sequence quality	alignment quality	pintail quality	SILVA taxonomy	Options
AB637255	*uncultured bacterium*	1497				Bacteria▸Firmicutes▸Bacilli…	
AB637257	*uncultured bacterium*	1497				Bacteria▸Firmicutes▸Bacilli…	
DQ346606	*uncultured compost bacterium*	1506				Bacteria▸Firmicutes▸Bacilli…	
HQ441581	*uncultured bacterium*	1535				Bacteria▸Firmicutes▸Bacilli…	
JQ738748	*uncultured bacterium*	1500				Bacteria▸Firmicutes▸Bacilli…	
JQ793548	*uncultured Ammoniphilus sp.*	1502				Bacteria▸Firmicutes▸Bacilli…	
JQ825124	*uncultured bacterium*	1498				Bacteria▸Firmicutes▸Bacilli…	
JX079438	*uncultured bacterium*	1528				Bacteria▸Firmicutes▸Bacilli…	
JX133257	*uncultured bacterium*	1500				Bacteria▸Firmicutes▸Bacilli…	
Y14579	*Ammoniphilus oxalaticus*	1497				Bacteria▸Firmicutes▸Bacilli…	

http://www.arb-silva.de/aligner/

SINA alignment and classification

1. Take input 16S sequence
2. Identify 10 closest matches in database
3. Classify OTU for input sequence (common lowest classification)

Bacteria▸Firmicutes▸Bacilli▸Bacillales▸Paenibacillaceae▸Oxalophagus▸
Bacteria▸Firmicutes▸Bacilli▸Bacillales▸Paenibacillaceae▸
Bacteria▸Firmicutes▸Bacilli▸Bacillales▸Paenibacillaceae▸Ammoniphilus▸
Bacteria▸Firmicutes▸Bacilli▸Bacillales▸Paenibacillaceae▸Oxalophagus▸
Bacteria▸Firmicutes▸Bacilli▸Bacillales▸Paenibacillaceae▸uncultured▸
Bacteria▸Firmicutes▸Bacilli▸Bacillales▸Paenibacillaceae▸Oxalophagus▸
Bacteria▸Firmicutes▸Bacilli▸Bacillales▸Paenibacillaceae▸uncultured▸
Bacteria▸Firmicutes▸Bacilli▸Bacillales▸Paenibacillaceae▸uncultured▸
Bacteria▸Firmicutes▸Bacilli▸Bacillales▸Paenibacillaceae▸Oxalophagus▸
Bacteria▸Firmicutes▸Bacilli▸Bacillales▸Paenibacillaceae▸Ammoniphilus▸

Bacteria▸Firmicutes▸Bacilli▸Bacillales▸Paenibacillaceae▸

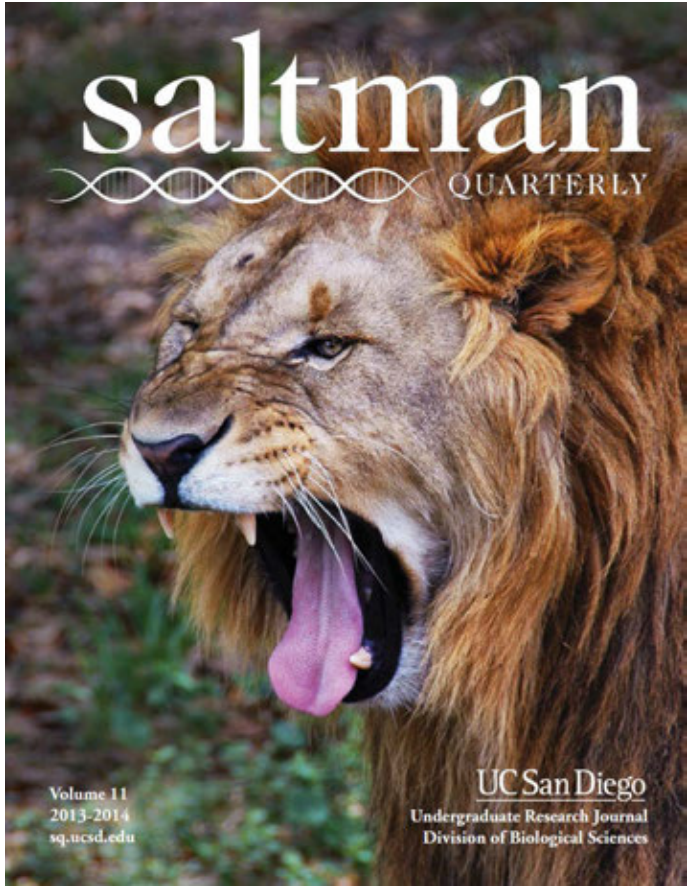
Microbial biodiversity in soil

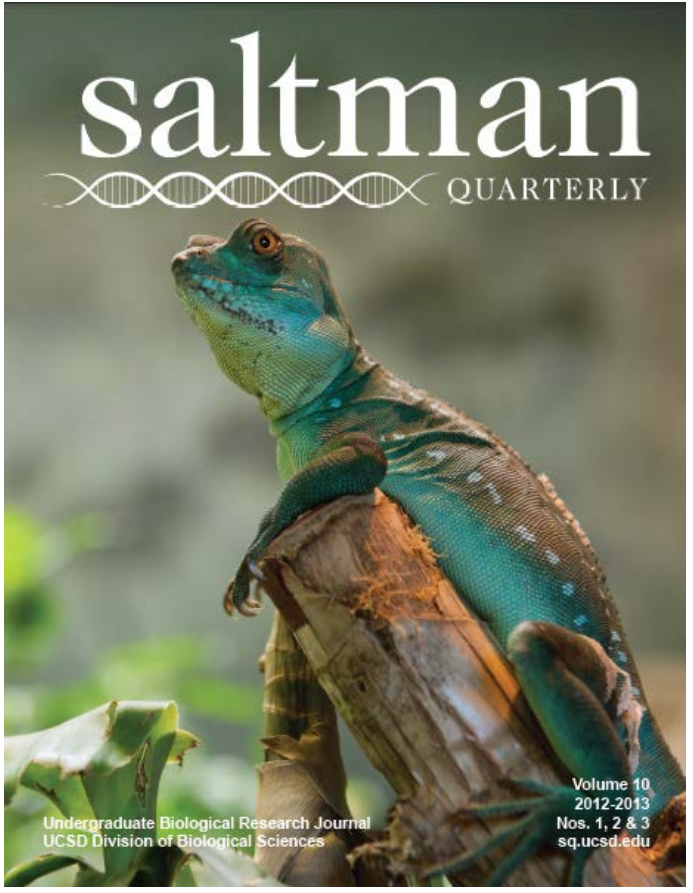
Native plant species

Invasive plant species

Soil properties:	moisture, pH
Functional biodiversity:	Ecoplate: AWCD, richness, Shannon H, Shannon E, carbon source categories
Genetic biodiversity:	16S sequencing: richness, Shannon H, Shannon E

Lab reports in the format of research journal papers





Data analysis in Excel and sequence alignment

Ecoplate data
- Week 3 handout
- Data: http://goo.gl/OwuhJu
- Excel datasheet: http://goo.gl/6lxhTE

16S data (already trimmed)
- Week 8 handout
- http://goo.gl/BDbIht

Workshop 3

RABLE 2015

Ecological and Evolutionary Perspectives on a Tri-trophic System

Dr. Jessica Pratt and Colleen Nell
Department of Ecology & Evolutionary Biology, UCI

Workshop Abstract:

A tri-trophic system, one that incorporates three levels of a food chain, can be easily demonstrated using plants, aphids, and their predators (real or simulated) to teach a variety of ecological and evolutionary concepts in an interactive setting. A Plant-Aphid Study System is simple to set up in the classroom with minimal equipment and can be maintained by students in very little time each week. Aphids are exceptional study subjects for the classroom because they are very easy to keep, reproduce asexually with very fast generation times, have unique anatomical features, and interesting interactions with other organisms in nature. Many students are familiar with aphids as pests but have never examined them closely to determine what makes them successful. This workshop will cover the following topics: 1) selecting and obtaining a Plant-Aphid Study System, 2) materials and time needed to maintain a Plant-Aphid Study System, 3) examples of short-term and long-term lab activities using a Plant-Aphid Study System, and 4) outside resources for additional activities, background information, and interesting popular and formal science articles related to aphids and tri-trophic systems.

This tri-trophic study system can be used for single lab investigations on a variety of topics including plant structure-function relationships, food chain interactions, evolutionary adaptations of plants and insects, aphid population growth and density dependence, and insect biology and ecology (among other topics). It is also ideal to use for a series of investigations that include those previously mentioned topics as well as long-term student-designed experiments that manipulate the plant-aphid environmental context and examine the effects on plant traits and aphid population growth. Workshop participants will complete two hands-on lab activities with aphids and plants during the workshop – *Ecology, Behavior, and Adaptations of Aphids* and *Stop Poking Me! – An Aphid Predator Simulation Lab*. All workshop materials will be provided in editable form for attendees to download.

Workshop Schedule:

10:15 → Introduction to research using tri-trophic systems
10:25 → Selection and maintenance of Plant-Aphid study systems
10:35 → What is an aphid? *Ecology, Behavior, and Adaptations of Aphids* exercise.
10:55 → Discussion
11:05 → Aphid dissection video
11:15 → Break
11:30 → Overview of short and long-term activities using a Plant-Aphid study system
11:40 → *Stop Poking Me! – An Aphid Predator Simulation* exercise
12:00 → Presentation of results and discussion of data analysis with students
12:10 → Discussion of activities, assessment, and adapting to your classroom
12:25 → Wrap up

Ecological and Evolutionary Perspectives on a Tri-trophic System

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Selecting and Obtaining a Plant-Aphid Study System:

Pea plants and pea aphids are recommended by many websites including http://insected.arizona.edu/home.htm, which provides a detailed description of how to obtain and maintain the pea aphid system. Pea plant seeds can be purchased at any greenhouse, nursery, or garden supply center. Pea aphids can be mail ordered from supply companies. A list of suppliers can be found here: http://insected.arizona.edu/gg/suppliers.html. Pea aphids are a common pest species, so care needs to be taken to not release pea aphids into the wild.

Native plants and aphids should be fairly easy to come by with a simple trip to a local farm or arboretum, or chat with any gardener you know. Using local plants and aphids might be trickier, but more interesting to students. Keep in mind that many aphids are specialists and feed only on a particular type of plant. Some common Southern California native plant-aphid pairs that are fun to work with because they are big and brightly colored include the two below.

Uroleucon macolai on *Baccharis salicifolia*

Aphis nerii on milkweed (*Aesclepias spp.)*

The Predators:

Ladybird beetles (a.k.a. ladybugs) are one of the most common aphid predators. Ladybird beetles can be ordered online and are often found at local gardening centers as they are commonly used in gardens and farms to defend plants from pest aphids and other herbivorous insects. All students know what a 'ladybug' is but few recognize the important role they play as insect predators in natural systems. Of course, many exercises can also be completed using various tools to simulate a predator (e.g. paintbrush, forceps).

Materials Needed to Maintain a Plant-Aphid Study System:

In the interest of not 'reinventing the wheel', please use the Resource Sheets found at http://insected.arizona.edu/gg/resource/default.html. These contain detailed and excellent lists of materials needed and links to suppliers and prices that will work for many lessons and activities.

Acknowledgements- Many of the ideas and information included in this workshop resulted from conversations with Dr. Kailen Mooney (UC Irvine) and Catherine Prenot. Ms. Prenot developed an aphid lesson module for her high school classroom and we have included much of that information in this workshop.

Ecological and Evolutionary Perspectives on a Tri-trophic System

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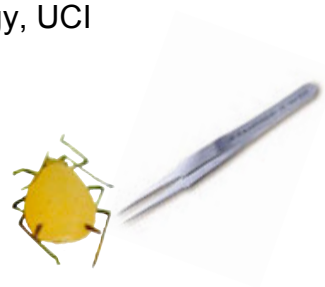

Stop Poking Me! – An Aphid Predator Simulation Lab

Introduction:
All organisms have adaptations that allow them to better survive in the environment in which they live. These adaptations can include both physical characteristics such as size, camouflage, bad-tasting or toxic chemicals, and behavioral characteristics. Some behavioral adaptations allow organisms to avoid being consumed by their predators. For example, some animals are active only at night to avoid potentially threatening predators that are awake during the day. In this activity we will examine anti-predator behavioral adaptations in aphids.

Research Question: Do aphids demonstrate predator avoidance behaviors when their colonies are attacked?

Experimental Design:

1. Each student group should obtain the following:
   - Two milkweed plants covered with aphids
   - A "predator" (paintbrush, straw, or forceps)
   - Two pieces of labeling tape
   - A stopwatch

2. Write a hypothesis about what will happen to the aphids when they are faced by a "predator".

   IF aphids have behavioral adaptations to avoid predators **THEN** _____

   _____

   Based on your hypothesis write a prediction that could support your hypothesis Given the "predator" you chose. Predator:_____

   Prediction: _____

   _____

3. What are some possible behavioral adaptations aphids could exhibit to a predator?

   _____

   _____

4. Follow the steps on the board for completing the experiment for your "predator" an record your data in the table on the next page.

Ecological and Evolutionary Perspectives on a Tri-trophic System

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	Experimental Plant – Record the percent of aphids exhibiting each behavior.			
Trial	Wiggled Abdomen	Walked Away	Jumped off Plant	Other (describe)
1				
2				
3				

	Control Plant – Record the percent of aphids exhibiting each behavior.			
Trial	Wiggled Abdomen	Walked Away	Jumped off Plant	Other (describe)
1				
2				
3				

Results and Conclusions

1. Were the results from all three trials the same? Yes or No
2. If not, why do you think this is? -

   _____

   _____
3. Based on your results do you support or reject the hypothesis? Support Reject
4. What can you conclude about aphid behavioral adaptations ?

   _____

   _____

Communication of Results:

1. Use the provided poster template to create a poster in powerpoint to communicate the results of your experiment to the rest of your class. Be sure to include background information, your hypothesis and predictions, your experimental design, and your results and conclusions.

Ecological and Evolutionary Perspectives on a Tri-trophic System

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Ecology, Behavior, and Adaptations of Aphids

In the following exercise you will observe aphids on their host plant and in a "Petri-dish habitat". Record your observations of aphid anatomy and behavior and answer the questions below as you follow the directions.

1. Make a "Petri dish habitat" for your aphid: cut out one circular piece of paper towel, moisten it and place it in a Petri dish.
2. Cut a small leaf from the plant and place in your Petri dish on top of the paper towel.
3. Using a fine paint brush, nudge one large aphid until it moves from its location on the plant. Gently place the aphid on the leaf in your Petri dish.
4. Utilize hand lenses and dinolite microscopes to diagram your aphid as detailed and accurately as possible.

5. Watch the aphid for at least three minutes. What does it do? Describe.

5. Without the use of text books or other resources (except for your sizable craniums) name, describe, label and predict the function of at least three parts of the aphid's anatomy. Be creative, but make sure that your reasoning makes sense in terms of what behaviors you see in your aphid.

	Name	Description	Predicted Function
1.			
2.			
3.			

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Follow-up Questions:

1. If you were an aphid, what challenges might you face in your environment?

2. How do aphids move?

3. How do aphids eat?

4. Explain how the structure of an aphid may help it survive in its environment.

7. Define parthenogenesis and telescoping generations.

8. What adaptations or features of aphids might make them successful agricultural pests?

9. What questions are you left with at the end of this inquiry that you would like to investigate?

Teaching ecological and evolutionary perspectives on a tri-trophic system

Jessica Pratt & Colleen Nell

RABLE Workshop

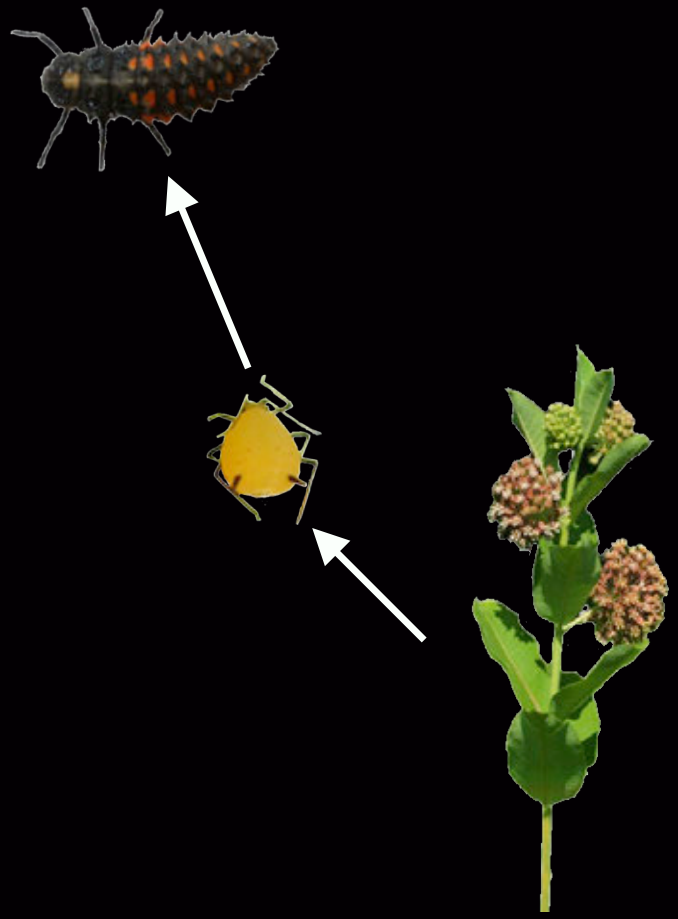
February 21, 2015

Community Ecology

Study of distribution, abundance, demography and interactions between co-occurring species

Research Focus

Plant- Insect Interactions



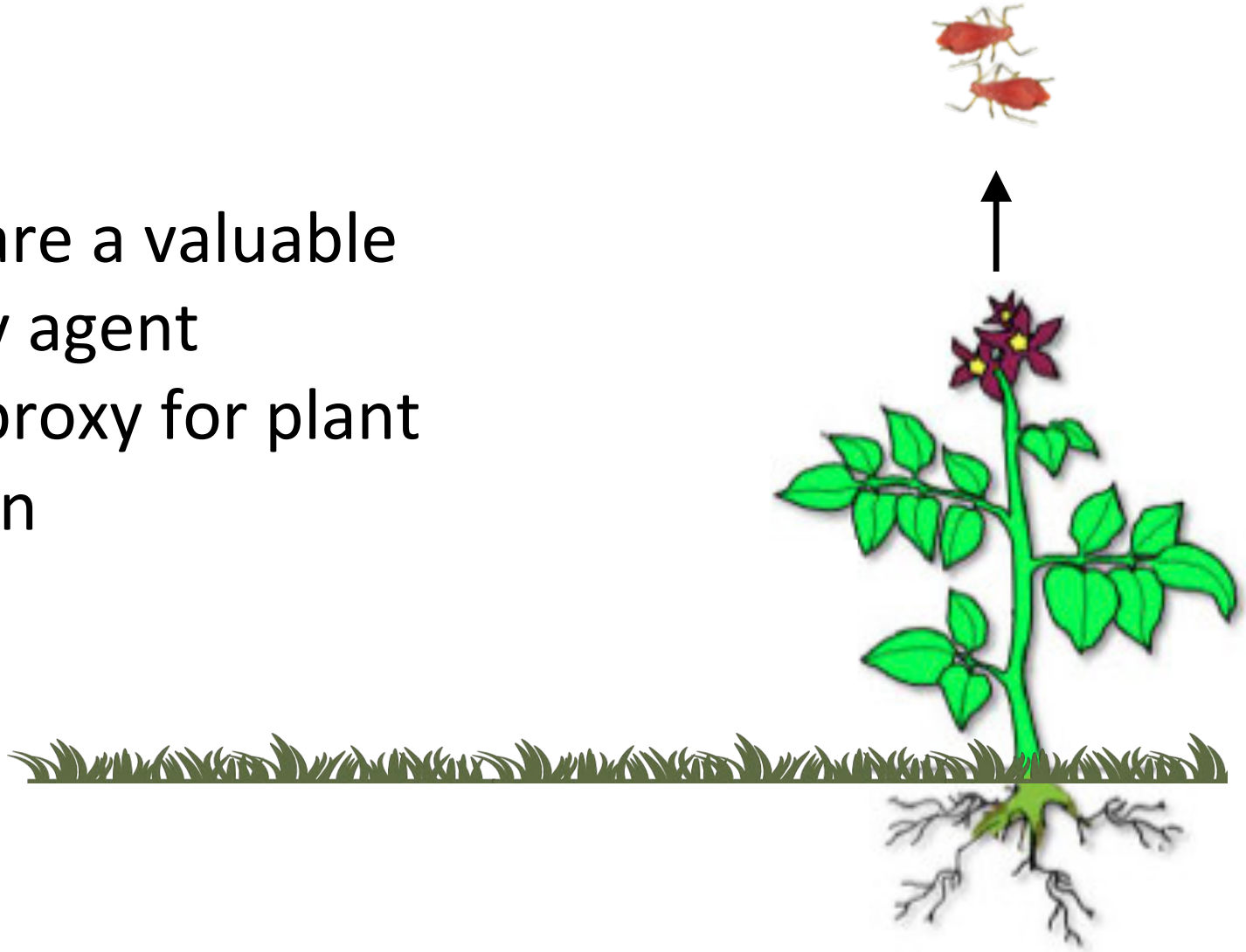
- What is the relative importance of bottom-up vs. top-down controls over plant-insect interactions?
- How do plant-insect interactions change along environmental gradients?

Experimental Approaches

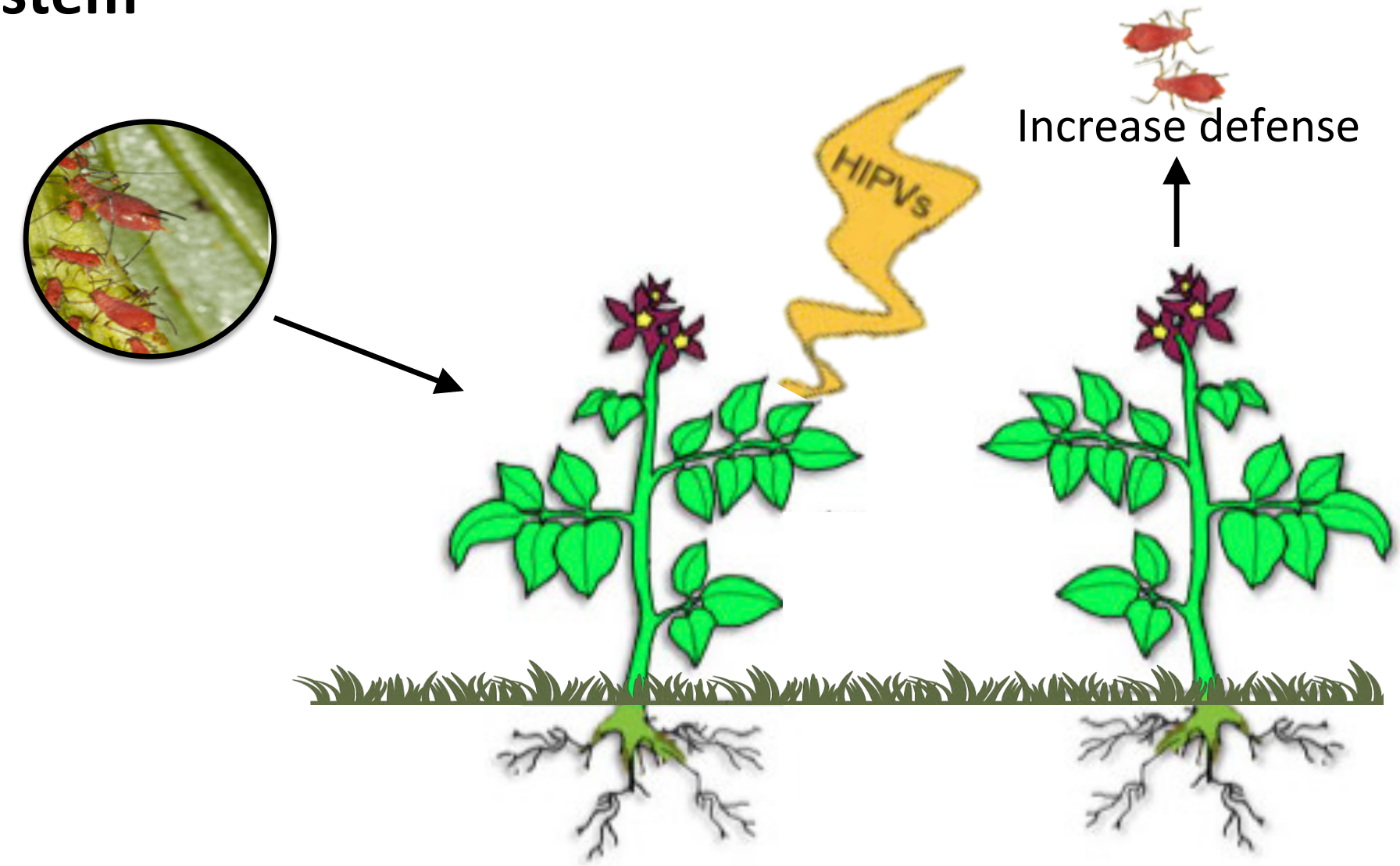
Tritrophic Interaction Studies

Research using a Plant-Aphid study system

- Aphids are a valuable bioassay agent
- Simple proxy for plant condition



Research using a Plant-Aphid study system

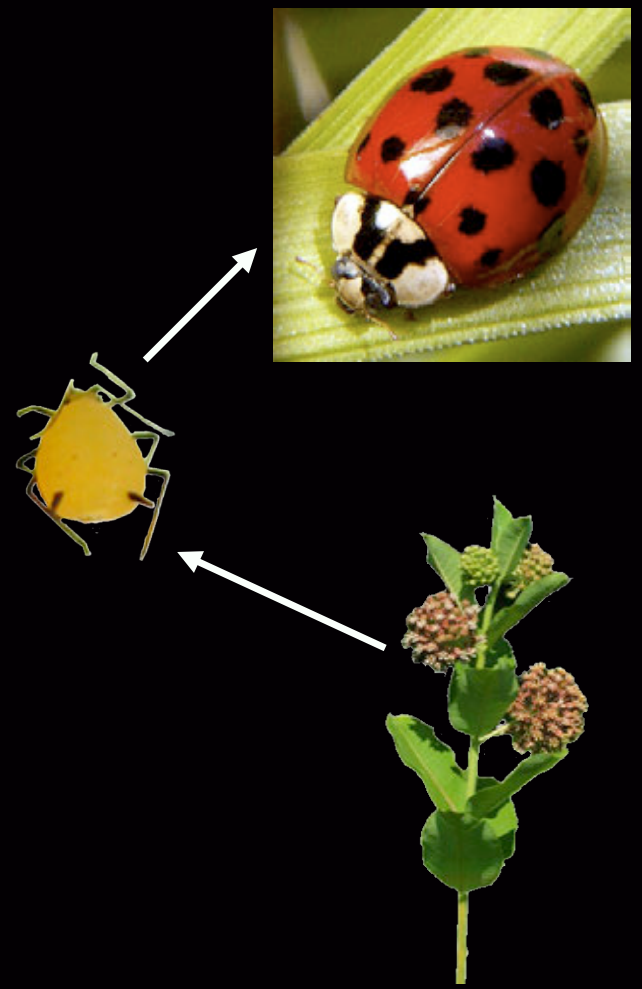


Plant-plant communication

A Tritrophic System

The Plant -- Aphid -- Ladybug System

How can this system be used in the undergraduate classroom to teach a variety of ecological and evolutionary topics?



Selecting & Obtaining a Plant-Aphid Study System

- Pea plants/aphids are recommended by many websites including:

http://insected.arizona.edu/home.htm

- Native plants/aphids should be fairly easy to come by with a simple trip to a local farm or arboretum, or chat with any gardener you know!

Aphis nerii on
milkweed

Uroleucon macolai on
Baccharis spp.

Materials Needed: Plant Propagation

- Two plastic containers or pots
- Plastic plant trays
- one large bucket or bowl for mixing soil
- all-purpose potting soil
- watering can
- Sharpie and plant tags
- Seeds
- Fluorescent, full spectrum, or 'grow light' bulbs and fixture
- 24-hour appliance timer

Materials Needed: Aphid Rearing

- Healthy plants
- Fine-tipped paintbrushes
- Mesh insect cage (optional)

Materials Needed: Predators

- Bag or box of ladybugs


Milkweed Growth Experiment
Ms. Poderoso & Mrs. Pratt
7th Grade Life Science, Periods 2 & 4
TREATMENT GROUP
Water and Fertilizer


P2 G13 A 2yr 2
P2 G14 A 2yr 2
P2 G9 A 2yr 2

Ecology, Behavior, and Adaptations of Aphids

- Follow the directions on your handout to do the following in your group:
  1. Make a Petri-dish habitat for your aphids
  2. Using a hand lens or dissecting scope, observe the aphids in their Petri-dish habitat and on their host plants
  3. While doing so complete the first side of the lab worksheet.

Aphid Initiation

What is an aphid?

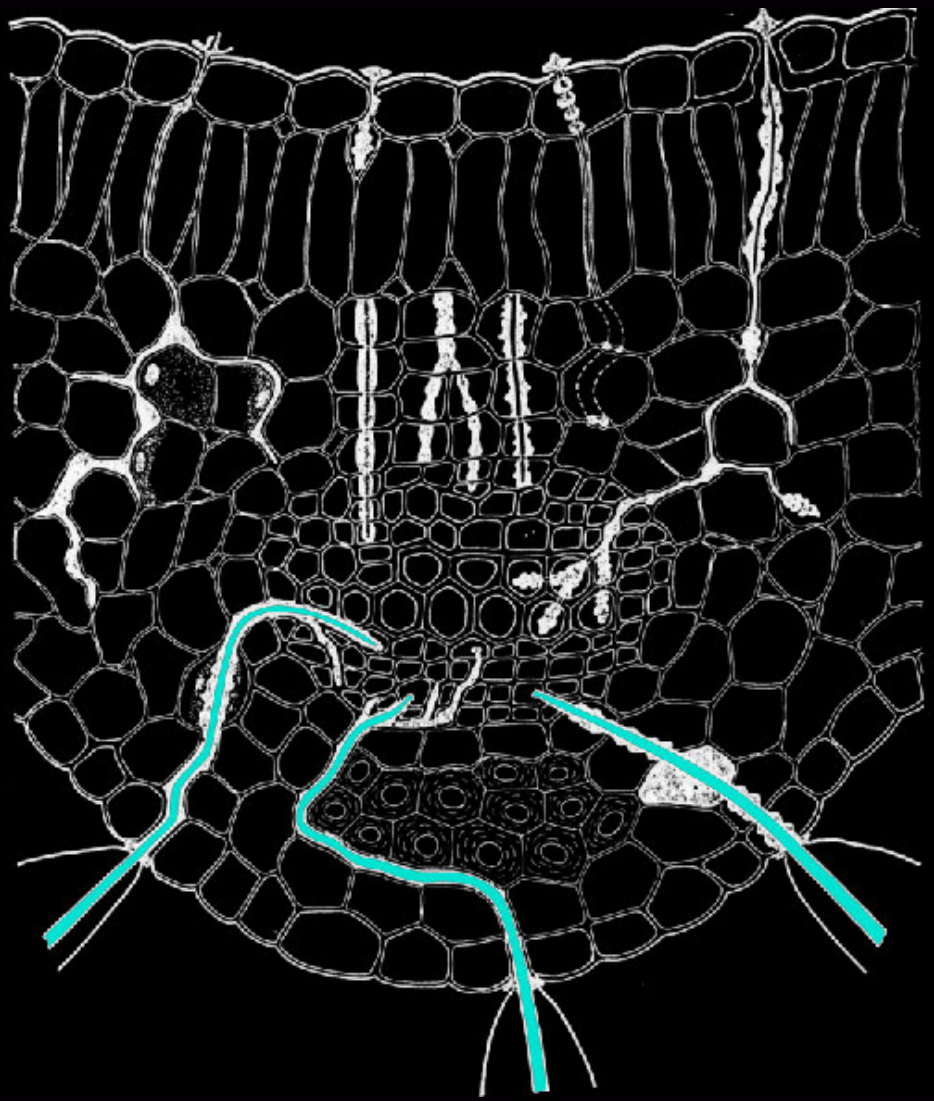
- **Herbivorous** insects
- Often considered pests
- Important food source for other insects & birds

How do aphids feed?

- Aphids have a long straw-like mouthpart called a **stylet** that they use to feed on **phloem** sap

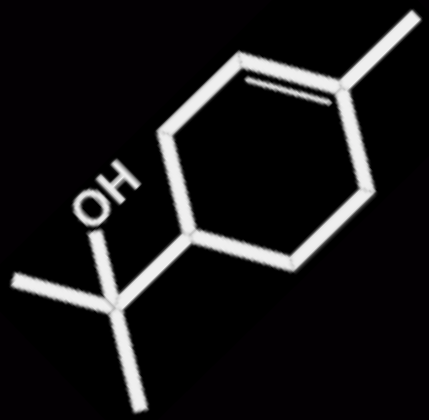
How do aphids feed?

- All aphids feed by inserting their mouth parts between plant cells to reach the phloem sap.



Aphids & their interactions….

…with plants



Plants produce chemicals to defend themselves from herbivores like aphids

Aphids & their interactions….

…with ants

Many aphids have a **mutualism** with ants

Aphids & their interactions….

…with ants

Aphids secrete **honeydew** to feed the ants.

The ants protect the aphids.

Aphids & their interactions….

…with predators

Aphids secrete poisons from their **cornicles** to defend themselves

Aphid Reproduction

- Give birth to live young via **parthenogenesis**
- Have **telescoping generations**
  - The baby aphid developing inside the mother already has its own babies developing inside it.

Complete Follow-up Questions
on back side of worksheet

Discussion Questions

- What should students know beforehand?
- What preparation is required?
- What other questions related to aphids would you be interested in investigating?

Aphid Dissection Video

15 minute break

Potential Classroom Activities

- Seed Structure & Function – Germination & Tissue Differentiation
- Student-directed Experiment –
  - Scientific Method, Experimental Design
  - Data Collection, Plant trait measurements
  - Analysis and presentation of data
- Aphid Initiation Activity – Aphid biology, plant-aphid interactions
- Population Explorations – of aphids in the classroom and in the field
- Plant-Aphid Systems Jigsaw Activity
- Aphid-predator Simulation
- Power of Predators

Experimental Design

Jessica Pratt
GK-12 Program
10/15/09

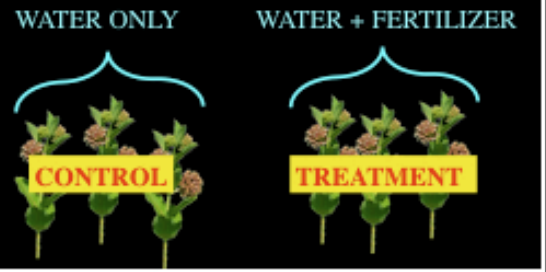
1

Scientific Method

1. Question
2. Observations
3. Hypothesis / Predictions
4. Design an Experiment
5. Data Collection
6. Analysis- Results
7. Conclusions

2

P2 Question: Does adding nutrients to milkweed increase growth?



3

Components of a GOOD Experimental Design

Has a CONTROL

Has REPLICATION

All other factors are STANDARDIZED

The experiment is REPEATABLE

Data Collection

- We are interested in **Plant Growth**

Independent variable: our TREATMENT

Dependent variable: what we MEASURE

Record the following information in your lab notebooks:

- Experimental Question
- Hypothesis / Prediction
- Experimental Design
  - List your CONTROL, TREATMENT, INDEPENDENT VARIABLE, DEPENDENT VARIABLE, STANDARDIZED FACTORS


Milkweed Growth Experiment
Ms. Poderoso & Mrs. Pratt
7th Grade Life Science, Periods 2 & 4
TREATMENT GROUP
Water and Fertilizer


P2 G13 A 2yr 2
P2 G14 A 2yr 2
P2 G9 A 2yr 2

Measuring Plant Growth

Jessica Pratt
GK-12 Resident Scientist
October 29, 2009

1

Your Lab Notebook

- This is the MOST IMPORTANT part of the long-term project.
- You will rely on information in your lab notebook when you do your final poster.
- You should already have two entries, one from the day we planted the seeds and one from the day we.
- You will not be allowed to participate in the experiments if your lab notebook is not up to date

2

Day 1 Entry- the planting day

1. Date
2. List your plant ID labels
3. State what you did-

   For example: "Two seedlings of the common milkweed and two seedlings of the CA milkweed were planted in pots".
4. List any other important observations.

3

Record the following information in your lab notebooks:

- Experimental Question
- Hypothesis / Prediction
- Experimental Design
  - List your CONTROL, TREATMENT, INDEPENDENT VARIABLE, DEPENDENT VARIABLE, STANDARDIZED FACTORS

4

Data Collection

- We are interested in **Plant Growth**

Measurements and UNITS:

1)Plant Height in centimeters (cm)
- Measure from the soil surface to the top of the plant
- Make sure the plant is upright

2)Number of leaves (#)

5

Data Table

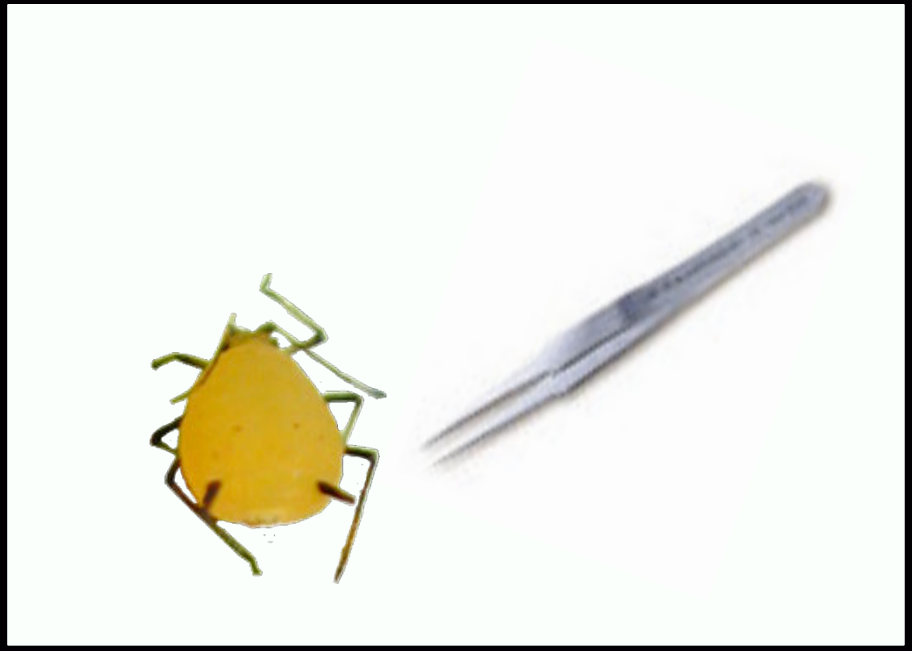
- Make one data table for each plant on a separate page.
- Title your data table with the plant ID.

Table 1. Growth data for P1G1 A.fas 2

Date	Height (cm)	Change in Height	# of Leaves	Change in # of Leaves
10/29/09	6.4	0	3	0
11/ 12/09	8.1	8.1-6.4 = 1.7	6	3

6

Stop Poking Me!
An Aphid Predator Simulation



Discussion Questions

- How could you adapt these activities for a class that you teach?
- How could you use this system in a large lecture hall / introductory bio or ecology class?
- What topics do you cover that could be taught using this system?

Thank You

NSF GK-12 Program at UCI (Grant DGE-0638751)

The Mooney Laboratory at UCI

Workshop 4

RABLE 2015

LAB 3

Neurophysiology – Electrical Activity of Neurons

The following lab manuals were adapted and written by Veronique Boucquey, Derek Huffman, Joyce Lacy of the Dept. of Neurobiology and Behavior at the University of California Irvine.

Presenters: Natalie Goldberg, Andre White, Veronique Boucquey, Julia Overman, Lauren Javier and Andrea Nicholas

Summary

In Lab 4, you will investigate the electrical properties of neurons experimentally using a classic preparation, the cockroach leg. Therefore, the exercise for Lab 3 will introduce you to cockroach anatomy, action potentials, and electrophysiology methodology. This week you will complete the cockroach leg preparation, listen for spontaneous and evoked activity, and use the recorded neural signal from one leg to modulate another leg's neural activity.

Goals

- Complete the cockroach leg preparation
- Listen to spontaneous and evoked neural activity
- Use the recorded neural signal from one cockroach leg to modulate another leg's activity

Background

Electrical Properties of Neurons

As you learned in Bio Sci N110, neurons are electrically active cells that rely on their electrical signaling capabilities to transmit information throughout the nervous system. The measure that is used to define the electrical state of a neuron is called the **potential** (short for transmembrane potential or membrane potential), which is the potential/voltage difference between the inside and the outside of the neuronal membrane. There are several different types of neuronal potentials, including resting potentials, synaptic potentials, receptor potentials and action potentials. These potentials all result from the differential distribution of ions on either side of the neuronal membrane, which sets up both concentration and electrical gradients across the membrane, and is affected by the opening and closing of particular ion channels.

Please review your notes from Bio Sci N110 and/or the chapters on the electrical properties of neurons in any introductory neurobiology textbook and make sure that you understand the fundamentals of resting potentials and action potentials. Your understanding of these concepts is critical to your understanding of what you will be doing and the interpretation of your results in the next two laboratory exercises.

TA Notes for Labs 3 & 4:

- Make sure that all of the equipment is clean before class and that there is no residual Vaseline! Vaseline can act as an insulator and mess up the recordings/experiments!
- Assign one student in each group to handle the Vaseline. Vaseline should only be placed on the exposed wound! Once this is completed, have this student change gloves!!
- Make sure to pin along the midline of the leg! In most cases, there is a light (or dark) colored line runnig down part of the midline in the coxa part of the leg (find the trochanter). Usually pinning on there, gives great CAPs!

Methods for Recording the Electrical Activity of Neurons

There are four principal methods that are used to record the electrical activity of neurons:

Extracellular recording measures the voltage change along the outside of a cell, providing a "reflection" of what is happening on the inside of the cell. This technique measures the voltage difference between two recording electrodes placed outside of the cell. This is the method you will be using in the next two labs.

Intracellular recording measures the voltage difference between the inside and outside of the cell membrane. This technique is difficult and requires inserting one recording electrode into the cell, penetrating the cell membrane without compromising neuronal health, and placing the second recording electrode outside of the cell.

Patch-clamp recording measures electrical currents (ion flow) through single ion channels in a neuronal membrane. This very delicate technique involves isolating a patch of membrane small enough to contain only one or two ion channels. This patch of membrane can be pulled away from the cell, and ion flow through the isolated channels in the membrane patch can be measured.

Optical imaging allows direct visualization of the voltage difference across the cell membrane, providing both spatial and temporal resolution of the membrane potential. This technique involves application of certain voltage-sensitive dyes, which change color or other properties depending on voltage, followed by evaluation with microscopy.

Single Action Potential

The action potential (Fig. 3-1, steps 5-7: peak/trough), which appears as a **biphasic** (two-part) **waveform** (steps 6 & 7: peak/trough), is the rapid and brief depolarization that is conducted down the length of axons in order to convey information from one place to another in the nervous system. This fundamental neural signal results from a stereotyped pattern of opening and closing of voltage-gated Na^+ and voltage-gated K^+ channels. More detailed properties of the action potential will be addressed in the next lab.

1 & 2 Inhibitory hyperpolarization

3 & 4 Sub-threshold depolarization

5 Threshold depolarization

6 Action potential
rising: V-gated Na^+ channels open;
recovery: V-gated K^+ channels open

7 After-hyperpolarization (K^+ channels still open)

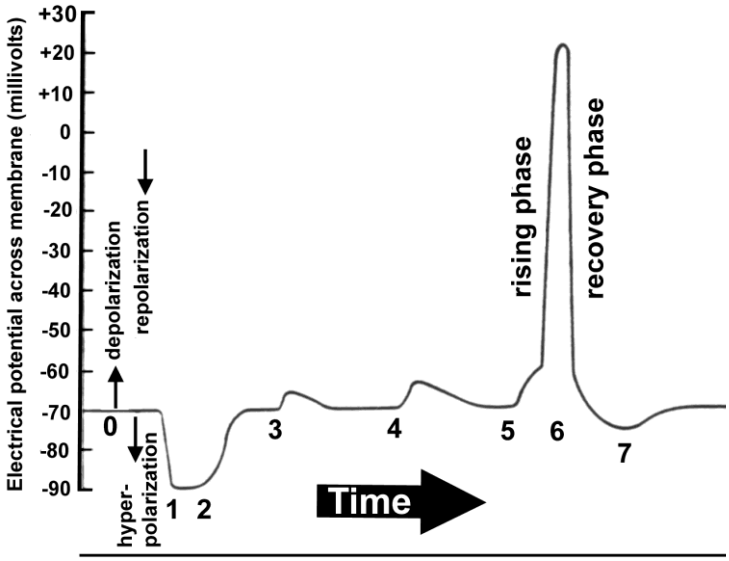


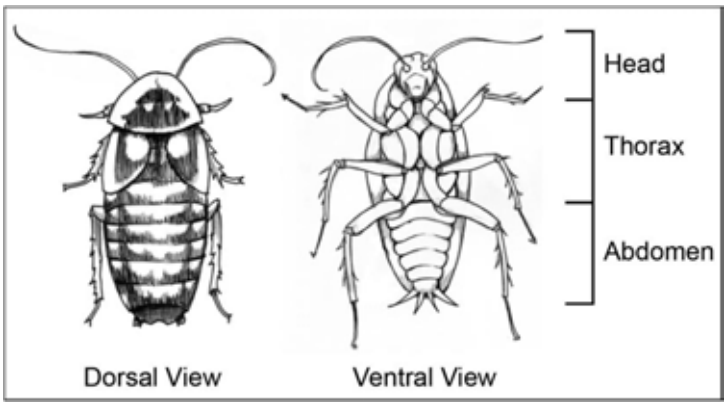
Figure 3-1. Potential Changes and the Action Potential in a Single Neuron

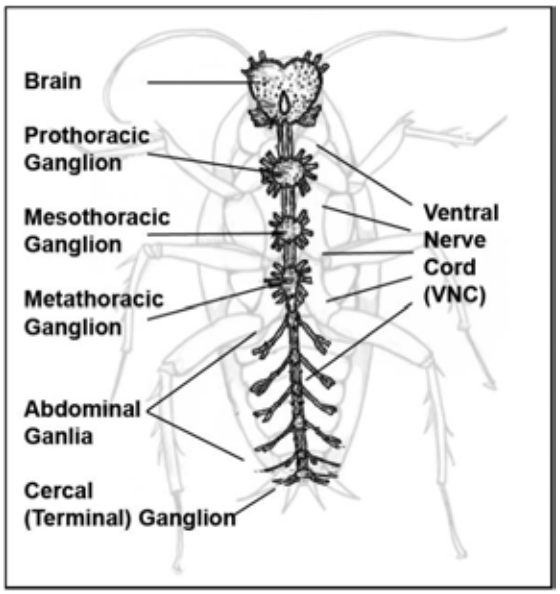
The cockroach preparation

History of Cockroach Preparations:
Cockroaches have been used for decades in neuroscience research—interestingly, many publications on the physiology of cockroach legs have come from investigations of the efficacy of pesticides (e.g., Cornwell, 1968). Cockroach legs live for many hours after being severed, providing a stable preparation to study many neuronal properties. For example, after being severed, cockroach legs exhibit **spontaneous activity**. Spontaneous activity refers to neuronal firing without external stimulation. By stimulating spines (hairs) on the leg, **evoked activity** (that is, activity evoked by external stimulation) can be observed. Evoked activity can often decrease as a result of prolonged stimulation. These changes are referred to as **sensory adaptation**. Sensory adaptation can be useful for signaling changes in the environment. For example, this morning when you put your watch on, you felt the cold of the metal and the pressure of the strap, but now you probably do not notice your watch is there.

Listening for Spikes (Action Potentials):
In the late 1950s Hubel and Wiesel performed a series of experiments in which they showed visual stimuli to cats while recording from visual cortex. The images were shown to cats on a projector using transparent slides. They amplified the output of the neuronal response through a speaker, thus allowing them to hear when a cell became highly active. Interestingly, they discovered that cells in primary visual cortex do not respond to large objects but rather to lines (e.g., Hubel & Wiesel, 1959). Their results were actually discovered by accident—they were attempting to get the cells to respond to the objects on the slides; however, they eventually heard spiking activity when the edge of their slides passed through the cell's preferred line direction and spatial location. This only happened when they were removing the slides, so if they were only recording the spiking activity with an oscilloscope (without listening to the spiking activity as well), it is possible that they would have stopped recording in between stimulus presentations. Therefore, it is possible that if they were not listening to the neurons, they may have never discovered the properties of primary visual cortex. After hearing the neuronal behavior, they quantified it by recording the responses of neurons and showing increased evoked activity to very simple visual stimuli in the visual cortex. In today's lab, you will be listening to spikes, much as Hubel and Wiesel did in their Nobel Prize winning experiments. Today you will be able to hear spontaneous and evoked activity, as well as possibly observing sensory adaptation.

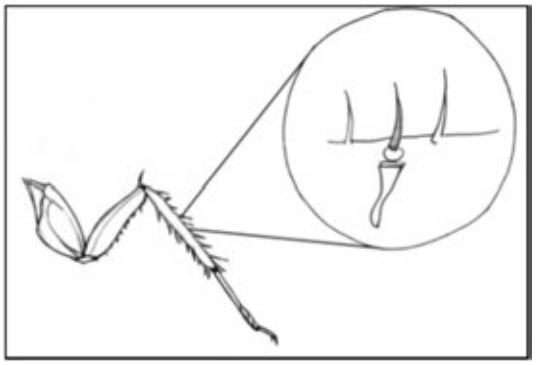
From backyard brains: Cockroach Anatomy and Senses
(http://www.backyardbrains.com, open source material)

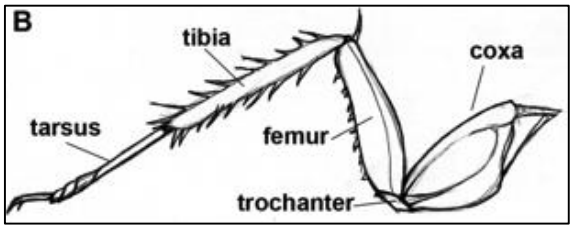




Each segment of the cockroach contains a region of the Ventral Nerve Cord (VNC), a collection of neurons that send information to the muscles of the body, while receiving information from the sensory organs of the periphery. This information is relayed to and from the brain using action potentials and synapses.

When observed up close, you can see how the cockroach leg is covered with large spines along the tibia and femur. Each spine has a neuron wrapped around it, which sends action potentials (APs) to the VNC and eventually the brain. The pattern and frequency of APs sent will allow the VNC to distinguish a strong external stimulus from a weak one. Which hair cells are being stimulated will determine where the cockroach perceives the stimulation is located.





EXPERIMENT 1: Listening for spiking activity using the Spikerbox

Equipment: Spikerbox, stopwatch, dissecting tray, 2 sets of insect pins with wire attachment, blue pad, toothpick, Vaseline, scissors, amplifier, audio cable, plastic cup, cockroach

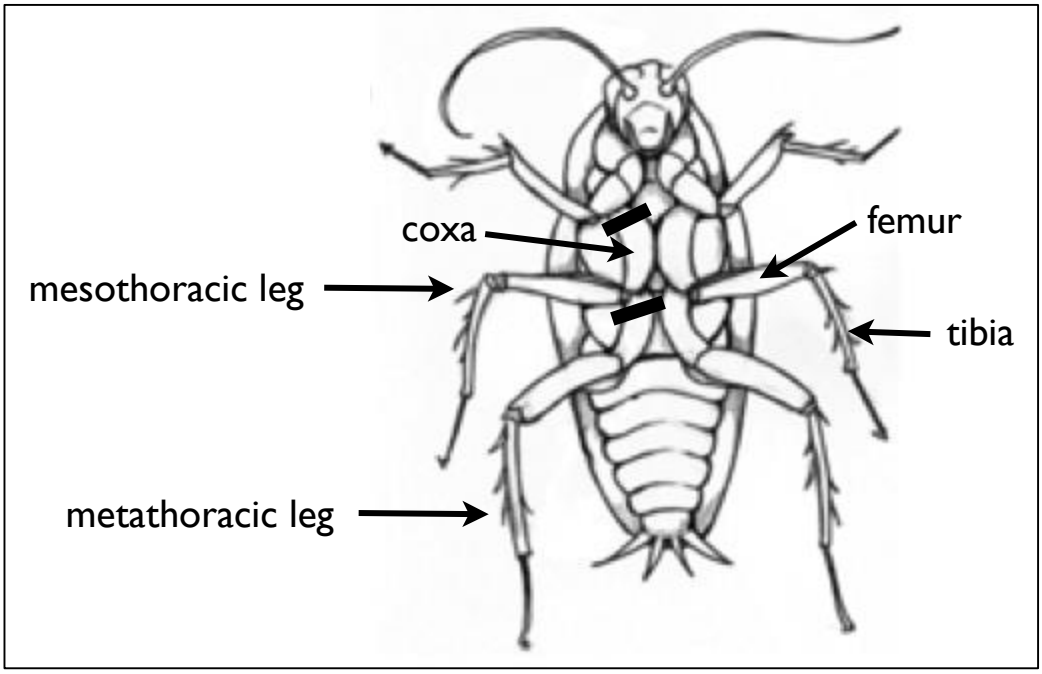
Procedure

1. Obtain cockroach from bin (pick it up by gently pinching its sides with your thumb and middle finger- you can do it!)

2. Place cockroach in one of the cups. To anesthetize the cockroach, place in freezer for about 10 minutes, or until it stops moving. Use a stopwatch.

3. Place cockroach on its back on a paper towel on the dissecting tray.

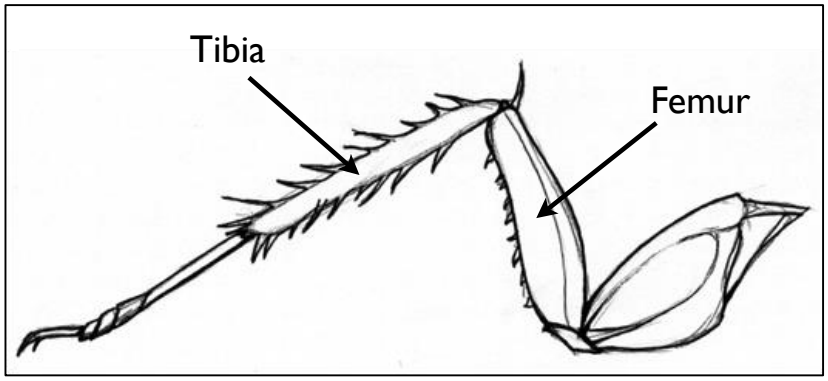
4. Remove 4 legs (see diagram): REMOVE THE MESOTHORACIC (middle) LEGS FIRST



   Gently pull the mesothoracic (middle) leg away from cockroach body. Cut the mesothoracic leg at the *highest point possible* (closest to the body, above the coxa- see dark line on diagram) so that you have all three segments of the leg intact (coxa, femur, tibia). Using the toothpick, place a pea-sized amount of Vaseline on the exposed wound of the leg. Make sure to place a good amount of Vaseline on the leg. If you do not, the leg will die. Repeat for other mesothoracic leg. Repeat for the metathoracic (hind) legs.

5. Sacrifice the cockroach: roll up cockroach in paper towel, tape, and place in freezer.

6. Pin ONE of the metathoracic legs (see arrows in diagram below): Pin the leg using the insect pins with wire attachment. Pin in the femur and tibia (one pin in each). Try to pin down the midline of the leg segments. The pins CANNOT touch each other- this will create a short in the system. The pins will go through the leg and into the blue mat. These are your *recording* electrodes.



7. Plug the wire coming from the pins into the Spikerbox (green to green plug).

8. Plug the audio cable into the left plug on the Spikerbox (black plug). Plug the other end of the audio cable into the “input” plug on the amplifier.

9. Turn on Spikerbox (flip the switch).

10. Turn on amplifier.

11. Listen for spontaneous spiking (these will sounds like popcorn pops).

12. Use clean toothpick to lightly brush the cockroach leg, listen for spikes (lots of popcorn pops).

13. Touch the bottom of the tarsus (foot) and listen. Brush different directions & listen: Do you hear different types of spiking activity? Keep toothpick on one barb & listen for changes in spiking activity (does it decrease eventually?)

14. If spiking is hard to hear, it may be that the leg is still quite cold, and therefore less spiking activity is occurring. If spiking is still hard to hear after waiting a few minutes, try moving one of the insect pins to a slightly different location. Make sure you have enough Vaseline on the leg. If all else fails, try pinning the other metathoracic leg.

15. Turn off amplifier and Spikerbox.

16. Fill your plastic cup with ice and water. Place ONE of the mesothoracic legs in the cup of ice water for 15 minutes (use stopwatch). **NOTE: move on to Experiment 2 after the leg has been in the ice water for 15 minutes!**

Thought Questions:

1. What method of recording are we using to hear spikes?
   Extracellular recording

2. Why would spiking activity attenuate when you continuously stimulate one barb?
   Sensory adaptation

3. Are the spikes you are hearing when brushing the leg coming primarily from motor neurons or sensory neurons? Why?
   Most likely sensory neurons responding to brushing of the spines. From chapter material: Each spine has a neuron wrapped around it, which sends action potentials (APs) to the VNC and eventually the brain

4. Why might stimulating the barbs in different directions produce different spiking activity?
   For the cockroach to differentiate between where the stimulation is coming from in its environment

EXPERIMENT 2: Temperature effects on spiking activity

Procedure

1. After the 1st mesothoracic leg has been in the ice water for 15 minutes, you will take it out of the ice water and pin it according to the steps in Experiment 1. Restart the stopwatch.

2. Un-pin the metathoracic leg that you were using in Experiment 1 and set aside for Experiment 3.

3. Using your second insect pin with wire attachment, pin the 2nd mesothoracic leg according to the steps in Experiment 1.

4. Plug the 2nd mesothoracic leg (non-ice water) into the Spikerbox. Listen for spikes.

5. Plug the 1st mesothoracic leg (ice water) into the Spikerbox. Listen for spikes.

6. Turn off amplifier and Spikerbox.

Thought Question:

1. Do you notice a difference between the 1st and 2nd mesothoracic legs? If so, (a) characterize the difference(s), (b)what do you think causes the difference(s)?
   (a) The second leg has less spiking activity
   (b) The cold from the ice water decreased membrane fluidity of the cells (remember Bio93), so that for some of the cells (or all cells if you hear NO spikes) a low enough temperature is reached that the membrane has become rigid and therefore the membrane-bound ion channels cannot work properly.

EXPERIMENT 3: Neuroprosthetics

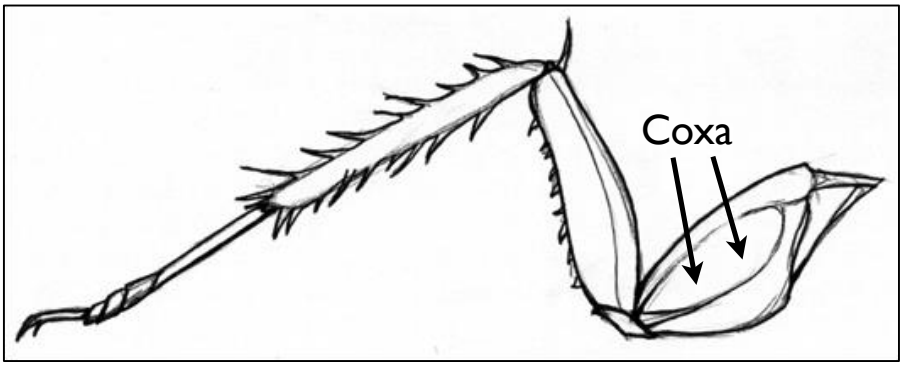
Use the recorded neural signal from one cockroach leg to modulate the other.

Additional equipment: stimulation cable (lead with black and red grabbers), insect pins, 2nd metathoracic cockroach leg

Procedure

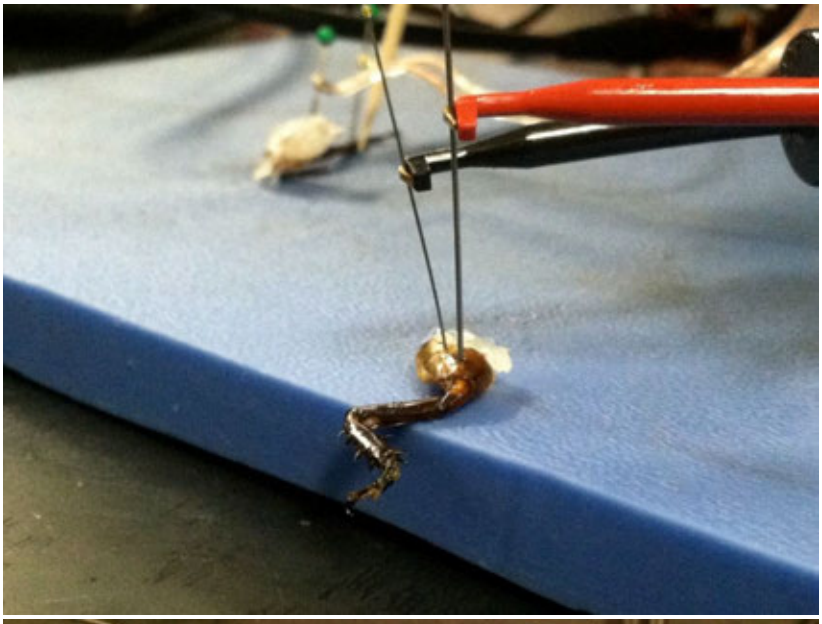
1. Re-pin the original metathoracic leg (from Experiment 1). Check that your original leg (leg #1) is still producing spiking activity by turning on the amplifier and listening for spikes. This leg will be providing the neural signal to stimulate the other leg.

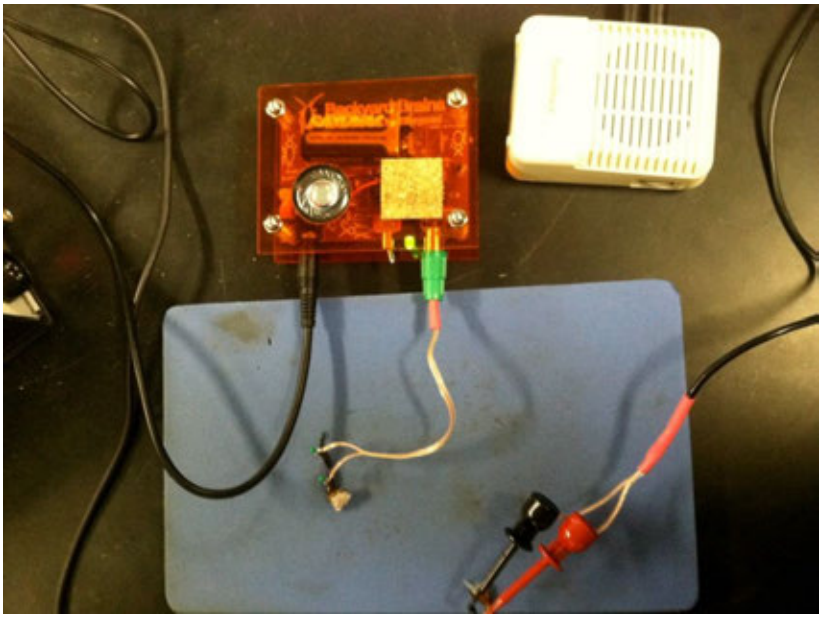
2. Now you will pin the 2nd metathoracic leg: (see diagram below) Pin the leg using the insect pins *WITHOUT* wire attachment. Insert both pins into the coxa. Try to pin down the midline of the coxa. The pins CANNOT touch each other- this will create a short in the system. Make sure you have the tibia hanging off the blue mat- this is so that it can move without restriction! This leg will be stimulated by the neural signal from leg #1. The pins will go through the leg and into the blue mat.



3. Turn off the amplifier.

4. (See picture below) Attach the black and red grabbers to the insect pins in the 2nd leg. These are your *stimulating* electrodes. Attach the other end of the simulating cable to the "output" plug on the amplifier.





5. Turn the amplifier on at lowest setting.

6. Brush leg #1. Observe leg #2 to see if there is any movement. Slowly increase amplification until you see movement of leg #2 when you brush leg #1.

7. Turn off amplifier and Spikerbox.

Thought Question:

1. *Circle the correct answers*: The signal coming from leg #1 is most likely from **SENSORY**/motor neurons, which is then used to stimulate leg #2. When you observe movement of leg #2, you are most likely observing sensory/**MOTOR** neuron spiking.

LAB 4

Neurophysiology – Electrical Properties of the Cockroach Leg Preparation

Summary

In this lab, you will elucidate several electrical properties of a cockroach that relate to the conduction of action potentials along nerves. By analyzing compound action potentials, you will characterize many important properties of the cockroach leg, including excitability, movement, electrophysiological relationship, refractory period, and multiple fiber types.

Goals

- Become familiar with methods for generating and interpreting electrophysiology data
- Characterize several electrophysiological properties of the cockroach leg
- Utilize electrophysiological data to learn more about the organization of the cockroach leg

Background

In order to understand the experiments in this lab exercise and interpret your data, you must understand the fundamentals of the action potential (i.e. single action potential). It is assumed that you are already familiar with these concepts, so not all important points will be covered here. Make sure you understand the diagram of the action potential in Figure 3-1 on p. 3.2 of the previous lab, including the mechanistic basis of each stage/electrical event indicated in the diagram (steps 1-7). In addition, make sure you understand what **threshold** means and why individual neurons have a threshold for firing an action potential; what is meant by the description **all-or-none** when referring to single action potentials; and what role different ions and ion channels play in the different parts of the action potential. You should also be comfortable with the terms **depolarization** and **hyperpolarization**.

Single Action Potential

Single action potentials are recorded by placing a single electrode into an axon with a second electrode placed outside of the axon. The voltage difference between these two electrodes is recorded; hence, resting potential reflects the voltage difference between the inside and outside of the axon. Resting potential is negative due to the Na^+/K^+ pump. The "all" portion of the all-or-none property of the single action potential refers to the fact that the height of the overshoot of the single action potential is physiologically determined and will not increase or decrease in size with varying strength of stimulation. The "none" portion simply states that the threshold serves as a binary operator for firing an action potential—that is, the cell will only fire an action potential if it reaches threshold. Figure 4-1 shows that it takes a finite amount of time for an individual cell to change from resting potential to threshold (this concept will be important later in today's lab as we change the amount of time we stimulate the cockroach leg). The upstroke of the action potential is due to increased **conductance** to Na^+ (more Na^+ channels open) and the after hyperpolarization is due to increased conductance to K^+ (more K^+ channels open). Na^+ channels

are quickly closed while K^+ channels are slow to close, resulting in a relatively short depolarization followed by a relatively long hyperpolarization (See Figure 4-2).

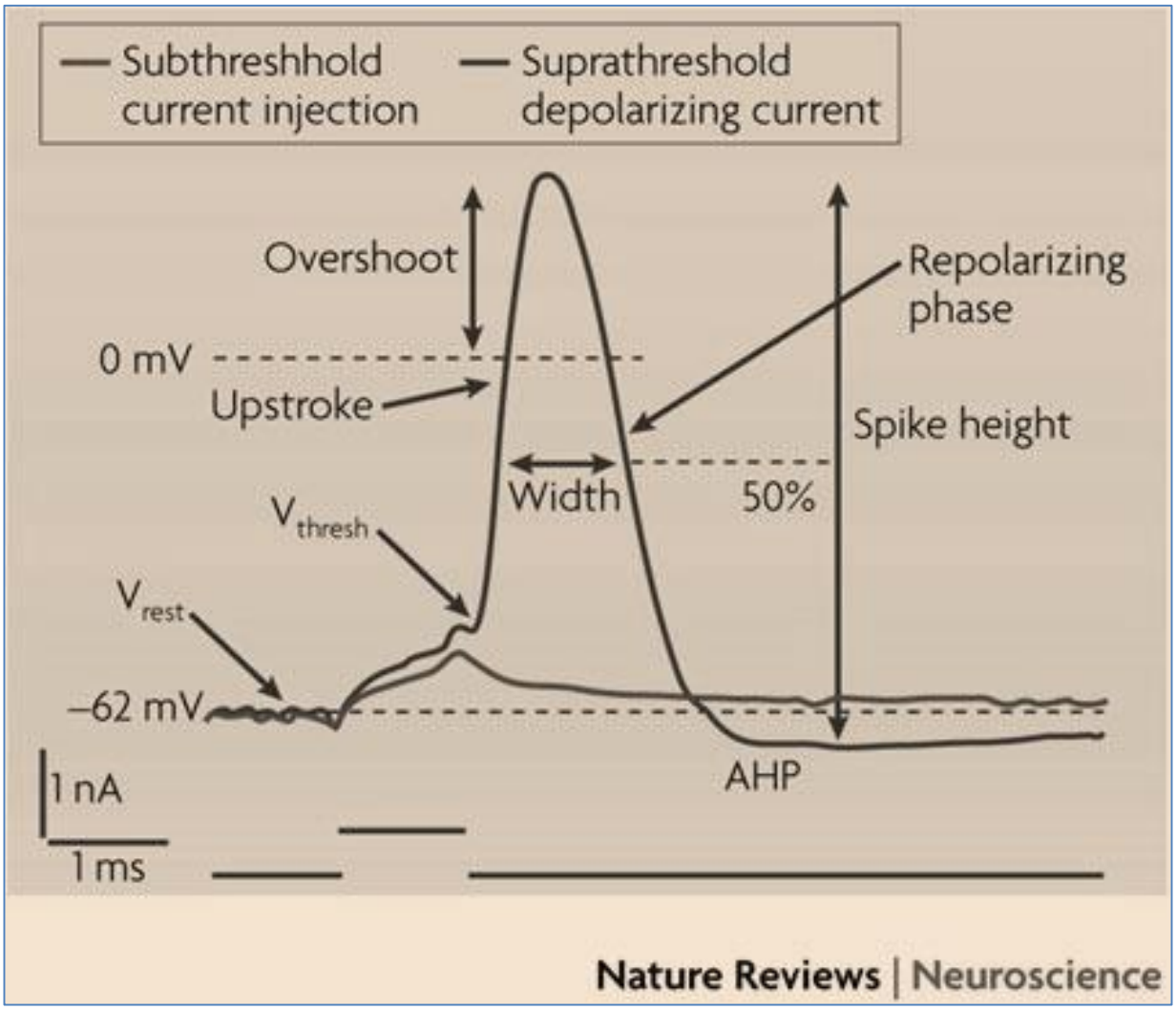


Figure 4-1. Dynamics of the Single Action Potential. It takes a finite amount of time for a single axon to change voltage from resting potential (V_{rest}) to threshold (V_{thresh}). The x-axis here depicts change in time. The line notch that is raised above the line at the bottom of this figure depicts the amount of time to change voltage from resting potential to threshold. AHP=After Hyperpolarization. Figure from Bean, B.P. (2008). The action potential in mammalian central neurons. *Nature Reviews Neuroscience(8)*: 451-465.

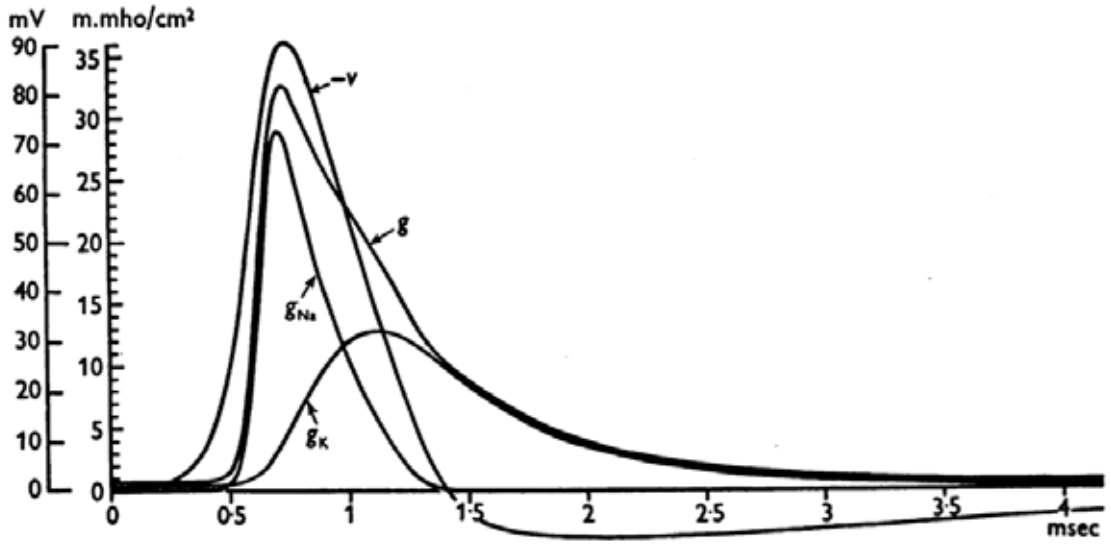


Fig. 17. Numerical solution of eqn. (31) showing components of membrane conductance (g) during propagated action potential ($-V$). Details of the analysis are as in Fig. 15.

Components of conductance change. The manner in which the conductances to Na^+ and K^+ contribute to the change in total conductance is shown in Fig. 17 for the calculated propagated action potential. The rapid rise is due almost entirely to sodium conductance, but after the peak the potassium conductance takes a progressively larger share until, by the beginning of the positive phase, the sodium conductance has become negligible. The tail of raised conductance that falls away gradually during the positive phase is due solely to potassium conductance, the small constant leak conductance being of course present throughout.

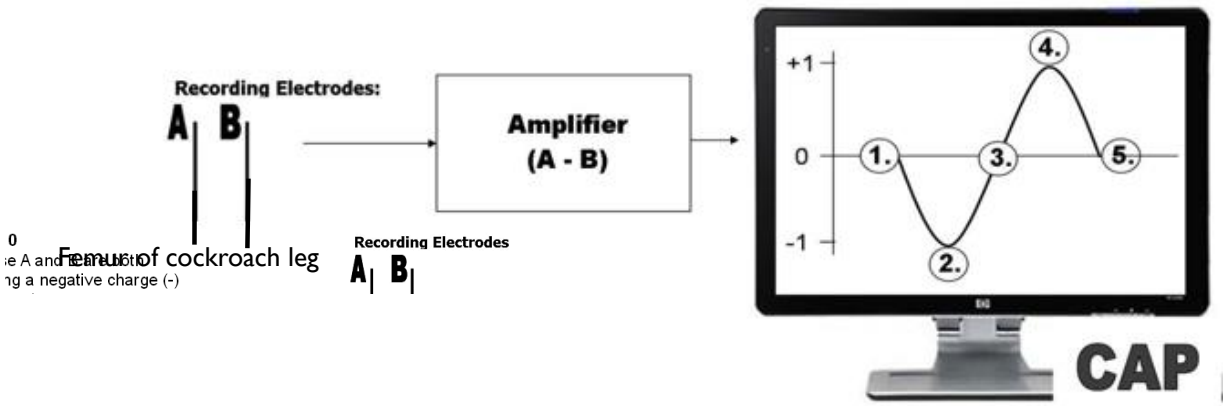
Figure 4-2. Components Underlying the Single Action Potential. Na^+ channels close relatively quickly while K^+ channels close relatively slowly, resulting in a quick upstroke of the action potential followed by a relatively long period of hyperpolarization. Figure and text from Hodgkin and Huxley (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. 117:* 500-544.

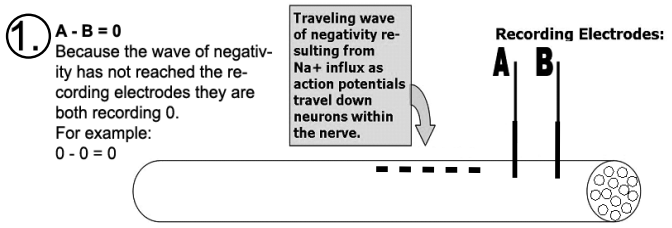
Compound Action Potential

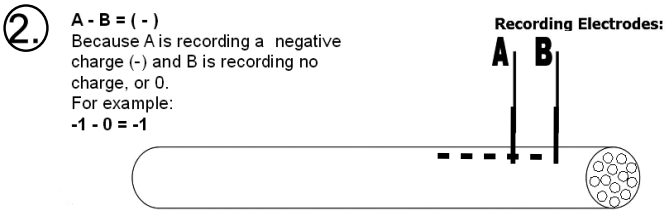
Extracellular recordings from a nerve can be used to distinguish either single action potentials in single axons or the sum of multiple single action potentials firing simultaneously in many axons that comprise a nerve. This recorded sum is called a **compound action potential (CAP)**. This week you will simultaneously stimulate many of the axons in a cockroach leg and observe the resulting compound action potential. It is important to note that compound action potentials are not all-or-none because they can increase size with increasing stimulus strength or duration of stimulus (violation of the "all" portion of the all-or-none property of the single action potential. See also the "Excitability" section below). While recording from more than one axon at once may seem like an indiscriminate technique, it is reliable and useful and offers the opportunity to explore many important aspects of action potential conduction. We will record the CAP through a differential amplifier. The amplifier is called a differential amplifier because it works by constantly comparing

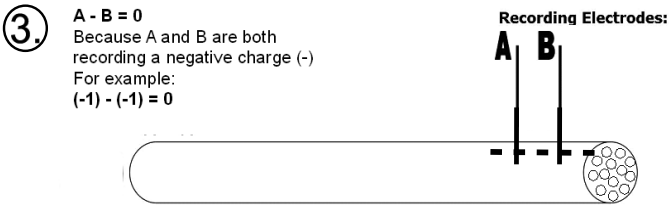
signals from two recording electrodes (A) and (B), subtracting one from the other (A-B), and then sending the result to the computer.

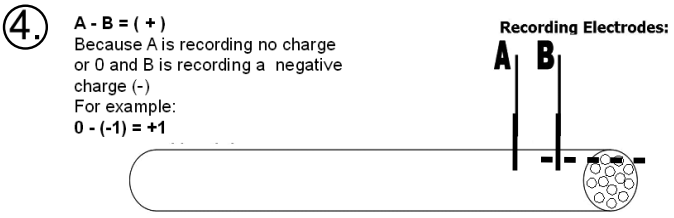
Here is an example of how a CAP is produced, step-by-step:











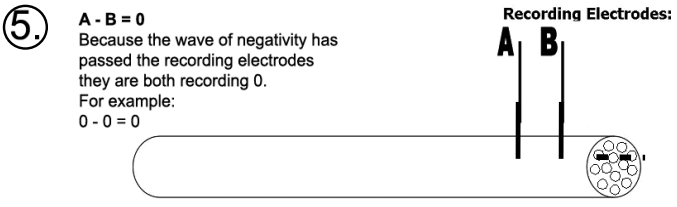
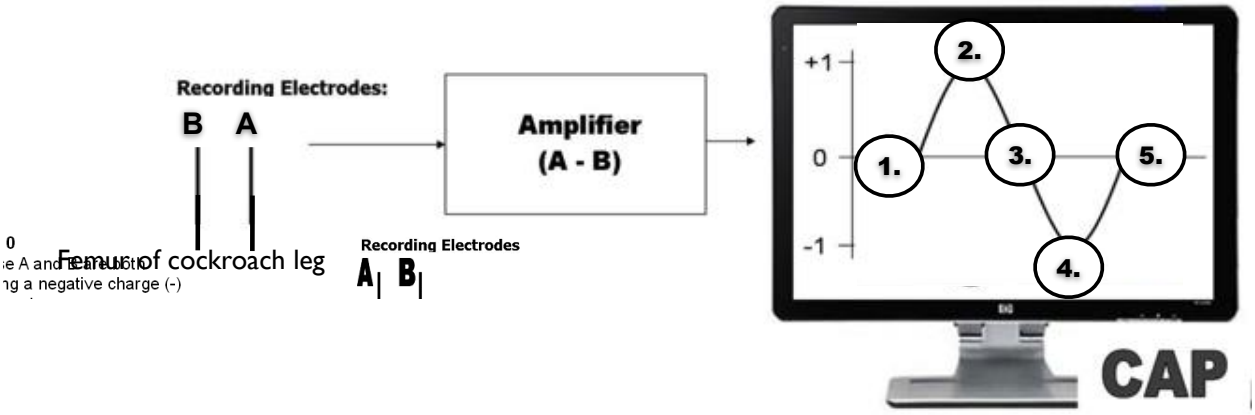
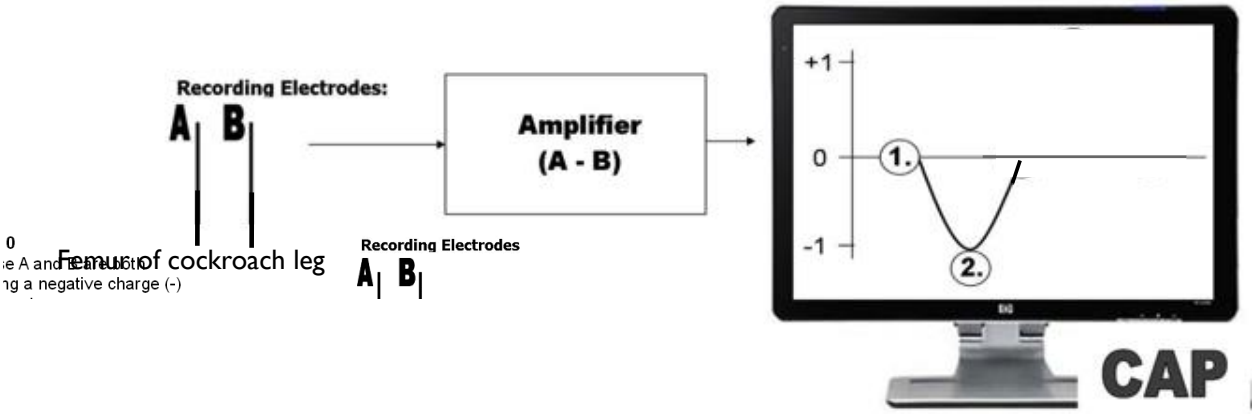


Figure 3-3. Recording a Monophasic CAP Using a Differential Amplifier

Other examples of CAPs:
Consider if the positions of your **recording electrodes A and B are reversed from the previous example**. Now the traveling wave of negativity will reach B first, rather than A first. This produces a CAP that is reversed in sign from the previous example. (Remember, the subtraction is ***always*** A-B).



Consider if recording electrodes A and B are not recording from same fibers (Note: recording electrodes A and B are in the original locations). We will observe only half of the CAP, because the traveling wave of negativity will only pass one electrode. An example of this type of CAP can be observed on page 4.14.



Stimulus Artifact

Today, you will be recording and characterizing electrical responses in a cockroach leg. To get your leg to respond electrically, you will be stimulating the leg with a pulse of electrical current from the stimulator. Before you examine the neural response, however, you need to learn to recognize the **stimulus artifact**. Some of the electrical current from the stimulating pulse is conducted passively down the leg and is picked up as a signal by the recording electrodes. This signal is the stimulus artifact; it is merely a sort of "echo" of the original stimulus and is not related to the neural response.

Refractory Period

The **refractory period** is the period of time after an action potential fires, during which action potential generation cannot be similarly repeated in the same membrane region. The refractory period has two phases: the **absolute refractory period** during which no amount of stimulation can trigger an action potential; and the **relative refractory period** during which a second action potential can be generated, but it either takes greater stimulation (single action potential) or yields a lower-amplitude action potential upon stimulation at the same level (CAP).

The absolute refractory period results from the inactivation of the voltage-gated Na^+ channels responsible for the depolarizing phase of the action potential. About one millisecond after they open, they close in such a way that no amount of stimulation can open them again until the membrane repolarizes and they reactivate. This important property makes the single action potential unidirectional. If the voltage-gated Na^+ channels can't be opened, a neuron cannot fire an action potential and is therefore refractory. Since the compound action potential reflects the summed aggregate activity of individual axons, the absolute refractory period of a CAP is defined as the period of time when all axons within the nerve are in their absolute refractory periods, and no amount of stimulation can elicit a CAP of any size.

The basis of the relative refractory period is different in a single action potential, which you are already familiar with, and the compound action potential, which you will be analyzing in this laboratory. After a *single* action potential, the voltage-gated Na^+ channels recover at slightly different times. When some, but not all, of the voltage-gated Na^+ channels have recovered and can be opened again, the neuron can fire another action potential if the stimulus intensity is increased. The relative refractory period of a *compound* action potential reflects the recovery of a subset of the axon fibers in the nerve. Since only some of the fibers have recovered and fire action potentials upon stimulation, the amplitude of the compound action potential is decreased during its relative refractory period.

Conduction

We have said that an action potential "travels" down the axon, but to understand this better, we must discuss **conduction** or how the charge travels through the neuron.

Three Types of Conduction Used by Neurons

Electrotonic conduction refers to the passive spread of membrane potentials through the cell. There is no active participation on the part of the cell to maintain the amplitude (e.g., no voltage-gated Na^+ channels opening, no action potential). Consequently these potentials decay over time and distance from their point of origin as current gradually leaks out of the cell. Electrotonic conduction is analogous to kicking a soccer ball across a grass field; if you kick it just once, the ball will travel forward, but it will slow down as it goes, ultimately petering out and stopping.

Active conduction involves the active maintenance/rejuvenation of the membrane potential as it travels through the cell. It occurs in the axons of neurons where voltage-gated Na^+ channels open as the membrane potential approaches threshold, thereby rejuvenating the membrane depolarization before it decays. The depolarization then spreads passively to the next patch of membrane, triggering the opening of its voltage-gated Na^+ channels, and so forth, all the way down the length of the axon. This process allows the action potential to travel long distances without any loss of amplitude and is the means of action potential **propagation in unmyelinated regions of axons**. Active conduction is somewhat analogous to dribbling a soccer ball down the length of a grass field; the ball continually gets one little boost right after another to maintain its forward movement over long distances.

Saltatory conduction is a combination of active and electrotonic conduction and is used in myelinated axons. **Myelin** is the insulating sheath that wraps around vertebrate axons. Along the length of a myelinated axon are periodic gaps in the sheath, called **Nodes of Ranvier**, where the majority of the voltage-gated Na^+ and K^+ channels are clustered. As the action potential travels down the axon, it advances electrotonically within the myelinated regions, and is actively rejuvenated by the activation of the voltage-gated channels at the Nodes. This process of saltatory conduction is analogous to passing a soccer ball from one person to another all the way down a grass field; one person kicks the ball, it rolls and begins to slow as it reaches the next person who then gives it another kick to get it to the next person, and so forth all the way down the field.

Properties Affecting Conduction Speed and Efficiency

Two axon properties that influence conduction speed and efficiency are **axon diameter** and **myelination**. Larger diameter axons conduct electrical signals faster than smaller diameter axons. This is because increasing the diameter lowers the **internal/axoplasmic resistance** (R_i), thereby making it easier for signals to move forward along the axon. Myelin, which ensheathes vertebrate axons, does two things. First, it increases **membrane resistance** (R_m), thereby increasing the axon's insulation and reducing leakage of the signal out of the cell as it travels. Second, it decreases **membrane capacitance** (C_m: the amount of charge captured and stored by a patch of membrane), thereby reducing the amount of time it takes to charge up myelinated regions of the membrane, and thus increasing the speed at which charge spreads through these portions of the axon. Invertebrate axons do not contain myelin. In order to have fast conduction velocities (important for survival), many invertebrate axons have evolved to have very large axons—squid and cockroach giant axons can get close to 1 mm in diameter! The large size of squid axons allowed researchers to readily study them, causing them to be extensively studied for their electrophysiological properties (e.g., Hodgkin and Huxley's Nobel Prize winning research utilized the squid preparation). Vertebrates have myelin, which increases conduction velocity while minimizing the amount of volume required for each axon, thus allowing more axons to be packed into a smaller volume. Figure 4-2 shows the comparison between regular and giant axons in the squid as well as the cockroach. Notice that even though larger axons increase conduction

velocity, these axons are relatively slow compared to the cat's myelinated axons (See also Table 4-1 for comparison of axon diameter between these species).

Conduction in a Nerve and Multiple Components

The cockroach leg contains axons of differing diameter. As discussed in the previous section (and as is evident in Figure 4-2) giant axons of the cockroach leg offer faster conduction velocity. The CAP is a summation of many axons which each fire single action potentials. Due to differing axon diameters, axons within the cockroach leg have differing conduction velocities. It is likely that you will view CAPs with **multiple components** (multiple deflections within the CAP) in today's lab, which are likely due to differing size of axons in the leg.

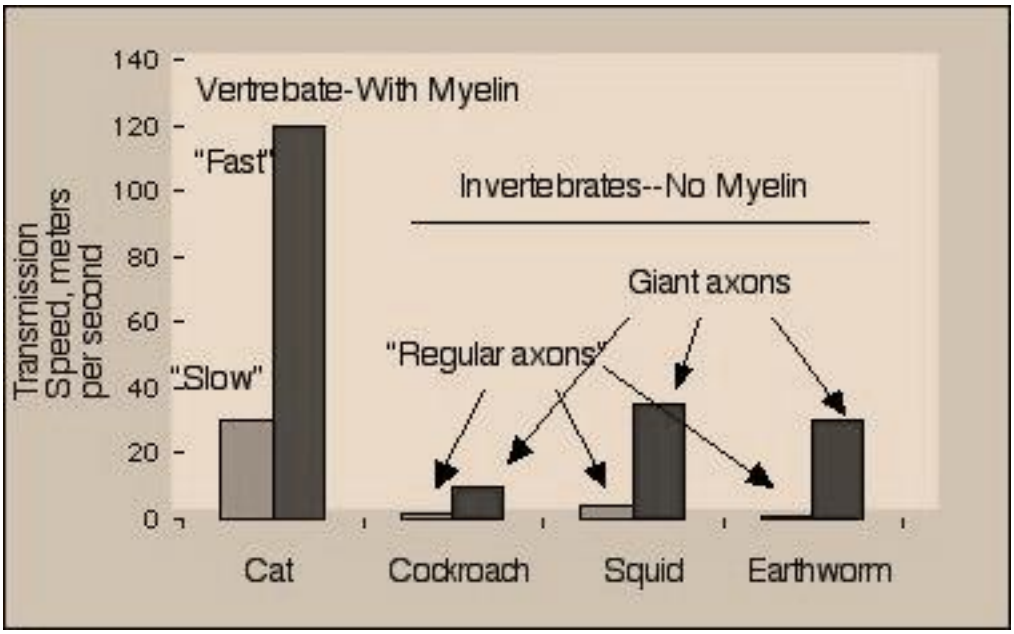


Figure 4-3. Conduction velocity differences between vertebrate and invertebrate axons. Note that although larger axons allow faster conduction velocities, these axons are relatively slow compared with myelinated axons found in mammals. Data from Bullock, T. H. and G. A. Horridge 1965. Structure and function in the nervous sytems of invertebrates. Freeman: San Francisco. Figure from http://www.animalbehavioronline.com/myelin.html

Table 4-1. Compare the conduction velocity of a small myelinated axon to a giant unmyelinated axon

Nerve Tissue	Temperature (°C)	Fiber Diameter (µm)	Velocity (m/sec)
Cat (myelinated)	38	2-20	10-100
Squid (giant, unmyelinated)	20	500	25

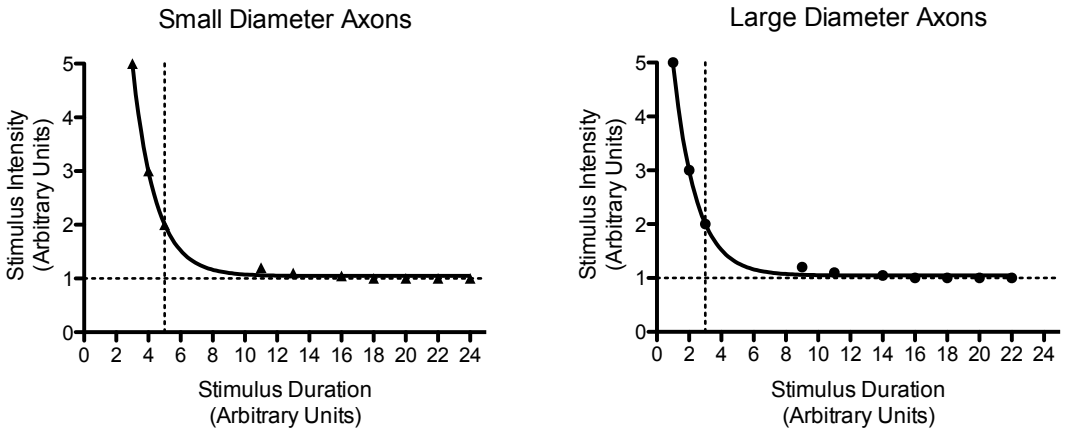
Excitability

The excitability of a nerve refers to how readily it generates an action potential, and this in turn is related to the nerve's threshold for firing. In contrast to that for a single action potential, threshold for a compound action potential is the stimulation point at which you barely get a measurable response out of the nerve. The threshold for a nerve depends on two things: the strength and the duration of the stimulus. This is analogous to boiling water; you can either put it on very high heat for a short time, or on lower heat for a long time to reach a boil. Figure 4-1 shows that the change in voltage from resting to threshold takes a finite amount of time. Increasing the duration of the stimulus results in greater probability that an axon will have enough time to reach its threshold. In this experiment, you will generate a stimulus strength-duration curve for reaching threshold in order to determine two key properties that relate to nerve excitability:

> **Rheobase voltage**: weakest stimulus that will elicit any response from the nerve (i.e., weakest stimulus that will bring the nerve to threshold).
>
> **Chronaxie time**: stimulus duration required to elicit a response when stimulating at 2x the rheobase voltage. This is a measure of nerve excitability; lower chronaxie times reflect greater nerve excitability.

The relationship between these parameters and nerve strength-duration curves is illustrated in Figure 4-4 below. In any particular nerve, fibers are not identical in diameter or internal resistance and thus the relative excitability of these fibers varies. *Fiber recruitment* is the process in which increasing the amplitude or duration of a stimulus increases the number of fibers activated. Fibers with faster conduction velocities are more excitable. These fibers require less current (either a weaker stimulus intensity at a given duration or a shorter duration at a given stimulation intensity). Thus, fiber recruitment starts with the largest diameter axons first since they have the lowest internal resistance followed by the smallest diameter axons that have the highest internal resistance. For myelinated fibers in vertebrates, the greater membrane resistance results in higher efficiency by decreasing the leak of the charge; hence, myelinated fibers are more excitable than unmyelinated fibers. Today we will only be dealing with the non-myelinated case. Lastly, for a given stimulus strength (intensity), a longer stimulation duration would be needed to bring the fibers that have a slower conduction velocity to threshold.

Figure 4-4. The Effects of Axon Diameter on Nerve Excitability. This figure depicts hypothetical fiber types. Rheobase voltage is equal to 1 (arbitrary units) in both figures (horizontal line). Chronaxie time (vertical line) is smaller for large diameter axons indicating that they are more excitable. This results from decreased R_i in the larger axons.



EXPERIMENT 1: Stimulating the cockroach leg using LabScribe

Equipment: stimulator box, dissecting tray, insect pins, blue pad, toothpick, Vaseline, scissors, 3 white leads with red, black, and green alligator clips, red lead with red alligator clip, black lead with black alligator clip, cockroach

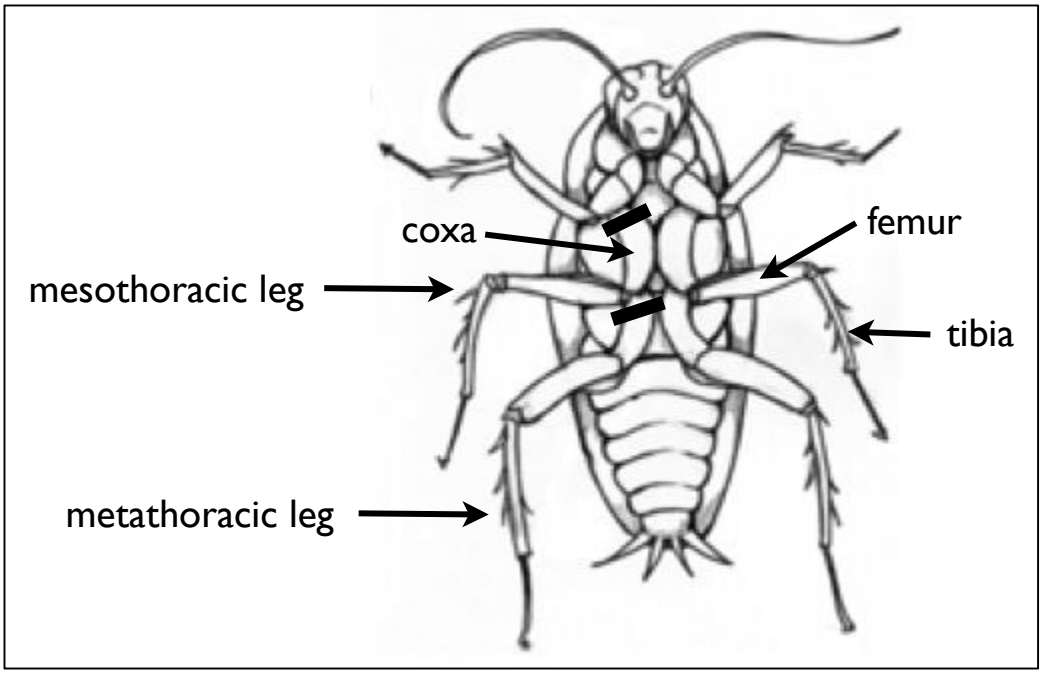
Procedure

1. Obtain cockroach from bin (pick it up by gently pinching its sides with your thumb and middle finger—you can do it!)

2. Obtain jar. Place cockroach in the jar and screw on lid. To anesthetize the cockroach, place in freezer for about 10 minutes, or until it stops moving. Use a stopwatch.

3. Carefully get cockroach out of the jar and place on its back on the paper towel on the dissecting tray.

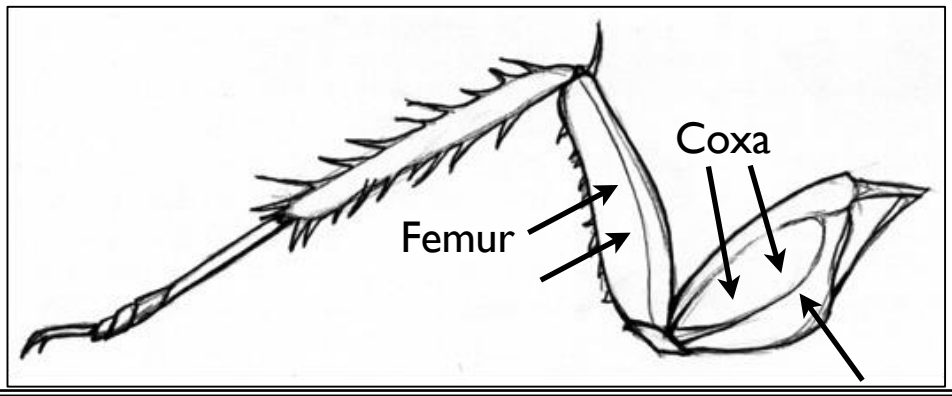
4. Remove 4 legs (see diagram): REMOVE THE MESOTHORACIC (middle) LEGS FIRST



5. Gently pull the mesothoracic (middle) leg away from cockroach body. Cut the mesothoracic leg at the *highest point possible* (closest to the body, above the coxa- see dark line on diagram) so that you have all three segments of the leg intact (coxa, femur, tibia). Using the toothpick, place a pea-sized amount of Vaseline on the exposed wound of the leg. Make sure to place a good amount of Vaseline on the leg. If you do not, the leg will die. Repeat for other mesothoracic leg. Repeat for the metathoracic legs.

6. Sacrifice the cockroach: roll up cockroach in paper towel, tape, and place in freezer.

7. Pin ONE leg using the insect pins (see arrows in diagram below): 3 pins in the coxa (2 down the midline, one more lateral), 2 pins in the femur down the midline. The pins CANNOT touch each other—this will create a short in the system. The pins will go through the leg and into the blue mat. Make sure you have the tibia hanging off the blue mat- this is so that it can move without restriction!



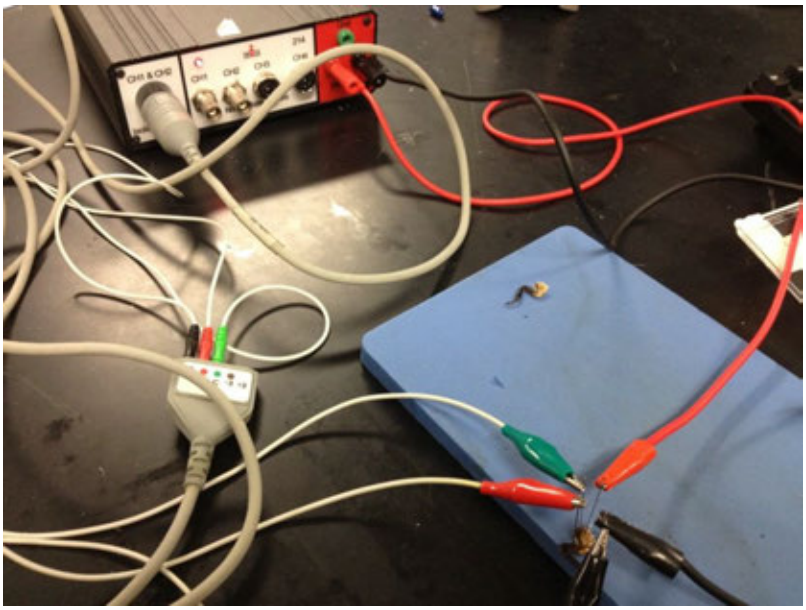
8. (See picture and diagram on following page) There are two leads (wires) coming from the stimulator box. Attach *red* lead (+) with *red* alligator clip to one of the pins down the midline of the coxa. Attach *black* lead (-) with *black* alligator clip to the other pin the down the midline of the coxa. These are your stimulating electrodes.

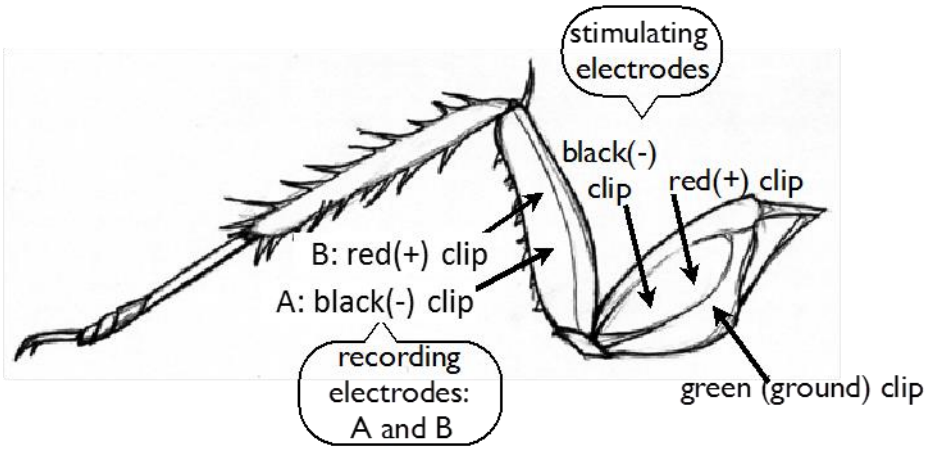
9. (See picture and diagram on following page) There are three white leads. Attach white lead with *red* (+) alligator clip to one of the pins in the femur. Attach white lead with *black* (-) alligator clip to the other pin in the femur. These are your recording electrodes. Attach white lead with *green* alligator clip to the pin on the lateral part of the coxa. This is your ground electrode.

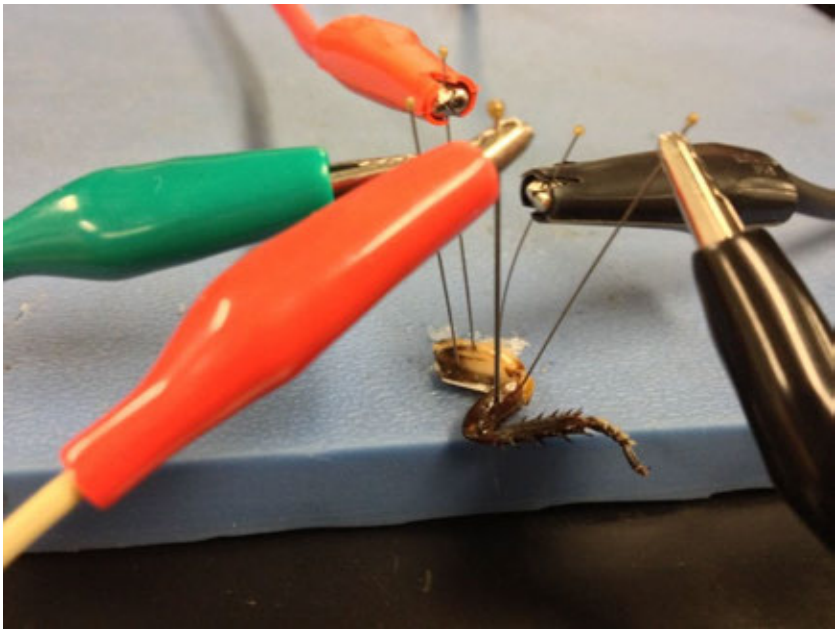
   No metal part of an alligator clip should touch another alligator clip's metal part- this will cause a short in the system.

10. Turn on stimulator box (switch on the back).

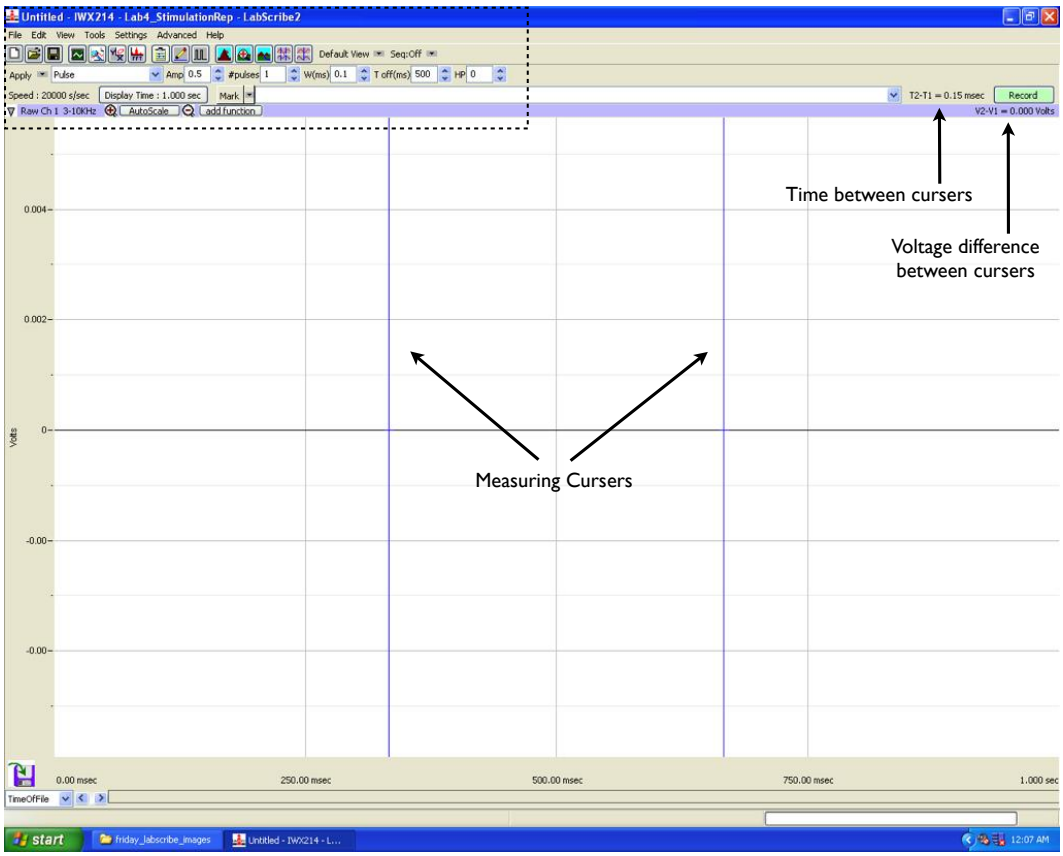
11. Open LabScribe2 from Desktop.

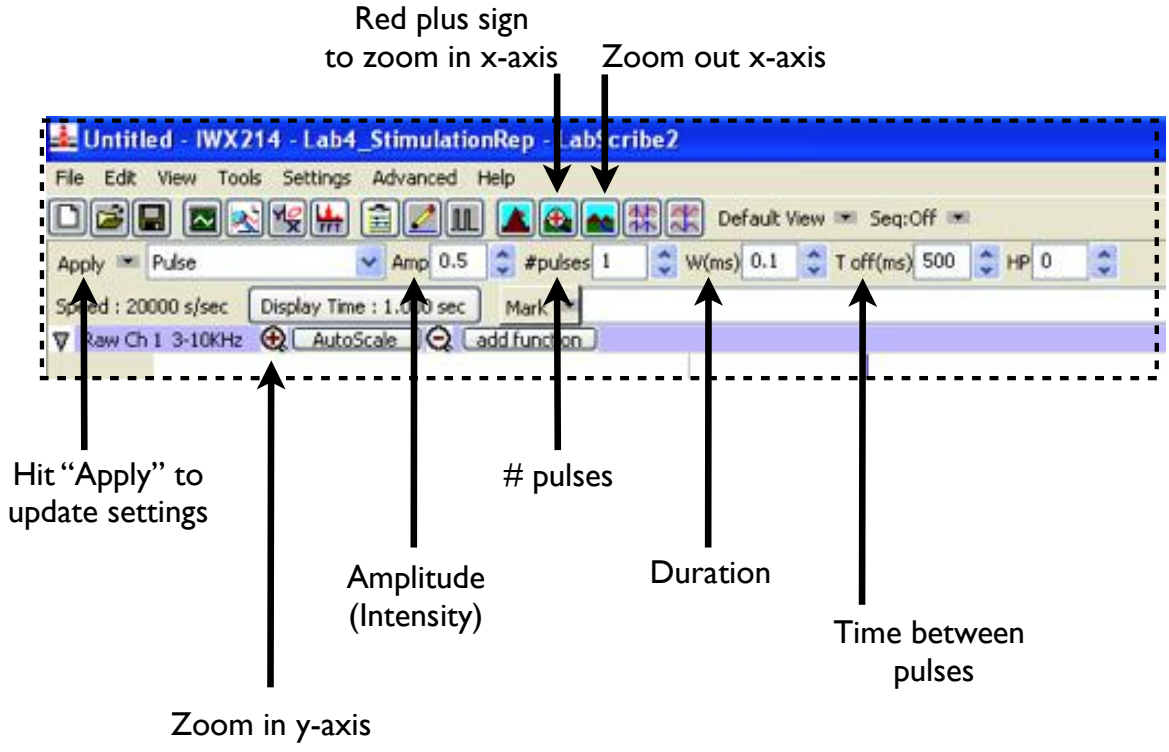






Labscribe2 screenshots





Eliciting a CAP

1. In LabScribe, under *Settings*, select Lab4_StimulationRep

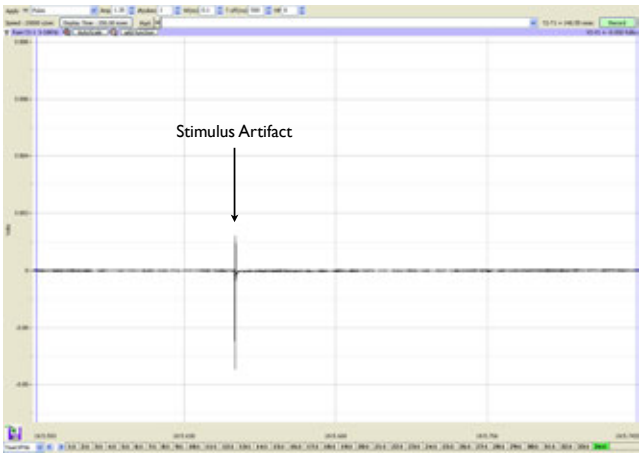
2. Under View, click (or unclick—this is a toggle checkmark) *Stimulator Panel.* This is where you can set the amplitude and duration of stimulation as well as the number of pulses. YOU MUST HIT APPLY AFTER EVERY CHANGE.

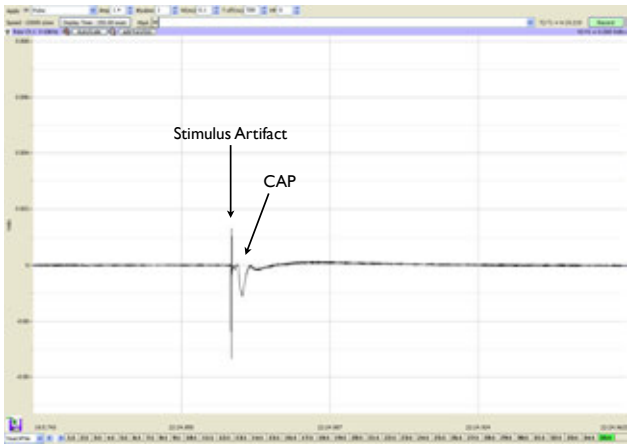
3. Initial settings: amplitude (Amp) = 0.5 # of pulses = 1 duration (W(ms)) = 0.1ms *(See screenshots of how to use LabScribe)*

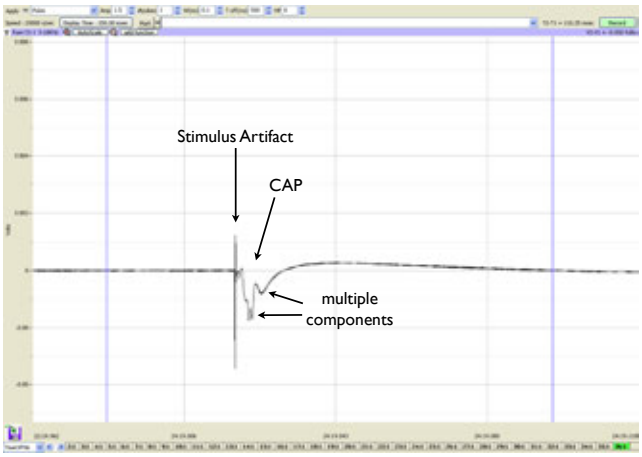
4. Click Apply then Record.

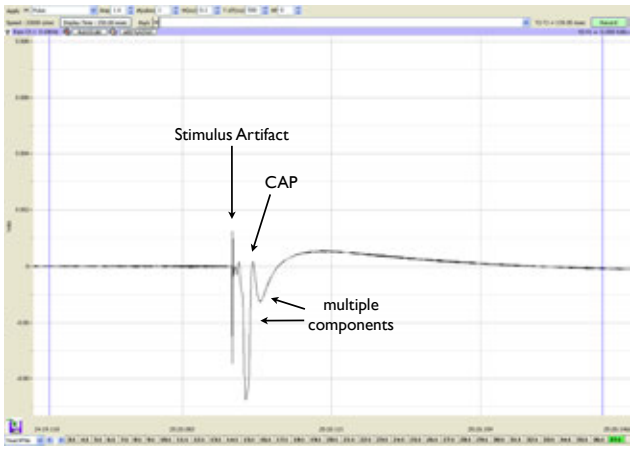
5. This setting does 10 sweeps and averages the 10 sweeps as it goes. DO NOT HIT STOP DURING A SWEEP. LABSCRIBE WILL CRASH. YOU MUST WAIT UNTIL THE PROGRAM FINISHES—IT WILL STOP ITSELF.

   Example Traces:









6. Use the blue cursers to zoom in on the x axis: move the two cursers to surround the area of interest (your stimulus artifact and CAP if you have one) and click on the red + sign to zoom in on the x-axis. To zoom in on the y-axis, double click on the y-axis or click the + sign next to 'AutoScale'. *(See Labscribe2 screenshots)* To zoom out on the x-axis click the mountain symbol next to the red + sign. To zoom out on the y-axis, click the – sign next to 'add function.'

7. WAIT 20 SECONDS before stimulating the leg again. Use a stopwatch.

8. If you have not seen a CAP, increase the amplitude to 1.0.

9. If you have not seen a CAP, increase the amplitude to 2.0.

10. If you have not seen a CAP, increase the amplitude to 3.0.

11. If you have not seen a CAP, increase the amplitude to 4.0.

12. If you do not see any CAP, try repining the stimulating electrodes and complete steps 1-9. It is possible neither electrode was near a nerve fiber. If this fails, pin another cockroach leg. ***ONCE YOU SEE A CAP DO NOT TOUCH/MOVE ELECTRODES***

Thought Questions:

1. Are the components that make up the first part of the CAP likely resulting from faster or slower fibers? Do you think they would have smaller or larger diameters?

Faster. Larger.

2. The CAP shows multiple downward and upward deflections, but sometimes the last deflection is much longer and has reduced amplitude compared to the others (see example traces on page 4.14. Observe the low amplitude curve at the tail end as it slowly returns to 0mV). What mechanism could be causing this? (hint: it has to do with channels)

K+ channels are slow to close to long hyperpolarization- see figure 4.2

EXPERIMENT 2: Amplitude-Duration Curve

Now that you have observed a CAP, DO NOT TOUCH OR MOVE ELECTRODES. You will now explore nerve excitability.

Procedure

1. Initial settings: amplitude (Amp) = 0.5 # of pulses = 1 duration (W(ms)) = 0.1ms

2. Click Apply then Record

3. This setting does 10 sweeps and averages the 10 sweeps as it goes. DO NOT HIT STOP DURING A SWEEP. LABSCRIBE WILL CRASH. YOU MUST WAIT UNTIL THE PROGRAM FINISHES- IT WILL STOP ITSELF.

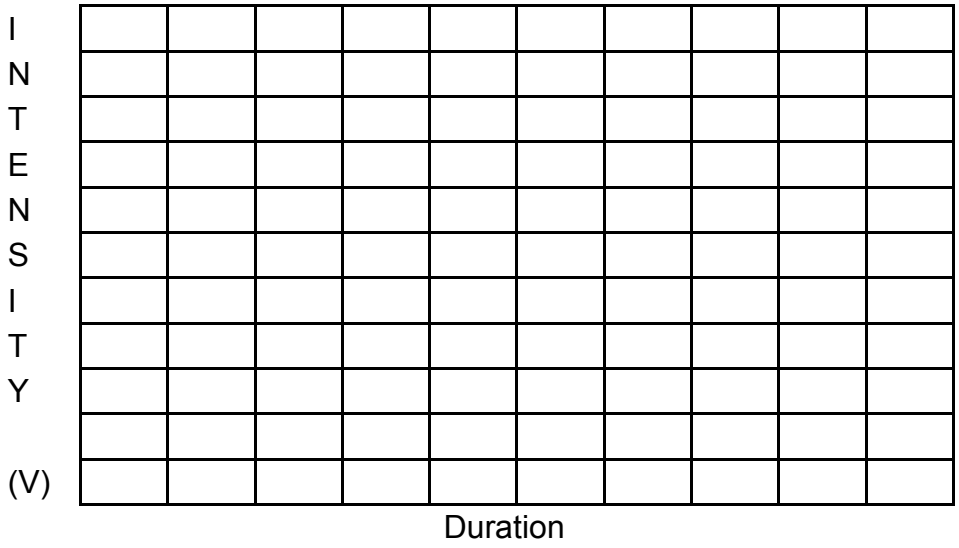
4. Gradually increase stimulus *intensity* (Amp) (HIT APPLY AFTER EACH CHANGE AND WAIT 20 SECONDS) until a CAP is observed. Record this stimulus intensity in the table below (this is the minimum stimulus intensity that is able to produce a CAP at a duration of 0.1ms).

5. Increase the duration by 0.1ms. Now decrease the stimulus intensity until you no longer see a CAP. Record, in the Table below, the lowest stimulus intensity at which you observed a CAP.

6. Repeat step (5) until you observe two subsequent durations (ex. Durations of 0.8ms and 0.9ms) that require the same intensity (ex. Amp = 0.54) to elicit a CAP.

Intensity (V)										
Duration (ms)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0

7. Plot the intensity and duration on the graph below.



8. Set the stimulus intensity (Amp) to the minimum stimulus intensity that is able to produce a CAP at a duration of 0.1ms (from chart above). Set the stimulus duration to 0.1ms.

9. Click Apply then Record.

10. In your lab notebook record (describe or draw) the stimulus artifact and CAP.

11. Change the stimulus duration to 1.0ms. DO NOT change the stimulus intensity.

12. Click Apply then Record.

13. In your lab notebook record (describe or draw) the stimulus artifact and CAP.

	Stimulus Artifact		**C. A.P.**	
Duration	**0.1ms:**	**1.0ms:**	**0.1ms:**	**1.0ms:**

Thought Questions:

1. *In reference to step #13*: Why does the stimulus artifact **NOT** increase in size vertically (i.e. why does it maintain the same intensity)?

Because the stimulus intensity is the same (only changing duration).

2. *In reference to step #13*: Why does the CAP increase in size vertically (i.e. why does the CAP increase in intensity)?

CAP amplitude is dependent on the number of axons firing. Increasing duration causes more axons to fire, increasing CAP size

3. What is the chronaxie time for your nerve fiber?

4. What is the rheobase voltage for your nerve fiber?

5. Why does increasing the duration of simulation allow for decreased intensity of stimulation to obtain the same CAP? (hint: see this chapter's material) Specifically discuss the ideas of nerve excitability and fiber recruitment.

From chapter: Fiber recruitment is the process in which increasing the amplitude or duration of a stimulus increases the number of fibers activated. Fibers with faster conduction velocities are more excitable. These fibers require less current (either a weaker stimulus intensity at a given duration or a shorter duration at a given stimulation intensity). For a given stimulus strength (intensity), a longer stimulation duration would be needed to bring the fibers that have a slower conduction velocity to threshold.

6. You may notice your chronaxie time and rheobase voltage differ from other groups. Without dissecting the cockroach leg, we cannot know the distance between the stimulating electrodes and the nerve fiber. If your stimulating electrodes were far from the nerve, would your rheobase voltage be high or low? Why?

High because need greater intensity to bring nerve to threshold because voltage has to travel further to reach nerve (loses voltage along the way)

EXPERIMENT 3: Correlate CAP size with degree of movement

For this experiment, one lab member will control the computer. The other three lab members must watch the cockroach leg- this will allow for greater reliability of measurement.

Procedure

1. Initial settings: amplitude (Amp) = 0.5 # of pulses = 1 duration (W(ms)) = 0.1ms

2. Three lab members watch the cockroach leg closely. Click Apply then Record.

3. This setting does 10 sweeps and averages the 10 sweeps as it goes. DO NOT HIT STOP DURING A SWEEP. LABSCRIBE WILL CRASH. YOU MUST WAIT UNTIL THE PROGRAM FINISHES- IT WILL STOP ITSELF.

4. Record the CAP intensity and degree of leg movement in the table below.
   To make measurements:
   Use the blue cursers to zoom in on the x axis: move the two cursers to surround the area of interest (your stimulus artifact and CAP) and click on the red + sign to zoom in on the x-axis. To zoom in on the y-axis, double click on the y-axis or click the + sign next to 'AutoScale'.
   Now place one blue vertical cursor just before the stimulus artifact. Place the other blue curser such that it intersects the highest (or lowest- whichever has greater magnitude) point of the CAP. In the upper right corner will be the difference along the y-axis (V2-V1) as well as the difference along the x-axis (T2-T1 or time) *(See Labscribe2 screenshots)*. Remember the trace you are observing is represented as the change in voltage (y-axis) over time (x-axis).

5. If you do not see a CAP at Amp=0.5, gradually increase stimulus *intensity* (Amp) (HIT APPLY AFTER EACH CHANGE AND WAIT 20 SECONDS) until a CAP is observed.

6. Once you observe a CAP, record the stimulus intensity (Amp), CAP intensity, and degree of leg movement in the table below.

7. Increase stimulus intensity in small increments until you see the tarsus move. (HIT APPLY AFTER EACH CHANGE AND WAIT 20 SECONDS). Record the stimulus intensity (Amp), CAP intensity, and degree of leg movement in the table below. (If you immediately see *both* the tarsus and tibia moving, try smaller increments of stimulus intensity to see *just* the tarsus move).

1. Increase stimulus intensity in small increments until you see the tibia move. (HIT APPLY AFTER EACH CHANGE AND WAIT 20 SECONDS). Record the stimulus intensity (Amp), CAP intensity, and degree of leg movement in the table below.

2. Continue until you observe three different stimulus intensities that induce movement of both the tarsus and tibia.

Stimulus intensity	CAP intensity	Leg Movement
0.5		

0=none
1=tarsus
2=tarsus + tibia

Thought Questions:

1. Why do we see a maximum CAP intensity? (i.e. At a certain point why does increasing stimulus intensity not result in larger CAPs?)

All the axons are firing so cannot recruit more for a larger CAP

2. Did you observe a relationship between CAP intensity and leg movement? Explain.

Monotonic relationship—as leg movement increases, CAP intensity increases. Eventually both hit maxima, perhaps with leg movement reaching its maximum first.

Sometimes groups will observe no leg movement- either the pinning could be keeping the muscle from moving, or we could be stimulating inhibitory fibers.

EXPERIMENT 4: Refractory Period

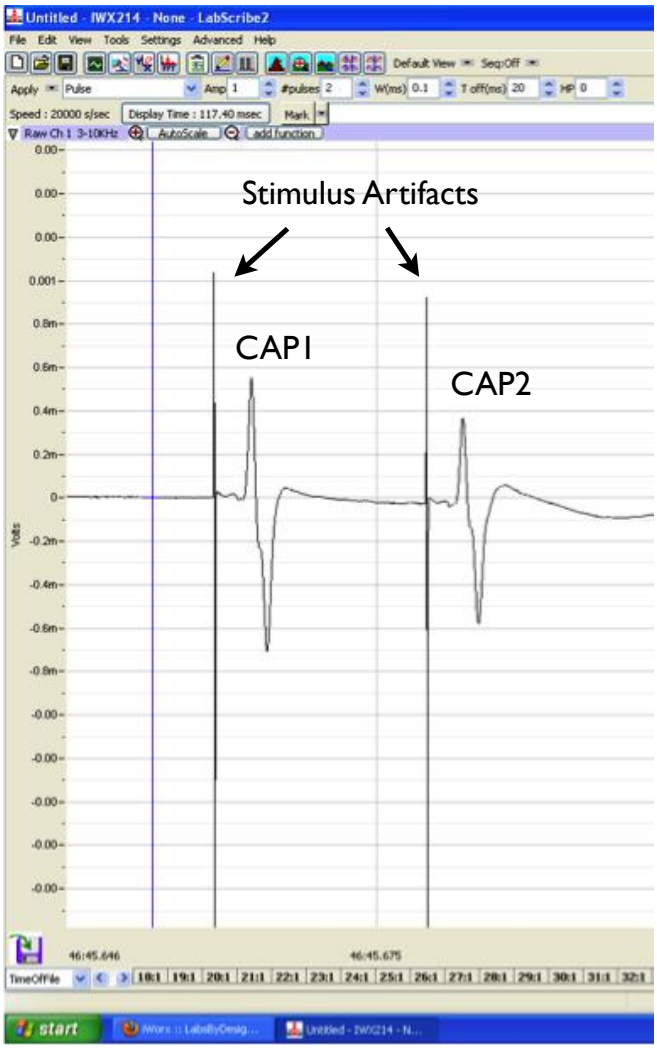
Procedure

1. Initial settings: amplitude (Amp) = an intensity that evoked a moderately sized CAP
   # of pulses = 2
   Toff (time between pulses) = 500ms
   duration (W(ms)) = 0.1ms

2. Click Apply then Record

3. This setting does 10 sweeps and averages the 10 sweeps as it goes. DO NOT HIT STOP DURING A SWEEP. LABSCRIBE WILL CRASH. YOU MUST WAIT UNTIL THE PROGRAM FINISHES- IT WILL STOP ITSELF.

   Example Trace:



4. Record intensity of first CAP (CAP1) and second CAP (CAP2) in table below:
   Use the blue cursers to zoom in on the x-axis: move the two cursers to surround the area of interest (your stimulus artifact and CAP) and click on the red + sign to zoom in on the x-axis. To zoom in on the y-axis, double click on the y-axis or click the + sign next to 'AutoScale'.
   Now place one blue vertical cursor just before the stimulus artifact of CAP1. Place the other blue curser such that it intersects the highest (or lowest- whichever has greater magnitude) point of CAP1. In the upper right corner will be the difference along the y-axis (V2-V1) as well as the difference along the x-axis (T2-T1 or time). Do the same for CAP2: Place one curser just before the 2nd stimulus artifact and the other curser at the highest (or lowest- whichever has greater magnitude) point of CAP2.

5. Change Toff to 300ms. Click Apply then Record. Record CAP1 and CAP2 intensities below.

6. Continue changing Toff according to the table below and record CAP intensities (HIT APPLY AFTER EACH CHANGE AND WAIT 20 SECONDS).

Toff	CAP 1 intensity	CAP 2 intensity
500ms		
300ms		
200ms		
100ms		
50ms		
20ms		
10ms		
5ms		
3ms		

Thought Questions:

1. Why does CAP2 become smaller than CAP1 as you decrease the time between stimulations?

Reached the absolute refractory period for some axons, therefore reached the relative refractory period for the CAP (many axons, some of which have not reached the absolute refractory period)

EXPERIMENT 5: Visualize the stimulus artifact and the CAP

1. Initial settings: amplitude (Amp) = an intensity that evoked a moderately sized CAP # of pulses = 1 duration (W(ms)) = 0.1ms

2. Click Apply then Record

3. This setting does 10 sweeps and averages the 10 sweeps as it goes. DO NOT HIT STOP DURING A SWEEP. LABSCRIBE WILL CRASH. YOU MUST WAIT UNTIL THE PROGRAM FINISHES- IT WILL STOP ITSELF.

4. Record what the stimulus artifact and CAP look like in the "Before" area below:

5. Change the polarity of the *stimulating* electrodes: In the "Amp" box, change the Amp to - (negative) (this effectively changes the stimulating electrode + to – and stimulating electrode – to +). In your lab notebook record (describe or draw) the effects of changing Polarity on the stimulus artifact and CAP in the "After" area below:

	Stimulus Artifact		**C. A.P.**	
Polarity	**Before:**	**After:**	**Before:**	**After:**

Thought Questions:

1. What is the difference between the way the stimulus artifact changed and the way the CAP changed when you switch the polarity?

Stimulus artifact flipped. CAP did not change. It is also possible that the CAP changes but it will not flip directions. If the CAP changes it is due to difference in the way it is being stimulated. SA flips because the charge flips.

2. Based on what you have learned, why does the CAP not change the same way as the stimulus artifact?

The stimulus artifact is a reflection of the stimulus. Flipping the polarity of the stimulus will flip the stimulus artifact. Action potentials can only be caused by depolarization of the axons, therefore it does not matter which stimulating electrode is positive or negative – if the axons are stimulated enough to produce action potentials, they will always depolarize (in the case of CAP not changing) or the way they are being stimulated is different so CAP might disappear or change in size (in the case of CAP changing).

Ayala | School of Biological Sciences
University of California, Irvine

Flipping a Course: Electrical Properties of the Cockroach Leg Preparation

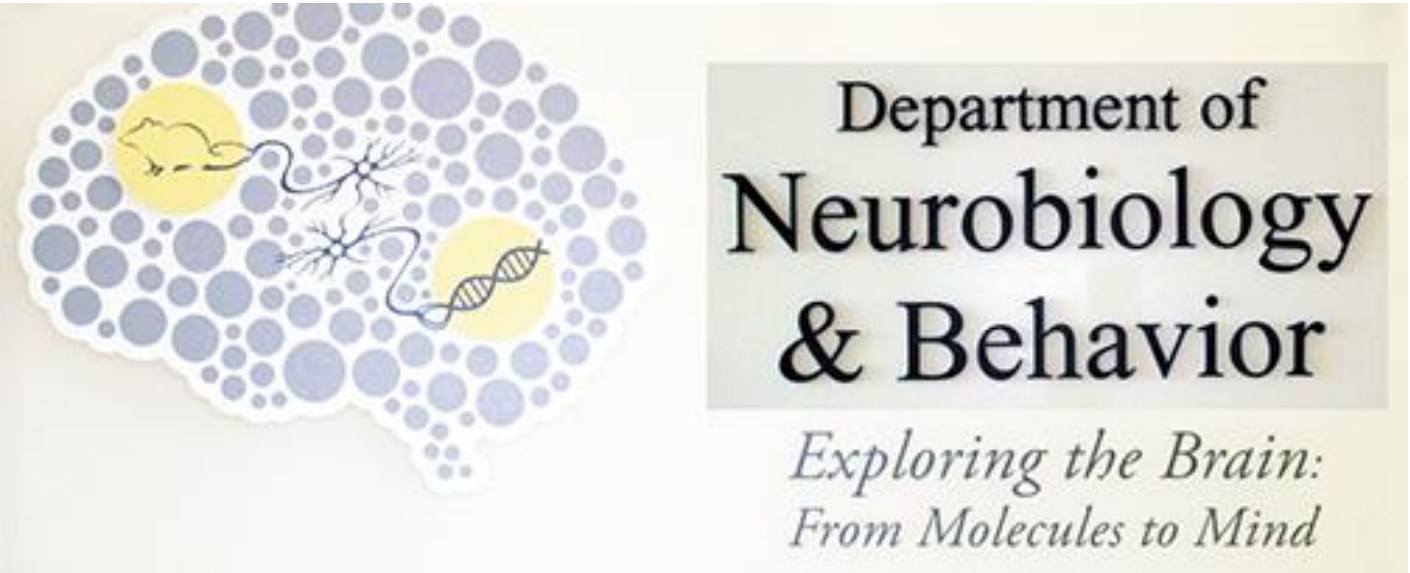
University of California at Irvine
RABLE Conference

Presented by: L. Javier, A. White, V. Boucquey, N. Goldberg, A. Nicholas

Department *of*
Neurobiology *and* Behavior
Exploring the Brain: From Molecules to Mind™

Flipping a Classroom

- Flip
- Ro
- Lab
- Ab

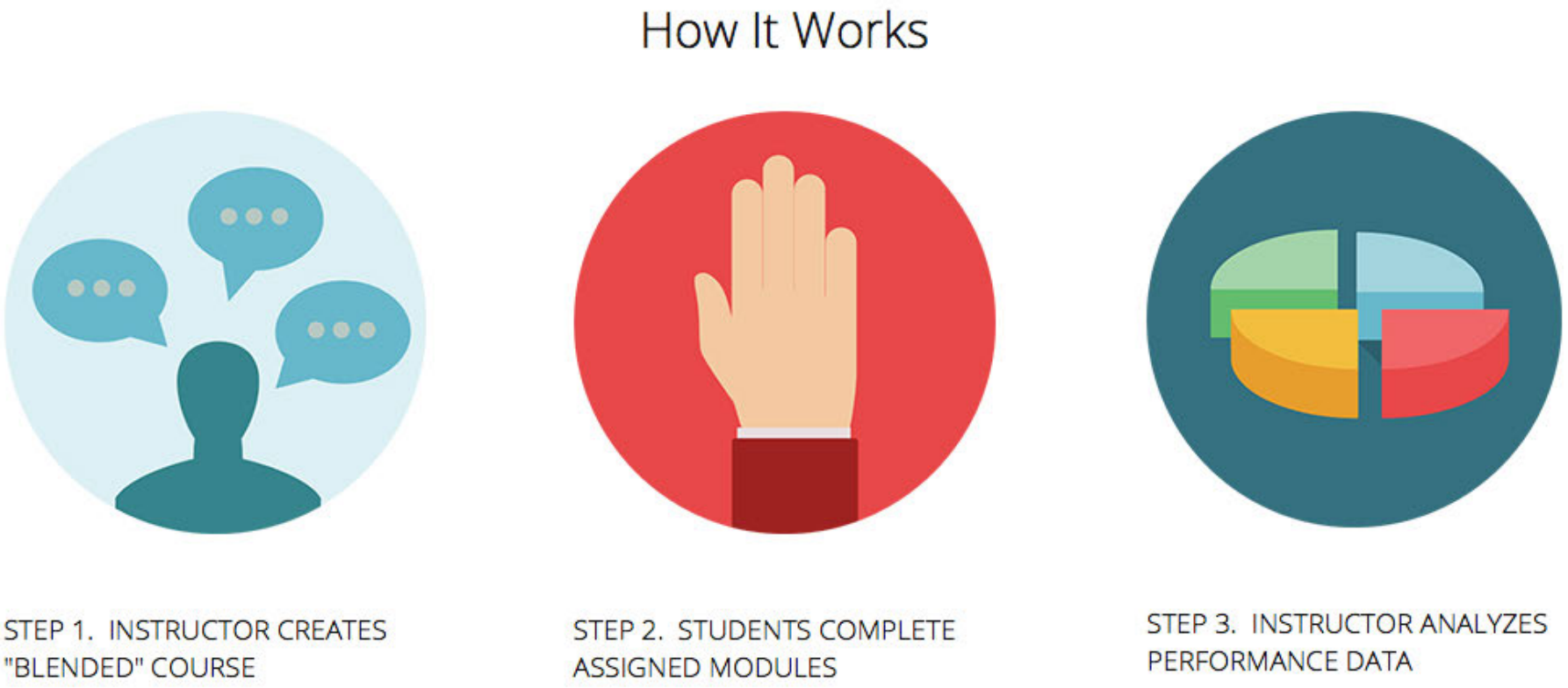


Lab Exercises

Experiment 1: Electrical Activity of Neurons

Experiment 2: Electrical Properties of the Cockroach Leg Preparation

Course Organization



- Compliments in-class activities

Experiment 1: Electrical Activity of Neurons

Background:

- Methods used to generate and interpret electrophysiology data
- History of cockroach leg preparation

Experimental Goal:

- Complete cockroach preparation (group work)
- Listen to spontaneous and evoked activity
- Use on cockroach to stimulate another leg
- Thought Questions

Rocketmix Module

Rocketmix:
https://my.rocketmix.com/InstructorHome.aspx

Experiment 2: Electrical Activity of Neurons

Background:

- Electrical properties of neurons
- Single action potential
- Compound Action potential (CAP)
- Use electrophysiology to learn about the organization of the cockroach leg preparation

Experimental Goal:

- Record and measure CAP
- Identify absolute and relative refractory period

Rocketmix

Advantages:

-Students have easy access to dynamic video presentation

-Primes students before class and tests their own understanding of the material

-Allows instructors to evaluate student understanding and ease of material

Training video for future instructors

Rocketmix

Limitation:

-Only as effective as the students utilization

-Rocketmix modules must be clear and precise

Workshop 5

RABLE 2015

Inquiry-based investigation of ectothermic metabolism

University of California, Irvine

Nancy Aguilar-Roca (nmaguila@uci.edu)

Over the course a traditional physiology course, discussions of metabolism often focus on mammals which are endothermic homeotherms. Students may not be exposed to heterothermic ectotherms, which are the vast majority of all living organisms. In recent years, research on heterothermic ectotherms has surged because of their vulnerability to climate change. The goal of this lab exercise is to add an open-ended inquiry element to a traditional protocol for measuring the effect of temperature on an ectotherm (the Madagascar Hissing Cockroach, *Gromphadorhina portentosa*). *Gromphadorhina* colonies are easily maintained in small spaces, genders are easily distinguished, adults are large enough to be individually marked for tracking, and it's possible to create custom diets with controlled quantities of supplements. Prior to this lab exercise, students will learn about metabolism and the differences between ectotherms and endotherms, and they will design their experiment. This system is flexible so that a multitude of different experimental designs are possible. The experimental treatments that have been used so far are the effects of chronic caffeine, green tea and a sports supplement on O_2 consumption and CO_2 production. For a class of 227 physiology students (in 11 lab sections), we set-up multiple colonies of Gromphadorhina with 2 replicate colonies of each supplement. Each lab section put the results of their experiments into a common database (a Google spreadsheet), and each TA was given the opportunity to structure a post-experiment activity for their own students.

Potential Learning Goals

- compare and contrast endotherm, ectotherm, homeotherm and heterotherm.
- explain the relationship between environmental temperature and the metabolic rate of an ectotherm (Madagascar Hissing Cockroach, *Gromphadorhina portentosa*).
- develop and test a hypothesis about the effect of a dietary or pharmacological substance on metabolic rate

Materials

- Madagascar Hissing cockroaches
- Metabolic chambers and gas analyzers or manometers
- Dry dog or cat food
- Coffee grinder or mortar & pestle
- Assorted supplements (liquid or powdered)

Cockroaches

Madagascar hissing cockroaches are fairly easy to keep. They will thrive (and probably reproduce) in a container with a mix of foods (dog/cat food, small pieces of apples, lettuce, carrots), places to hide (egg cartons work well) and a heating pad or heat lamp (~25-30°C).
http://www.petco.com/caresheets/invertebrates/Cockroach_MadagascarHissing.pdf
http://agweb.okstate.edu/fourh/aitc/lessons/extras/cockroach.pdf

Cockroaches also need a water source. Water beads are a good option because they don't spill and you can monitor how fast they are used.

Potential suppliers of madagascar hissing cockroaches (and most of these also sell water beads/crystals)

New York Worms: http://www.nyworms.com/index.html
Rainbow mealworms: http://rainbowmealworms.net
Ken the Bug Guy: http://kenthebugguy.com
West Coast Roaches: http://westcoastroaches.com

Cockroaches are large enough for marking individuals if students want to track weight changes. Students can create their own marking system with dots of acrylic paint on the carapace.

Custom food for experiments

The exact ratio of dried dog/cat food to liquid will vary with the brand of food so you may need to experiment a little to find a ratio that will work for you. We've been using 1g food plus 1 ml water or juice.

Put your desired quantity of dried food into the coffee grinder (or mortar) and grind it.
Add the appropriate amount of liquid to create a paste (roughly the same consistency as play-doh). Cut the tip off of a 10ml syringe and put the food into the syringe. You can extrude the paste from the syringe into regularly shaped pellets. (A pastry bag with a frosting tip would work too). Let the pellets dry out.

If you are adding a powdered supplement, add a known quantity to the ground dog/cat food before making the paste. Students can change the ratio of supplement to ground food to generate dose-response curves. If you are adding a liquid supplement, add it to the water (or juice) for making the paste. Again, you can vary the ratio of water to supplement to generate dose-response curves.

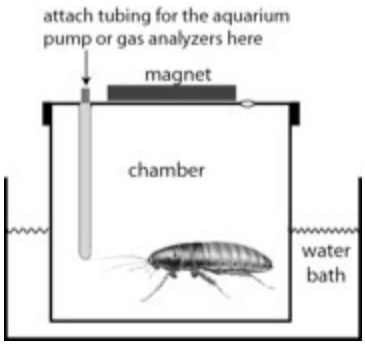
Before giving the roaches the supplemented food, they should be starved for 1 to 2 weeks (but with access to water beads). Deciding how long to feed the supplement to the roaches before measuring metabolic rate is a factor that students will have to decide on.

Measuring metabolic rate

The protocol described below can be accomplished in a 3-hr lab period.

Glass jars are best for metabolic rate measurements (many plastics are oxygen permeable). I use 500ml glass canning jars.

In this set-up the lid of the jar has 2 holes: 1 has a tubing connector to attach to the gas analyzer and the second one is to allow for air-flow to the gas analyzer. (I put a magnet on top to prevent it from floating in the water bath).



I put 2-3 similar sized cockroaches into the chamber, close the jar, and cover the openings with tape, so that the chamber is completely sealed and no fresh air can enter.

Record the time at which the chamber was first sealed shut and put the chamber into a water bath. Let the sealed chambers incubate for at least 60 minutes at temperatures above 23°C, and at least 90 minutes at temperatures below 23°C.

When ready, stop the timer and record the time to the nearest minute. Uncover both openings and attach the tube from the gas analyzers to the connector on the lid of the chamber. The other opening must be uncovered, to allow fresh air into the chamber as the analyzers remove the experimental air.

Record the data from the gas analyzers. It should take about 2 minutes get these readings. Record the lowest $\%O_2$ and the highest $\%CO_2$. Disconnect your chamber, remove the lid, and return the roaches to their cages.

Calculations

Vo_2 (ml O_2/min) = 0.9 * (Fo_2_Starting - Fo_2_Ending) * (Chamber Volume) / min

Fo_2_Starting = fraction of oxygen in the air = 0.2095

Fo_2_Ending = what you measured (percent divided by 100).

0.9 is to convert from ATPS to STPD (assuming room temperature is 21°C and atmospheric pressure is 760mmHg)

Relative-Vo_2 = Vo_2 / mass

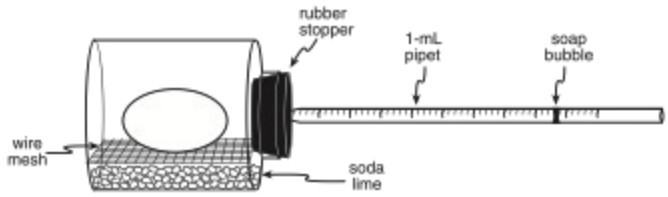
Vco_2 (ml O_2/min) = 0.9 * (Fco_2_Ending) * (Chamber Volume) / min

Fco_2_Ending = what you measured (percent divided by 100).

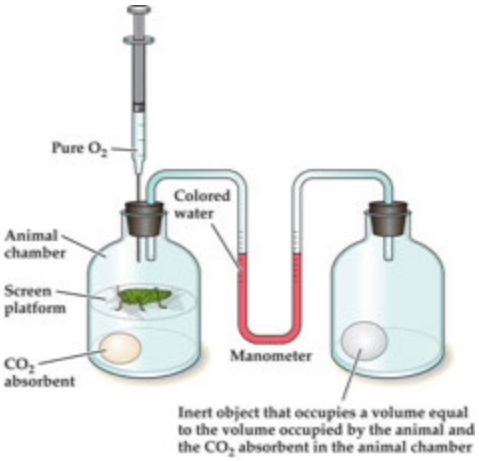
If you don't have gas analyzers, you can use a manometer. Manometers can be made with varying levels of sophistication, but they all consist of a closed chamber with a piece of water filled-tubing attached. As the animal inside the chamber respires, Soda lime or (potassium hydroxide) absorbs CO_2 and the volume of oxygen inside the chamber decreases. The water in the tubing will move as the gas volume in the chamber changes.

The simplest version is below. It consists of a jar on its side and with a one-hole stopper with a 1-ml pipet (Hiebert and Noveral, 2007). The organism can be kept away from the soda lime with wire mesh. The volume of oxygen consumed is read directly from the movement of the

water. By removing the soda lime, you can measure CO_2 produced (Vco_2). This apparatus works best in an incubator because the volume of air in the chamber will change with variations in ambient temperature.



In the second manometer set-up shown below, the second chamber corrects for temperature-induced changes in air volume. As the animal consumes oxygen, the fluid in the manometer will shift towards the chamber with the organism. At set-time points, air (or pure oxygen) is injected into the chamber until the fluid in the manometer returns to its original position. The volume of air injected is the volume of oxygen consumed. As with the simple manometer above, you can determine CO_2 production by removing the soda lime.
http://sites.sinauer.com/animalphys3e/boxex07.04.html
http://www.nuffieldfoundation.org/practical-biology/measuring-rate-metabolism



Noveral, J. and S. Hiebert. 2007. Are chicken embryos endotherms or ectotherms? A laboratory exercise integrating concepts in thermoregulation and metabolism. Adv. Physiol. Ed. 31: 97-109. http://advan.physiology.org/content/31/1/97

Workshop 6

RABLE 2015

BRINGING *CHLAMYDIA* AND CONFLICT TO THE CLASSROOM

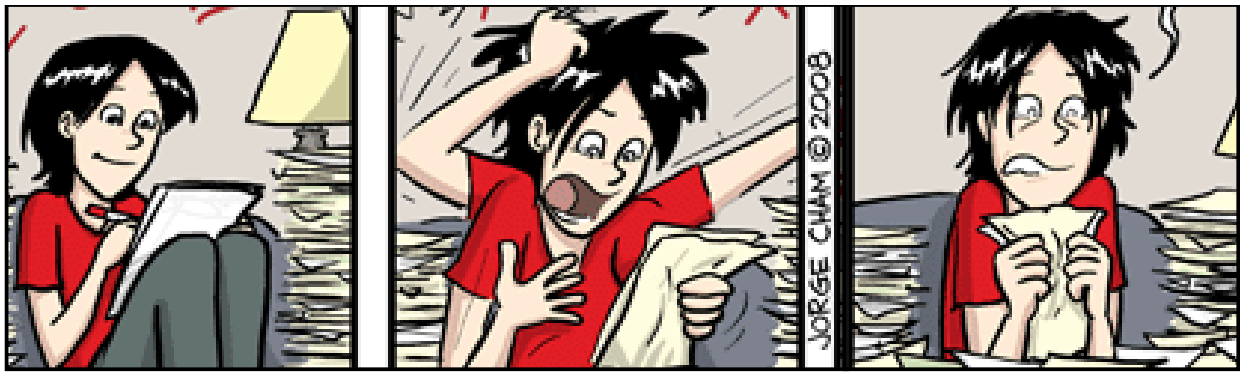
AN INTERACTIVE DISCUSSION ACTIVITY TO ASSESS STUDENTS' ABILITY TO READ PRIMARY LITERATURE

Bekah Le, Charles Yi, Vaishali Jayashankar, Seong Kim, and Rabi Murad

University of California, Irvine Dept. of Developmental and Cell Biology GAANN Fellows

UCI Ayala
School of Biological Sciences

How to read a science paper…?

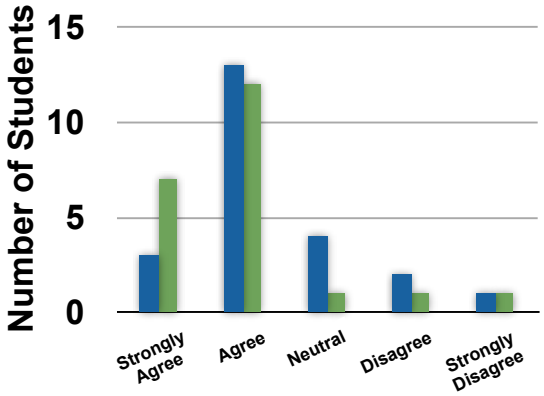


- Rationale for this workshop:
  - The ability to read and analyze primary literature is a necessary skill for students pursuing health science and research careers.
  - Students don't often get to practice this skill, especially in large classrooms.
  - Students should learn about the "realities" of research and the process of discovery.

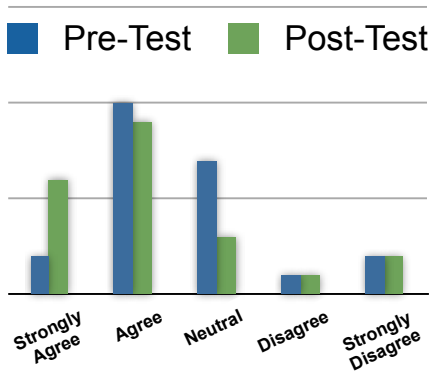
D140: “How to read a science paper”

- This workshop presents a module that stemmed from an upper division elective course developed by three Ph.D. candidates.
- Small class size – 15 students per discussion.
- Modules consisting of:
  - Research seminar given by current graduate student.
  - “Journal Club” style discussion.
  - Presentations, figure facts worksheet (Round, 2013) and pre- and post-module online quizzes.
- Class sessions devoted to Dev Bio techniques.

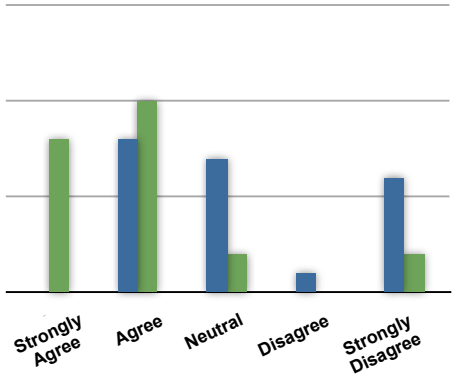
Students self-reported increase in confidence



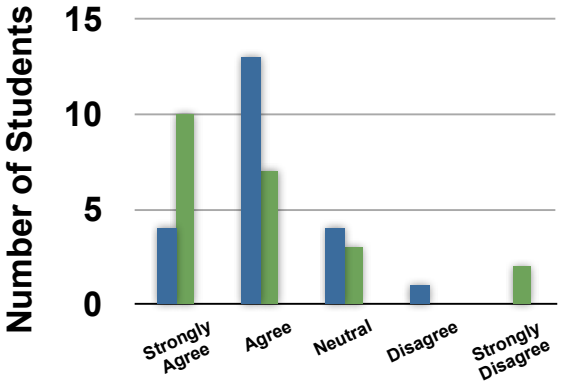
*Generally, I feel confident about working in a biological research laboratory**



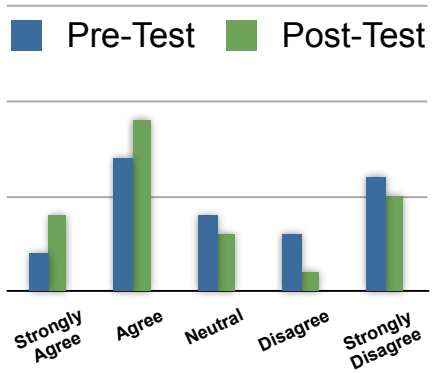
*I am sure I could conduct independent biological research**



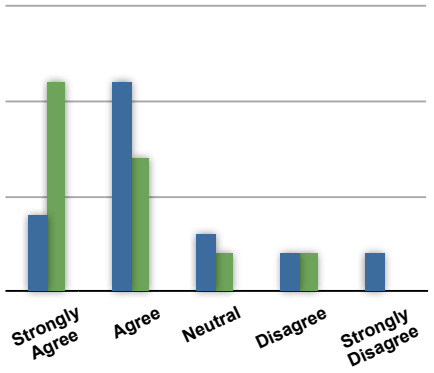
*I can handle reading and interpreting primary scientific literature**



*I generally feel confident explaining biological research to my PI**



*I generally feel confident explaining biological research to non-scientists**



*I generally feel confident explaining biological research to my peers in small groups**

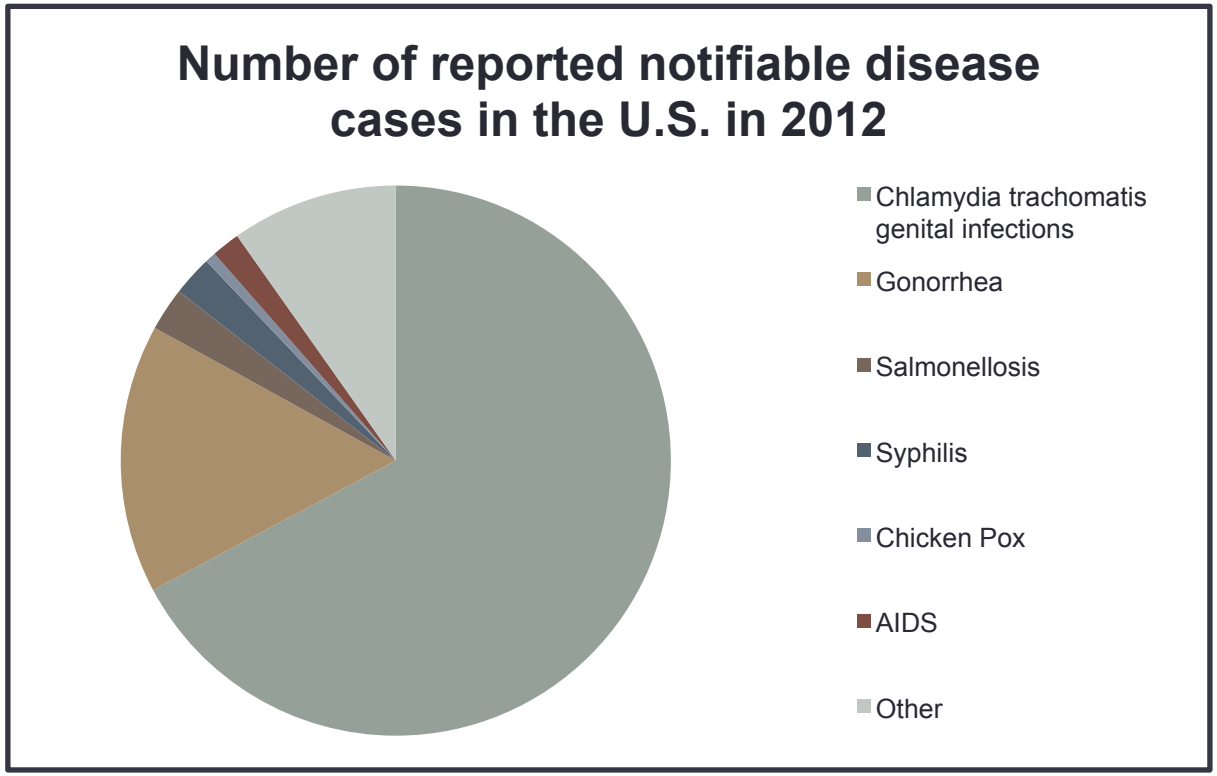
* P-value ≤ 0.05 (Wilcoxon signed-rank test)

Structure of today's workshop

- Present a module that can be incorporated into an existing course:
  - Introduction to the topic and basic techniques
  - Individual reading of one of two papers (shortened for workshop)
  - Discussion of same paper in small groups
  - Discussion of both papers in combined groups
  - Whole class discussion of both papers
- Closing discussion on module and modifications.

Chlamydia trachomatis causes significant disease

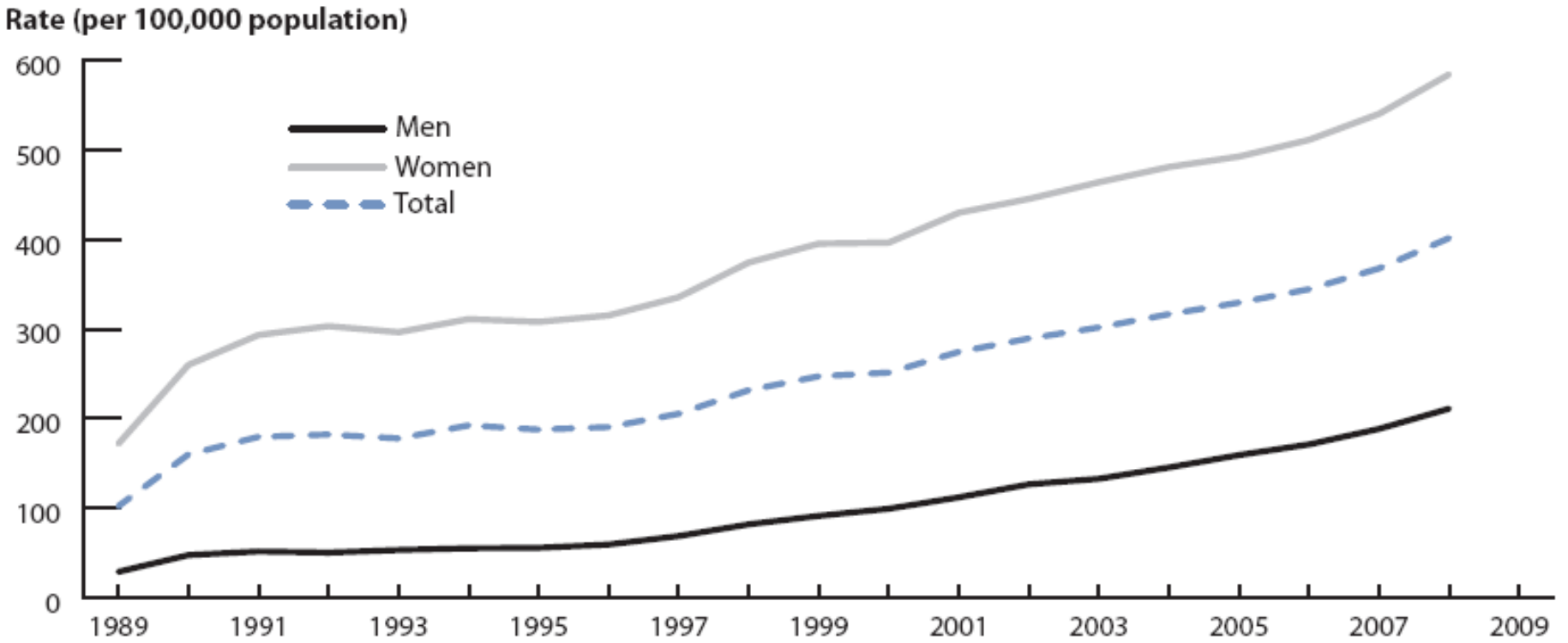
- Most commonly reported notifiable disease in the U.S. (regular, frequent information about individual cases is necessary for prevention and control of disease)



CDC, 2014

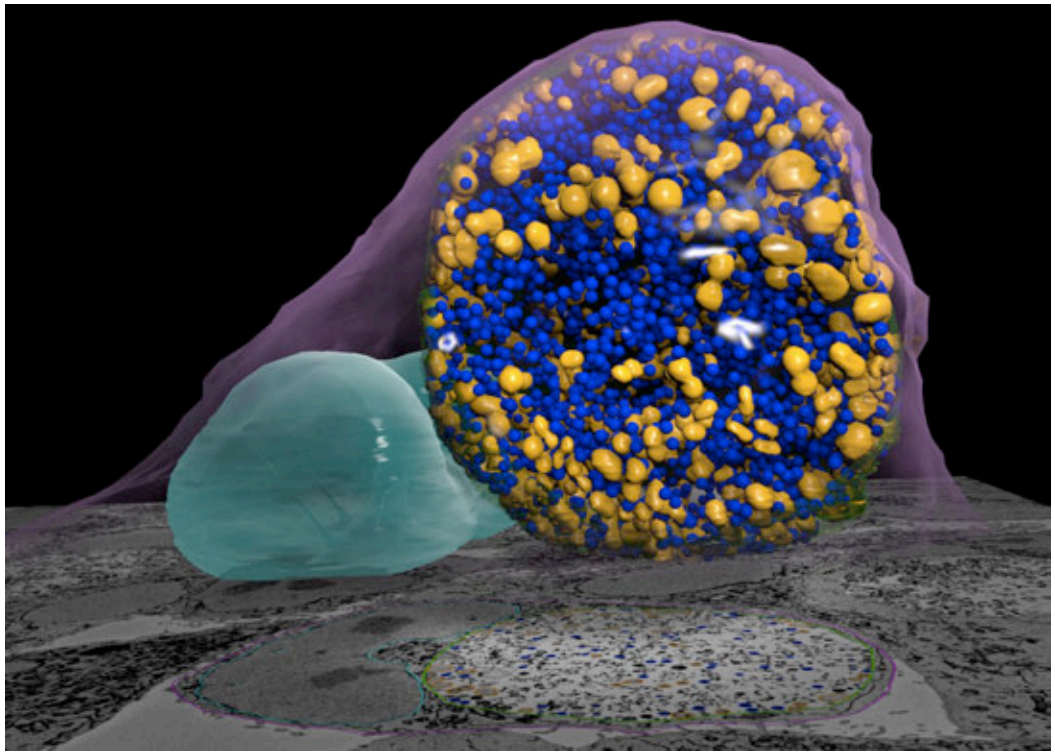
The number of reported *Chlamydia* genital infections are increasing annually in the U.S.

Figure 1. Chlamydia—Rates: Total and by sex: United States, 1989–2008



CDC, 2009

C. trachomatis relies on a host cell for survival



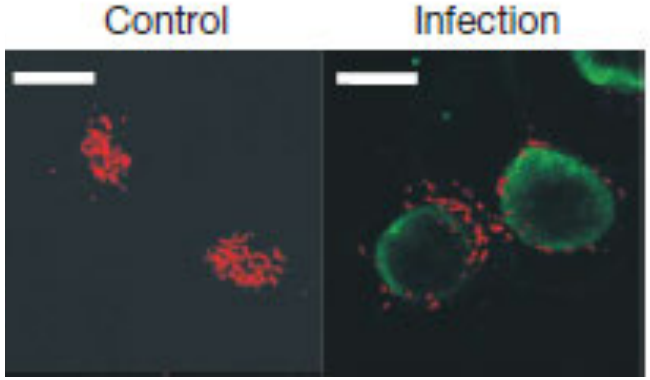
J. Lee, UC Irvine

- Obligate intracellular bacterium
- Modulation of host cell pathways promotes bacterial growth
  - Avoidance of fusion with lysosomal pathway
  - Acquisition of host lipids and nutrients
- Host cell complements minimal chlamydial genome

Chlamydia induces specific host-pathogen interactions

Golgi reorganization

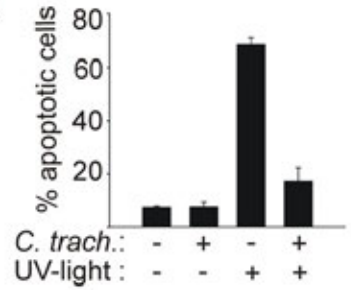
Heuer et al., *Nature*, 2009



Enhance lipid acquisition

Resistance to apoptosis

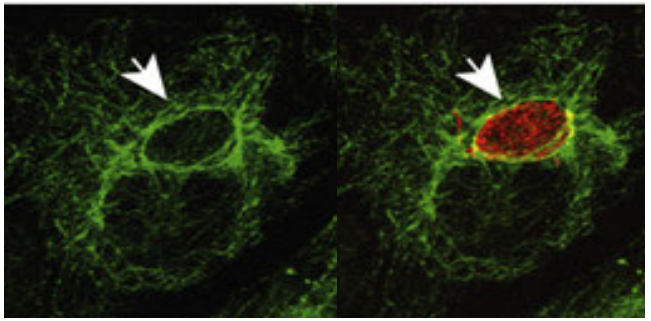
Fischer et al., *J Exp Med*, 2004



Preserve host cell and ensure bacterial replication

Intermediate filament re-organization

Kumar and Valdivia, *Cell Host Microbe*, 2008



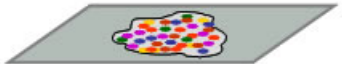
Support the chlamydial inclusion

How does *Chlamydia* induce host-pathogen interactions?

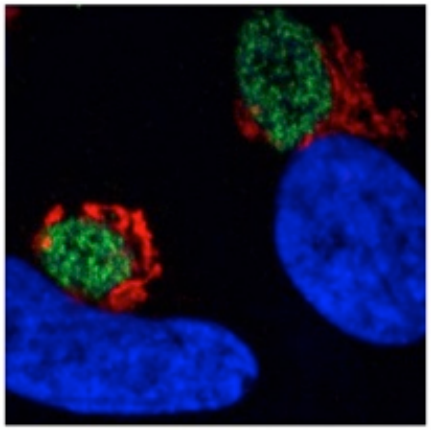
- Secreted effectors
  - Chlamydial proteins that can be translocated into the host cell cytoplasm
  - Modulate normal cellular proteins and processes

- Others?

Immunofluorescence Review

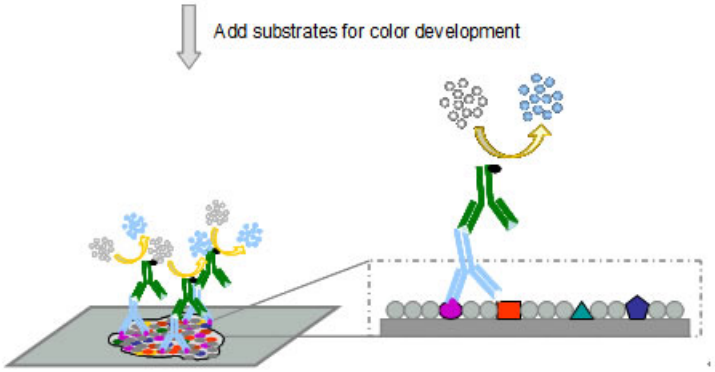


1. Begin with fixed cells or tissue sections
2. Block non-specific protein binding
3. Add primary antibody against protein of interest
4. Add tagged secondary antibody against primary antibody
5. Add color substrate for enzyme tags or visualize fluorescent tags



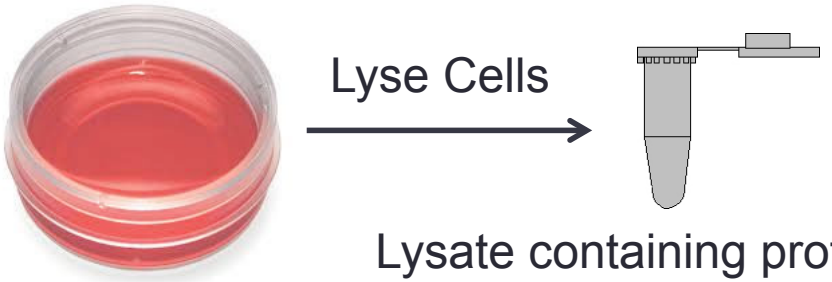
Chlamydial MOMP
Golgi ManII
DNA

Chen et al., *PLoS Pathog*, 2012

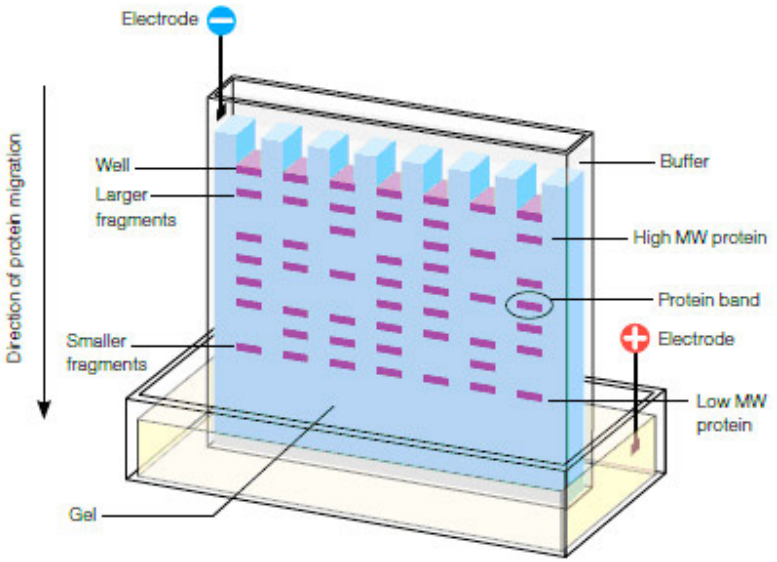


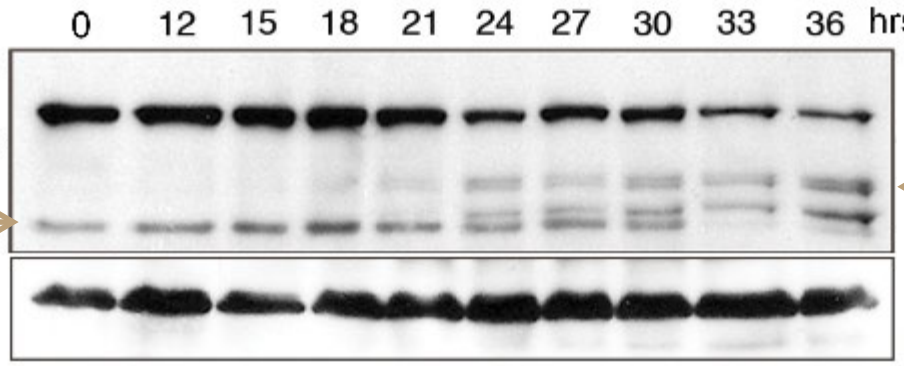
Proteintech Group

Western Blot Review



1. Collect lysate from cells
2. Denature proteins
3. SDS-PAGE
4. Transfer to membrane
5. Blot with specific primary antibodies to protein of interest
6. Blot with secondary antibodies to visualize proteins
7. How do you control for loading errors?





J. Lee, UC Irvine

Please read your assigned paper individually

Heuer et al

Chen et al

Please form groups based on paper and color

 **Heuer et al**

Heuer et al 

 **Chen et al**

Chen et al 

Please discuss your responses to the questions with your group members

Please merge with a group that has the other paper

Please exchange the following information by comparing the following information:
hypothesis, key results and conclusions

Questions addressing both papers

- **Please spend 10 minutes to answer the following questions:**

- State the hypothesis being tested by the authors (one sentence, do not copy from the authors' text)
- Based on the data presented, what conclusion can be drawn from each individual paper?
- From reading both of these papers, what conclusion did you draw about the role of golgin-84 cleavage play in chlamydia infection?
- Can you think of an interpretation that will explain both sets of data?

Whole group discussion

- Can you think of an interpretation that will explain both sets of data?
- What are the significance of these papers? How do these differing results impact the field?
- What can you do to resolve different research findings in literature?

A potential “end of activity” assignment for students:
Write a paragraph on what you learned about critically reading scientific literature.

How could this module be implemented in lecture and lab courses?

Suggestions for implementing this module

Choosing papers

- Interesting biological or pathological problem
  - Classic paper or current papers that are controversial/relevant
- Paper could cover topics and techniques from lecture and lab courses
- Articles with straightforward techniques and clear questions would be ideal

Other Modifications **(please see our lesson plan)**

- Students can read the paper before coming to class
- Assignment based on the paper could be assigned
- When the questions are handed out can be varied

Thank you for attending and please fill out the provided evaluation form!

Workshop 7

RABLE 2015

LAB 7
Behavioral Neuroscience- Habituation in *C. elegans*

Adapted and Written by Annie Ciernia, Drew Headley, Alex Pevzner, and Lauren Javier

Workshop presented by Julia Overman, Lauren Javier, Natalie Sashkin Goldberg, Andre White, Veronique Boucquey, and Dr. Andrea Nicholas

Summary

Across generations evolutionary adaptation shapes species to better fit their environment, but within a single lifetime individuals can learn new behaviors that further enhance their chances of survival. To do this, organisms must internalize the current state of the environment so that they can anticipate and optimally respond to future conditions. Such relationships between stimuli and their consequences can be learned as the organism interacts with its environment. For example, a child might learn after getting stung by a bee to be wary of insects. In this example the child gains an understanding of the relationship between bees and their ability to sting and cause her pain, and can use this information to avoid bees in future situations. The neural basis of this type of phenomenon is largely the concern of behavioral neuroscience. For this exercise, we will focus on the role of the nervous system in mediating the learned behavior of *C. elegans*, a small worm that displays many of the same acquired behaviors as larger, more complex creatures, such as mammals. Towards this end, a framework for understanding learning and memory with respect to behavior will be explained.

Goals

- Introduce the fundamentals of behavioral neuroscience
- Become familiar with principles of experimental design for behavioral analyses
- Investigate the link between genetics and behavior using a model organism, *C. elegans*
- Characterize mechanosensory habituation in *C. elegans* through interpretation of experimental results

Figure 7.1. Ivan P. Pavlov, the discoverer of classical condition, with one of his dogs.

Background

Associative Memory

Fundamentally, all behavior can be reduced to a collection of linkages between stimuli in the environment and the responses they evoke in an organism. Any physical change in the environment is a potential stimulus, and any change in the outward state of the organism is a potential behavior. If a particular stimulus reliably elicits a particular response, we infer the existence of a linkage between them. Put another way, we say that the stimulus is *associated* with the response. For example, if every time you open the cupboard where you keep your dog's treats, he runs into the room drooling, you can infer that he has associated the sound of the treat cupboard opening with getting fed a treat. Three different steps are necessary for such an association to be expressed: (1) subjects must detect the stimulus (in our example, your dog must hear the cupboard open), (2) link the perception of the stimulus to the response (the sound of the cupboard opening is linked with the response – running into the room drooling), (3) and perform the response (run into the room and drool). Experience can allow for the creation of new behaviors by modifying any of these steps.

Long-term changes in behavior often reflect memory, but not necessarily associative memory. Typically, a memory is only considered associative if it relates a stimulus to a response; this relation is called a stimulus-response (S-R) association. Thus, a memory is only associative if it reflects a change at step two, the linkage between perceiving a stimulus and responding. A change limited to stimulus detection (step one) would not be considered associative. The same is true for a change limited to the subject's responding (step three). Changes that are limited to stimulus detection and response production are non-associative. Using our example from above, if your dog lost his hearing and could no longer perceive the CS, his failing to run into the room drooling would not reflect a new or altered associative memory.

Table 7.1. Terms and definitions used in associative training along with examples of each

Term	Definition	Explanation / Example
Association	A linkage between either a stimulus and response, or a stimulus and another stimulus.	For example, in high school, you probably associated the school bell (stimulus) with the end of class and getting up to leave (response).
Conditioned stimulus (CS)	An initially neutral stimulus that begins to elicit a response (CR) after pairing with a US	In other words this is a stimulus for which the organism must learn the appropriate response. (Like the school bell – you had to learn how to respond to this stimulus).
Unconditioned stimulus (US)	A stimulus that drives responding without prior training	For example touching a hot stove (US) causes you to automatically withdraw your hand (UR). This response is automatic and not learned.
Conditioned response (CR)	The response elicited by the CS after pairing with a US	In the school bell example the bell is the CS and the CR is your learned response – getting up to leave class.
Unconditioned response (UR)	The innate response produced by the US prior to training	Again: touching a hot stove (US) causes you to automatically withdraw your hand (UR). This response is automatic and not learned.

As a side note, it is worth mentioning that current theories of associative memory argue that besides S-R associations, subjects also learn that one stimulus predicts another stimulus, which has been termed stimulus-stimulus (S-S) association. Psychologists and neuroscientists often have to infer S-S learning, because only the experimental stimulus and the animal's response are observable. A variety of tests have been developed to judge whether an associative memory reflects S-S or S-R learning. Frequently, including in this text, the observation of associative memory will be reported in S-R terms, while the psychological interpretation of the associative memory will be given in S-S terms. Given the previous example, the S-R interpretation is that the sound of opening the dog treat cabinet causes your dog to approach you and salivate. However, the S-S interpretation is that your dog has associated the sound of the cabinet opening with the dog treats, and thus the dog behaves as if anticipating the reward. In spite of these two different ways of viewing associative memory, both S-R and S-S require the change to occur between stimulus detection and response production. Ultimately, the same standardized protocols can be used to induce associative and non-associative memory, no matter whether associations are S-R or S-S.

Associative and Non-associative training protocols

To study how different training protocols produce associative or non-associative memories, we must establish a common vocabulary. Stimuli fall into two categories. *Unconditioned stimuli* (US) induce an innate response that does not depend on prior experience. Such stimuli are intrinsically motivating; subjects will either approach or avoid them without training. For instance, touching a hot stove will elicit a withdrawal response. The heat from the burner is sufficient to drive a response; you do not need to *learn* to withdraw your hand. In this case the hot stove is the US. The other class of stimuli is known as *conditioned stimuli* (CS). These are not intrinsically motivational, because subjects will eventually stop responding to them, unless they predict a US. The initial response to the CS is known as an orienting response (OR), and is driven by a sudden and unexpected change in the environment. Imagine a creaking noise you hear in your new apartment at night. At first you would notice it and become alert and concerned, but when enough nights had passed with the creaking noise, and nothing bad had happened you would get used to the noise and stop responding. The creaking is an example of a potential CS, if it predicted the arrival home of an upstairs neighbor that played really loud annoying music (a US) then you *would* continue to respond to the creaking.

Training subjects to learn that a CS predicts a US can be done with *classical conditioning*. Classical conditioning is a form of associative conditioning that involves *pairing* the CS with a US. During pairing the CS is presented and then shortly thereafter the US occurs. Each instance of presenting the CS and US is called a *trial*. Typically, the strength of the prediction will increase with the number of trials. Over time the CS will come to elicit a *conditioned response* (CR), the subject will respond to the CS as if anticipating the US. An iconic example is Pavlov's dogs (Fig. 7.1), which were trained to associate the ringing of a bell with the presentation of beef. Upon hearing the bell, the dogs would begin salivating, as if anticipating the beef. If you then repeatedly present the bell without a food reward, the dog will learn that the CS no longer predicts the US. This form of new learning is called *extinction*. During extinction, a CS that was previously paired with a US is repeatedly presented alone (without the US). Thus, in extinction the previously acquired CS-US association is slowly "extinguished" after repeated CS only presentations (Fig. 7.2).

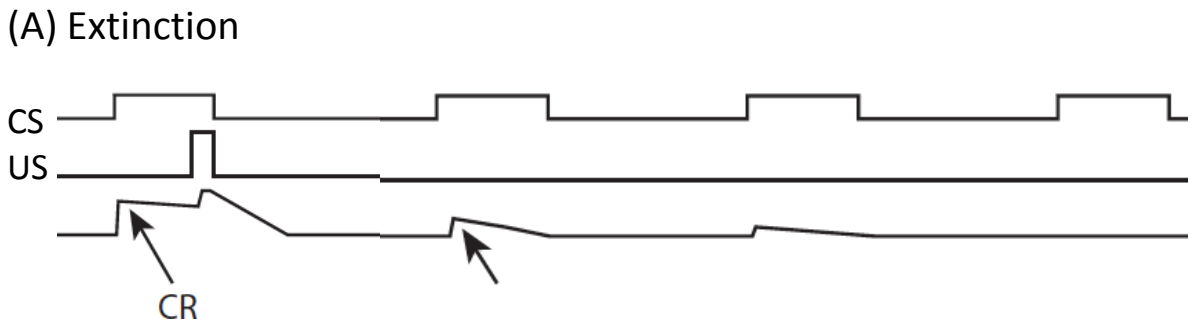


Figure 7.2. Extinction of Response

(A) Classical conditioning repeatedly pairs the conditioned stimulus with an unconditioned stimulus (US). The acquired response to the CS is known as a conditioned response (CR). When the conditioned stimulus is presented repeatedly without the unconditioned stimulus, the subject learns that the CS does not predict the US. As a result, the subject's conditioned response diminishes over trials, until finally, it no longer elicits a conditioned response. This is known as extinction.

Besides learning that one stimulus predicts another, such as in classical conditioning, in non-associative conditioning, subjects can also learn that stimuli are not predictive. Two commonly studied forms of non-associative learning are habituation and sensitization. During *habituation*, a novel stimulus (S) is repeatedly presented without a US. During repeated presentations the subject learns that the S does not predict another stimulus, and consequently the subject stops responding to the S (decrease in OR). Another form of non-associative learning is *sensitization*. During sensitization the presentation of a strong stimulus (S) enhances future responses to many different stimuli. For example, your dog will respond much more strongly to a normal pitch tone after it has been played a painfully loud tone. In this example, the dog has been sensitized by the loud tone (S) and will then respond much more strongly to otherwise innocuous stimuli (increase in OR).

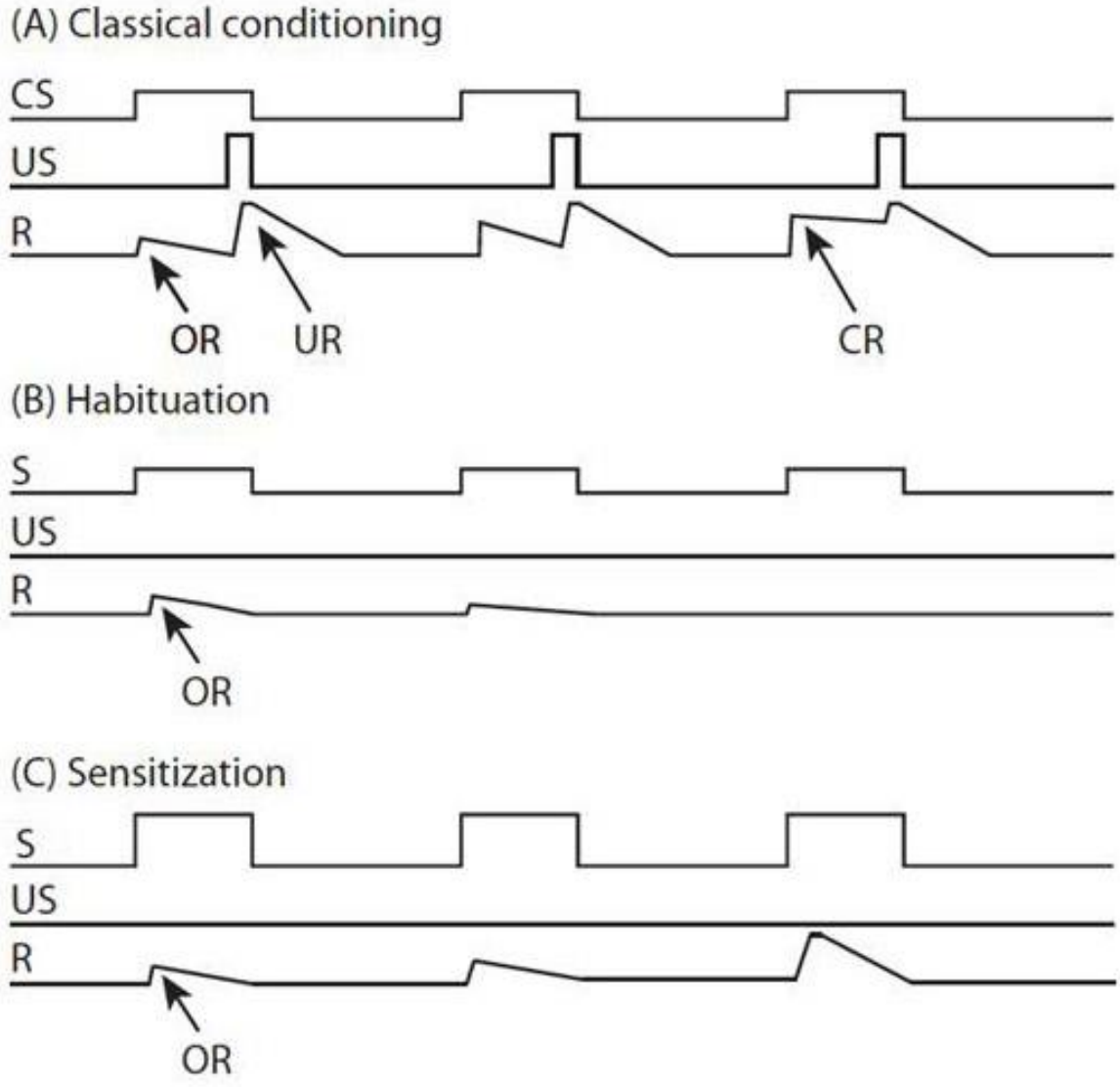


Figure 7.3. Training protocols us to study memory.

(A) Classical conditioning repeatedly pairs the conditioned stimulus with an unconditioned stimulus. Stimuli, such as the CS and US, elicit several different responses. The first CS presentation evokes an orienting response (OR), which is driven by the novelty of the CS. US presentation evokes a strong innate response (UR), reflecting its inherent biological significance. Responding to the unconditioned stimulus remains the same throughout training, while responding to the conditioned stimulus increases across training trials. The acquired response to the CS is known as the conditioned response (CR). (B) Habituation training lacks an unconditioned stimulus, and the repeated presentations of the novel stimulus (S) leads to decreased responding. (C) Sensitization presents a strong stimulus (S) without a US resulting in an increase in the orienting response over repeated S presentations.

Memory vs. performance distinction

The field of learning and memory is interested in understanding how the nervous system acquires, stores and recalls information for experiences (i.e. memory). Although intuitive, 'memory' cannot be easily defined or directly measured. Memory must be inferred from a change in the animal's behavior. Therefore, our capacity to study memory is conditional, not only on the animal being able to perform the task, but our ability to detect a change in the

performance. Such an indirect measure of memory is problematic for neuroscientists, because in the course of conducting experiments they must differentiate between the animal's memory for an experience and its ability to perform the appropriate action. This is known as the memory vs. performance distinction.

It is important to note that if the animal doesn't display 'memory' when tested one cannot automatically conclude that the animal didn't learn or that it forgot the events. Below is an example to illustrate this point. Rats will explore (i.e. sniff, rear, lick) the environment in which they are placed. However, if a mild foot shock is administered in that environment then the rats change their behavior from exploration to defensive freezing (immobility). This is a well-characterized fear response that is context specific. In an effort to understand the neurobiology supporting contextual fear, researchers decided to lesion specific brain regions and observe the rat's behavior after the lesion. One particular brain region that resulted in a significant decrease of freezing was the basolateral amygdala (BLA). Thus, it was concluded that the memory for contextual fear was in this structure. However, the memory vs. performance distinction was not given proper attention. Subsequent research demonstrated that animals with BLA lesions in fact were capable of remembering the context in which they received the shock, although they were not able to freeze in that context. If other fear related behaviors were measured (e.g. defecation, active avoidance, heart rate) then the animals showed a clear fear response.[1]

This example illustrates not only the importance of distinguishing between memory and performance in a task, but also the significance of measuring appropriate behaviors and conducting necessary controls. If freezing was the only measure taken then one would falsely conclude that the rats did not have a fear memory for the context. It was only through multiple independent measures that researchers were able to observe that the animal did remember the association between the context and shock. A critical control that was used to bolster this conclusion was demonstrating that BLA lesions prevented unconditioned freezing (freezing to cat hair, something that is not learned but is innate to rats).

Experimental Analysis of Behavior

Behavior is often difficult to quantify and may show a great deal of variability both between animals and within the same animal. To determine behavioral effects of a stimulus accurately, researchers must do their best to make objective, unbiased observations and measurements of the behavior. Several strategies can be used to achieve this, any of which may be employed in this laboratory exercise.

- To eliminate bias, some studies **blind** various aspects of the experiment so that not everyone knows what variables are being tested at a given point. For example, the experimenter may not know the specific gene mutation in their experimental subjects.
- Another strategy is to gather **multiple data points** for the same item and then determine statistical trends. This could mean that multiple individuals, each with his/her own biases and perspectives, score the same behaviors simultaneously, or that multiple subjects are used in the study.
- A third strategy relies on a **clearly defined set of criteria** for scoring behaviors so that, for example, different degrees of a behavior can be distinguished systematically.

C. elegans as a Model Organism

Caenorhabditis elegans are soil dwelling, bacteria eating nematodes (roundworms) that are approximately 1mm in length. *C. elegans* have a relatively short lifespan and reproduce quickly,

making them ideal experimental subjects. In the last forty years *C. elegans* has been used as a model experimental organism in neurobiology, developmental biology, and cell biology.

For neurobiologists, the adult worm's nervous system is an attractive model system for linking neuronal and molecular function to behavior. The adult worm's nervous system is composed of exactly 302 neurons, all of which have been identified and their interconnectivity mapped. Despite their relative simplicity, the components of the *C. elegans* nervous system are similar to more complex organisms such as humans. For example, over 5,000 genes in the *C. elegans* genome have corresponding human homologs, including important nervous system components.[2] Neurobiologists have also taken advantage of the sequencing of the *C. elegans* genome. Using this genetic information, researchers have made thousands of mutant worm strains. By targeting a specific gene and either knocking out or over expressing it, researchers have begun to examine the function of individual proteins. Combining the known neuronal connectivity with genetic manipulations has allowed researchers to begin linking molecules, neurons and behavior.

Adult *C. elegans* fall into one of two sexes: hermaphrodites or males (no females). Hermaphrodites possess all the necessary components for self-fertilization and are the most common sex of worm (~50-99% depending on the environmental conditions).[3] Hermaphrodite self-fertilization allows for the generation of genetically identical offspring while male mating (with hermaphrodites) produces greater genetic diversity. A single hermaphrodite worm can produce approximately 300 progeny by self-fertilization or 1200-1400 progeny by mating with a male. A single adult male can father about 3,000 progeny.[3]

Whether generated by self-fertilization or sexual reproduction, the resulting eggs hatch into the first larval stage (L1) in approximately 14 hours. Assuming favorable conditions, L1 larvae progress to become L2 larvae. L2 larvae then continue to L3, L4, and then adulthood. By 45-50 hours post hatching, an adult hermaphrodite lays its first eggs, completing its reproductive life cycle. Adult hermaphrodites continue to lay eggs for 3-4 days and then live for an additional 10-15 days.[3] However, if there is limited food, extreme temperatures, over crowding or other environmental stressors, L1 worms arrest development and enter the dauer stage. In this dormant stage, worms no longer feed and locomotion is dramatically reduced. Worms can remain in the dauer stage for up to four months and exit only when environmental conditions become favorable. When this occurs, dauer stage worms can develop into L4 larvae and then adults.

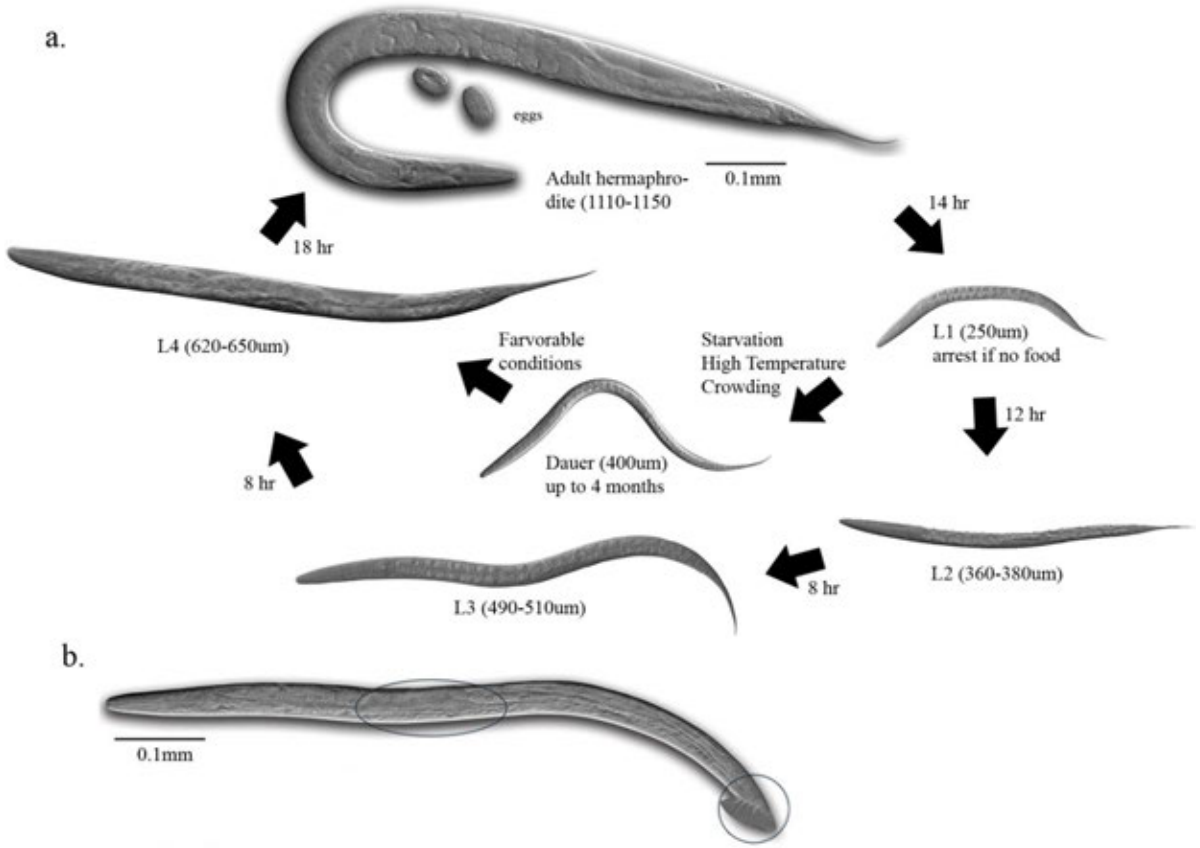


Figure 7.3. *C. elegans* Life Cycle.
a. *C. elegans* hermaphrodites undergo sexual or self-fertilization to produce eggs. In approximately 14 hours the egg hatch into L1 larvae which then develop into L2 larvae if food is present. L2 larvae will then develop into L3, L4 and finally adults. If conditions are not favorable, L1 larvae will arrest development and enter the dormant dauer stage until conditions improve. b. Male *C. elegans*. Note the lack of eggs (middle circle) and fanned tail (right circle) that distinguish the male from hermaphrodites. Adapted from WormAtlas.org.

Habituation in C. elegans

In this lab you will perform a series of experiments to characterize mechanosensory habituation in *C. elegans*. *C. elegans* respond to vibrations (which can be induced by tapping the side of the plate they live in) by reversing their swimming direction. This **tap-withdrawal response** shows multiple forms of non-associative learning including habituation, dishabituation, and sensitization.[4] In order to conclude that the decrease in the tap-withdrawal response with repeated stimulation is true habituation learning, you need to test for performance confounds. **Sensory adaptation** or sensory fatigue is evoked activity that can often decrease as a result of prolonged stimulation. Sensory fatigue is simply the inability of the animal to respond to a stimulus due to a deficit, not in memory, but in sensation. For example, suppose a specific set of sensory neurons are required for C. elegans to respond to a mechanosensory stimulation. Furthermore, suppose these neurons can only respond once every 15 sec or they stop firing. With our repeated tapping the worms would rapidly be unable to respond due to a sensory deficit independent of their memory for the stimulus. Similarly, motor fatigue, which is the lack of responding due to a deficit in the motor system underlying the measured behavior, can produce behavior similar to habituation.

To distinguish between sensory/motor fatigue and habituation, we will use **dishabituation**- the phenomenon in which a strong novel or noxious stimulus can result in the return of the habituated behavior to near its pre-training response level. Immediately, following training a strong novel or noxious stimulus is delivered and the worms are then given a set of test taps. Dishabituation is shown by an increased response above habituated levels observed at the end of the training trials. Dishabituation is performed immediately following the memory test, at a time point when sensory adaptation and motor fatigue are predicted to be maximal. Consequently, if habituated worms show strong dishabituation following the strong noxious stimulus, it can be concluded that the initial habituation was not due to sensory or motor fatigue and is likely true habituation learning. Dishabituation is similar to sensitization, except that dishabituation produces a recovery of the original response while sensitization produces a response that is stronger than the original.

Today's exercise will examine tap-withdrawal habituation in *C. elegans*. Small groups of *C. elegans* will be placed in petri dishes for these experiments. For habituation, the dish will be repeatedly taped, and the tap withdrawal (swimming reversal) response will be measured across trials. The final habituated response is predicted to be much smaller in magnitude then the initial response. In other words, after enough experience with the tapping vibration stimulus, the worms will decrease their swimming reversal response. We will be using two blocks of training trials separated by a 10min rest period (Fig 7.4). There is predicted to be "**savings**" between the two blocks of training trials. Savings refers to an enhanced rate of learning (i.e. habituation) during subsequent training. For example, if you learn a list of words and then forget them, you will be able to relearn them faster the second time then you did the first time. This difference in learning rate is savings. Following training, your worms will be given a 10min break and then 10 additional test taps. These taps are considered a "memory test" to assess how well your worms remember the initial training. Immediately following testing, you will dishabituate your worms to test for a potential performance impairment. In the final experiment of this lab you will examine a mutant strain of *C. elegans*, compare the mutant strain's habituation learning to wild type *C. elegans*, and look up the function of your mutant protein.

Fig 7.4. Timeline for training and testing procedures in Experiments 2 and 3.

LAB EXERCISE: Habituation in *C. elegans*

Experiments:

Experiment 1: Basic Microscopy & *C. elegans* Lifecycle Identification.

Experiments 2: Habituation & Dishabituation in wild type *C. elegans*

Experiment 3: Genetic Manipulations in *C. elegans*

Experiment 1: Basic Microscopy and C. elegans Life Cycle Identification

You will observe your experiments through a dissecting microscope similar to the example in Figure 7.5. Using the focus knob, lower the microscope body all the way down and then place your specimen on the stage plate. Look through the eyepieces, which can be swiveled to accommodate variations in distance between the observers' eyes, and turn the focus knob until the specimen comes into focus. You can then adjust the magnification with the zooming knob. The focus may need to be adjusted if you change the magnification significantly. If the focus is not sharp, you may need to adjust the diopter ring

on the eyepiece, which is similar to lenses used in eyeglasses to correct vision.

In this experiment you will be given a culture of *C. elegans* that are in various stages of their life cycle. You should identify at least one or two examples of each life cycle stage (except for male and daur stages) and take basic observations of worm behavior. Note the rough number of worms in each life cycle stage and how worm movement is dependent on stage. Refer the Fig 6.3 above and the hints below to identify each stage.

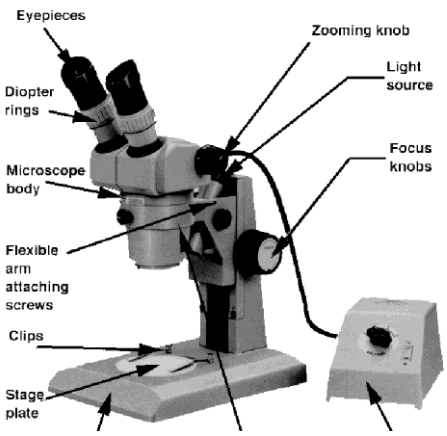


Figure 7.5. Dissecting microscope

How to identify each life cycle stage (refer to Fig 7.3):

- **Eggs**: small, ovular spheres, not moving
- **L1**: most recently hatched and smallest larvae
- **L2 & L3**: larger than L1 but smaller than adults and L4
- **Dauer**: smaller than all but the L1 larvae, thinner and more pointed than L1
- **L4**: almost as large as the adult hermaphrodite, lack internal embryos, clear crescent shaped patch near its center
- **Adult hermaphrodite**: largest worms, embryos inside, whip-like tail, move more slowly than the males,
- **Male**: slightly thinner and smaller body then the hermaphrodite, fan shaped tail, move rapidly and tend to stroke other worms by doubling back on themselves

*Note that under non-starvation conditions, wild type cultures will have only a few males and very few to no dauer stage worms.

Observations:

Experiment 2: Habituation

In this experiment you will work in pairs to study habituation in *C. elegans* (nematodes) using 60 training trials. Training trials will be delivered in two blocks of 30 trials each separated by a 10min rest period (60 total trials). Each trial will consist of observing the worm's initial behavior, delivering a tap stimulus, and recording any changes in the worm's behavior following the tap. The training trials will be delivered 10 sec apart, so make sure you are prepared to record the worm's behavior prior to beginning. One partner (Trainer) will deliver the tap stimulus and observe the worm's behavior. The second partner (Recorder) will run the stopwatch, announce when to deliver the tap, and record their partner's observations. For this experiment your TA or committee member will place 1-5 worms inside your petri dish. For this experiment you will be choosing one worm to observe. In response to each tap the worms are predicted to show a **tap-withdrawal response** –movement in the reverse direction following the tap (compared to the pre-tap movement). In this training scenario, the tap is a generic stimulus, neither CS nor quite US. The tap-withdrawal response is an **orienting response**.

In order to have a standardized set of scoring criteria, the worm's movements will be scored as the following:

R: Reversal- worm moves in the opposite direction compared to the pre-tap movement

M: Movement- worm is in motion in the same direction compared to the pre-tap movement

I: Inactive- worm is not in motion in any direction

Thought Questions:

1. Based on your reading from this chapter, what type of behavior do you predict the worms will show following the first training tap? Following the 10th tap? Following the 30th tap? Why?

Following the first training test the worms are predicted to show the tap withdrawal response (reversal swimming). This response is predicted to habituate during the course of training so that by the 10th trial there should be a decrease in the frequency of the withdrawal response and this response should be dramatically attenuated by the 30th tap. The "habituation" of the withdrawal response is thought to occur as the worms learn that the CS (tapping) does not predict a US (absent in this paradigm)

2. What advantage or disadvantage could tap-withdrawal response away from a tap confer to nematodes in their natural environment?

The withdrawal response to a novel stimulus such as a tap might confer an advantage to C. elegans by allowing them to avoid potentially harmful stimuli. For example, a vibration in the soil in which C. elegans live might signal the presence of a predator. A potential disadvantage of this response might result if environmental vibrations occurred near the source of food.

3. What advantage or disadvantage would habituation of this response confer to nematodes in their natural environment?

Learning to not respond to stimuli that do not predict a subsequent stimulus could confer a number of advantages in the wild. For example, if there is a constant drip of rainwater in an environment and this drip does not predict any subsequent events then there is no need for the worm to pay attention to it. Consequently, if the worm stops responding to the raindrops it can focus on other stimuli such as a potential predator or finding food. Without habituation, it would be difficult to distinguish biologically relevant from irrelevant stimuli, decreasing the organism's likelihood of survival.

Procedure:

Acclimation

1. Have your TA or committee member transfer 1-5 worms to your petri dish.
2. Place your petri dish on the dissection scope.
3. Observe the nematodes as they swim normally across the agar. Allow your worms to acclimate for 5 min while taking turns making observations. Note the directions of motion of the worms and approximately how often they turn and stop moving.

Observations:

Baseline Trials

1. The Trainer should choose one worm to observe for all the training trials. Based on the worm's baseline behavior, choose a worm that is moving frequently, is located near the center of the viewing field of the microscope, and is separated from other worms on the plate. Try to follow this worm throughout the procedure. If the worm swims out of the field of view choose another worm to score and make sure to note the change on your score sheet.
2. Conduct 5 baseline trials without tapping the side of the petri plate. These trials are designed to measure the baseline activity (prior to training) of your worm and to give you practice in observing and recording behavior.
3. For each Baseline Trial:
    a. Trainer: takes an observation of the worm's movement prior to tapping.
    b. Recorder: says tap, (but no tap stimulus is delivered) and then times 10 sec until the next baseline trial
    c. Trainer: takes a second observation immediately following the Recorder saying tap
    d. Recorder: writes down the pre and post tap behaviors in under the Baseline section of the Experiment 2 score sheet (found at the end of the lab).

For each trial there will be two measurements: one prior to "tap" and one immediately following "tap". **NO taps will actually be given! To count as a Reversal, the worm must reverse directions relative to the pre-tap movement. Consequently, if the pre-tap behavior is inactive, the post tap behavior CANNOT be a Reversal.

Training Trials- Two Blocks of 30 trials separated by a 10min "rest"

1. Training trials are identical to the Baseline trials except that the Trainer will deliver a single tap stimulus following the Recorder saying "tap."
2. Give 30 training trials, while taking observations of your chosen worm.
3. Immediately following the last training trial of block one start the stopwatch.
4. Allow your worm to "rest" for 10 min without moving the plate from the microscope stage.
5. While waiting construct a histogram of the scored behavior.
    a. Count the number of reversals in each set of 5 trials as shown on the score sheet. This gives you the frequency of reversals for each block of 5 training trials.
    b. Graph the frequency of reversal for each block of 5 trials. Remember that to count as a Reversal, the pre-tap behavior must be a Movement.
6. After 10min have elapsed repeat steps 1-5 for the second block of 30 trials.

7. Immediately following the last training trial, start the time for a second 10min rest period. Leave the worm on the microscope stage for this period.

10min Memory Test & Dishabituation

1. Following the 10min period, test the memory of your worms by repeating 10 trials exactly as was done during Training.
2. Record your results under the 10min test section of the score sheet.
3. Immediately following the memory test, dishabituate your worm by removing the petri dish from the microscope stage
4. Carry the petri dish to the vortex, place the dish on the top of the vortex pedestal and press firmly down so that the vortex activates. You should feel the plate vibrate.
5. Vortex the plate for 15 sec.
6. Following the dishabituation stimuli, immediately test your worm with 10 more trials (performed the same way as the memory test trials).
7. Calculate the frequency of reversals for each test and add these data points to your histogram.

Thought Questions:

1. Describe any general trends you notice in your in your histogram for the baseline and training trials. Do you have "habituation"? If so, by what trial block does it first appear?

"Habituation" would appear as a decrease in the frequency of the tap-withdrawal response over the course of the training trials. The onset of this decrease varies but should be noticeable by the end of the first training block.

2. Do you have "savings" in the rate of "habituation" between trials in block 1 and 2 (i.e. does the rate of "habituation" in block 2 appear greater than in block 1)?

Savings would be evident by an increased rate of habituation in block 2 compared to block 1. Savings can also occur for the first trial set (1-5) of block 2 (being lower than the first trial set (1-5) of block 1). The idea behind savings is that the worm "remembers" the habituation training from block 1 and hence habituates faster during the subsequent training trials. "Habituation" is in quotations here because without a control for performance there is no way to disambiguate true habituation learning from sensory/motor fatigue.

3. Compare the last block of training trials to the 10min memory test. Are the animals still showing a habituated response at test relative to training?

This is either yes or no depending on your data

4. Do your worms show dishabituation? How do your results from the dishabituation test influence your interpretation of the "habituation" observed during training and the memory test?

If the worms show dishabituation (withdrawal response above the habituated response levels at the memory test) then a decrease in the frequency of the withdrawal response can be attributed to true habituation learning and a habituated response during the memory test to true habituation memory. If there is not (or very little) dishabituation it is not possible to distinguish habituation learning/memory from sensory/motor fatigue in this experiment.

5. What other types of experiments could you use to determine if you have true habituation learning or sensory/motor fatigue?

In addition to dishabituation, other types of motor/sensory controls can be used. For example, training the worms with longer inter-trial intervals (60sec vs 10sec) has been used as a test for fatigue. Worms are trained with either 10 or 60 sec between trials. Animals with 10sec between trials habituate faster than 60 sec, but the rate of spontaneous recovery (return to pre-habituated levels) is also greater for short (10sec) interval. This rapid decrease in responding but faster recovery is opposite from what would be predicted by fatigue.

Experiment 3: Linking genetics and behavior.

In this experiment you will test a strain of worm with a single gene **mutation-** a change in the DNA sequence of one specific gene. Scientists often use mutations to try to understand a gene's function by observing changes in worm behavior and appearance when the gene is absent or non-functional. Some mutations may cause obvious physical changes while others may produce more subtle effects on behavior. You will be given one of four different genetic mutants to test for deficits or enhancements in habituation. In order to further examine your mutant, you look up the mutated gene on WormBase, an online database that contains the sequence for the entire *C. elegans* genome and the locations of all its predicted genes. You will also compare your gene to its human **homolog**- genes with conserved sequences across species and with potentially similar functions. Each one of the mutants tested in this class is either a loss-of-function or a deletion mutation in one of three different genes involved in glutamate signaling. Glutamate is an important neurotransmitter that is critical for learning and memory (see Lab 8 for more details on glutamate's role in long term potentiation). Your TA will give you the name of your worm's mutant gene that you can use to look up the protein your mutant gene encodes, its potential function in glutamate signaling, and its human homolog.

Procedure:

Examining your mutant worm

1. Obtain your mutant worms from your TA or committee member.
2. Observe your mutant worms under the dissecting scope. Note any differences between your mutant's behavior and the behavior of the wild type worms you observed in Experiment 1. Pay particular attention to any differences in the mutant's movements.

Observations:

3. Given your observations of your mutant's behavior and the results of your habituation training and testing in the wild type *C. elegans*, develop a hypothesis about what type of gene/protein mutation your worm might have.

Hypothesis:

4. Repeat the habituation training protocol used in Experiment 2 to test your mutant's response to the tap stimuli, habituation learning, habituation memory, and dishabituation performance. Record your results in the second score sheet and construct a histogram similar to that in Experiment 2.
5. Use Wormbase to find basic information about your gene. Go to http://www.wormbase.org and enter your gene name in the text box "search…" in the upper right hand corner. Click on the search (magnifying glass) button. Enter the strain you are interested in:

6. Examine the "Overview" section to answer the following Thought Questions.

Thought Questions:

1. What protein does your gene encode?

a. Nmr-1 deletion mutation: human homolog is the NMDA receptor
b. Glr-1 deletion mutation: human homolog is AMPA receptors
c. Eat-4 loss of function mutation: human homolog is the sodium-dependent inorganic phosphate cotransporter that is required for glutamate synthesis

2. What are its known function(s)?

a. Glr-1: glr-1 encodes an AMPA (non-NMDA)-type ionotropic glutamate receptor subunit; GLR-1 activity is required for mediating the behavioral response to light nose touch and the frequency with which animals change locomotory direction in response to sensory cues such as food; GLR-1 and GLR-2, a second AMPA-type ionotropic glutatmate receptor, can interact to form functional heteromeric channels; GLR-1 is expressed in motorneurons and interneurons, including four of the five pairs of command interneurons that are required for locomotory control; in the ventral nerve cord and nerve ring, GLR-1 localizes to perinuclear structures in cell bodies and to punctate structures that appear to be glutamatergic postsynaptic specializations; proper GLR-1 localization in the anterior ventral nerve cord of older larvae and adults requires activity of the class I PDZ protein LIN-10; GLR-1 is ubiquitinated in vivo and its abundance at postsynaptic elements, which may influence postsynaptic strength, is regulated by ubiquitination.
b. Eat-4: eat-4 encodes an ortholog of the mammalian BNPI vesicular glutamate transporter that affects chemotaxis, feeding, foraging and thermotaxis; eat-4 is expressed in specific neurons, including M3L and M3R which are known to be glutamatergic.
c. Nmr-1: nmr-1 encodes an NMDA-type ionotropic glutamate receptor subunit that affects the duration of forward movement which is important during foraging behavior, and also affects osmotic avoidance; the slow kinetics typical of NMDA-dependent currents are likely important for its effect on forward movement.

3. How might your protein's function(s) contribute to your observations? How might it contribute to any changes you observed in habituation?

In general glutamate is a critical component of the neural circuitry underlying memory formation. Eat-4 is required for loading glutamate into vesicles for presynaptic release. Without it, glutamatergic signaling is predicted to be disrupted potentially disrupting both sensory and motor function as well as memory formation. Glr-1 and Nmr-1 are both glutamatergic receptors that are critical for glutamate to activate downstream signaling cascades required for memory formation.

Homologous Proteins

7. Scroll down to the "Sequences" section. In the first column labeled "Transcript" click on the first entry.
8. View the "spliced + UTR" sequence. Select the entire sequence (from beginning of bracket until end of sequence) for your gene and click copy.
9. Open the website for the National Center for Biotechnology Information (NCBI) at http://www.ncbi.nlm.nih.gov/
10. Click on BLAST under "Popular Resources" on the right side of the page.
11.Under Basic BLAST click on "blastx" (Search protein database using a translated nucleotide query).
12. Paste your gene's DNA sequence from step 8 into the *Search* window.
13. Delete any non-nucleotide characters from the window.
14. In the Organism optional section type in "Homo sapiens (taxid:9606)" in the window.
15. Click on BLAST to send the query sequence to the NCBI database where the BLAST algorithm will attempt to find a match between your DNA sequence and a gene(s) in the human genome. This may take a few seconds or minutes.
16. Once the results window opens scroll down to Descriptions to see genes with homologous sequences to your mutant gene. The Query Coverage measure gives an approximate percent of homology.

In the Accession column, click on the link. Under the Summary section, is the information on that homolog's function(s). Use this information to answer the following Thought Questions:.

Thought Questions

1. What type of gene (if any) is the human homolog to your *C. elegans* mutant gene?

Glr-1 = AMPA receptors; Nmr-1 = NMDA receptors; Eat-4 = BNPI vesicular glutamate transporter

2. Do the *C. elegans* and the human homolog encode proteins with similar types of functions?

Yes, Glr-1 and Nmr-1 function similarly to AMPA and NMDA receptors respectively. The Eat-4 protein functions as a critical component of the machinery responsible for loading glutamate into presynaptic vesicles.

3. Given the behavior of your mutant worm, do you think the homologous human gene would be important for learning and memory in humans? Why?

Yes, all three mutants are predicted to have deficits in learning/memory and/or performance and the homologous genes in humans perform similar functions. Consequently, it is strongly predicted that all three of these genes will be important in human learning and memory.

4. What are some possible limitations of linking a single gene mutation to a behavior?

The function of any given protein is always a product of what other proteins are available to interact with it (the local environment). Consequently, manipulating a single protein can have dramatic effects on other proteins, which may or not result in a measurable behavioral change. For example, the loss of one protein product may be compensated for by a second protein and produce no measurable behavioral change even though it is involved in the behavior. Or under other conditions, the loss of one protein may further disrupt normal signaling of multiple processes producing a severe behavioral deficit. Removing a single piece of a very complex cascade of events may or may not disrupt behavior, but that doesn't mean that that protein isn't involved in memory or other processes. This is a very tricky topic for scientists to deal with.

5. What are some possible limitations of extrapolating a link between a gene and a behavior in animals to the same gene and a behavior in humans?

Even if a gene is homologous across species, that doesn't mean that all of that protein's functions are the same across species. In addition, behavior in animals compared to humans is often very different (think about the way we train and test habituation in worms compared to what you might do in humans). Therefore, scientists must exercise caution when extrapolating from animal work to humans.

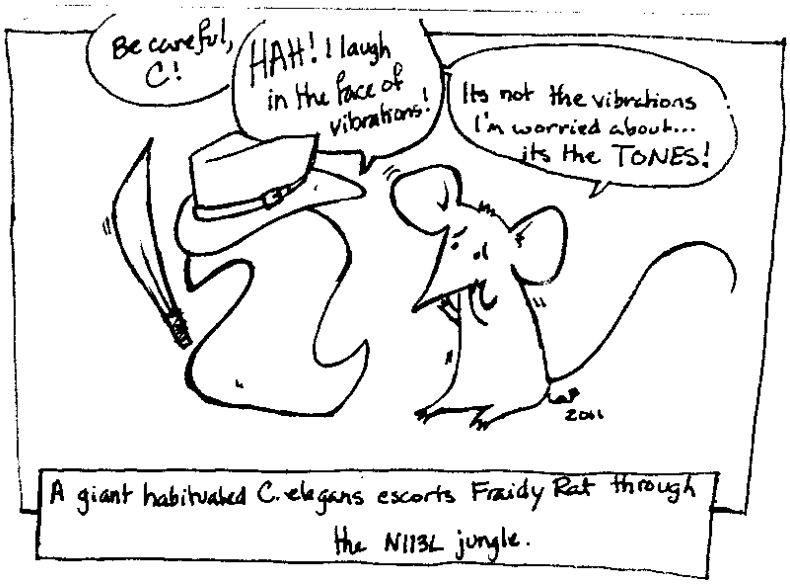


Figure 7.6. Drawing done by Charlotte Anderson, a student in N113L Winter 2011

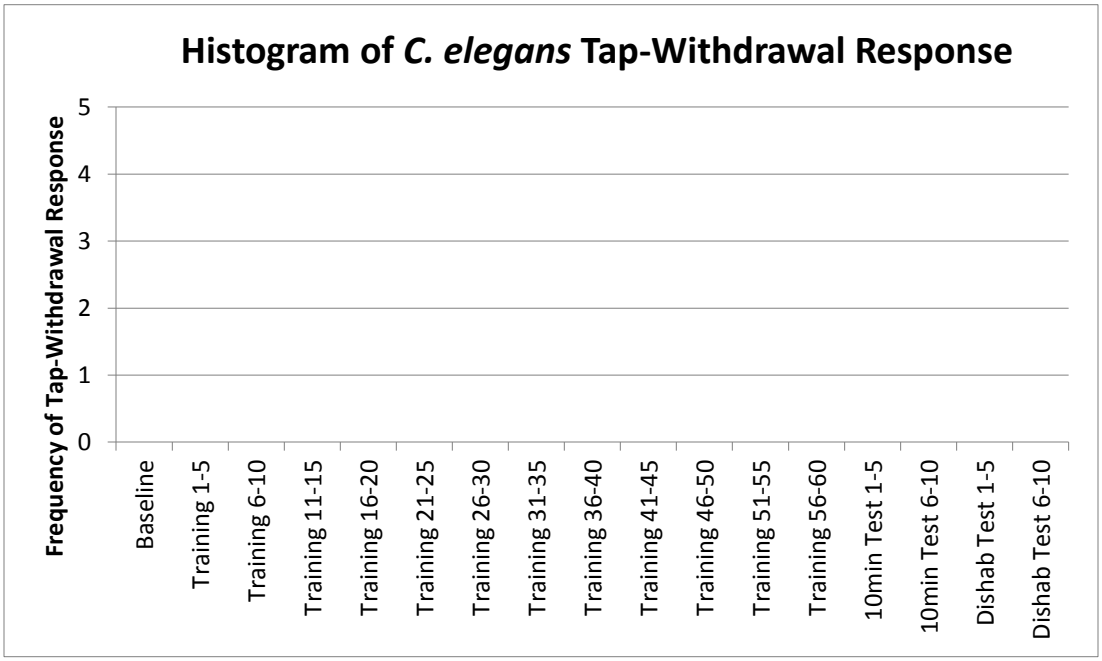
References

1. Vazdarjanova, A., & McGaugh, J.L. (1998). Basolateral amygdala is not critical for cognitive memory of contextual fear conditioning. *PNAS*, 95, 15003-15007.
2. Giles, A.C., & Rankin, C.H. (2009). Behavioral and genetic characterization of habituation using *Caenorhabditis elegans*. *Neurobiology of Learning and Memory*, 29, 139-146.
3. WormAtlas, Altun, Z.F., Herndon, L.A., Crocker, C., Lints, R. and Hall, D.H. (ed.s) 2002-2010. http://www.wormatlas.org.
4. Rankin, C.H., & Broster, B.S. (1992). Factors affecting habituation and recovery from habituation in the nematode *Caenorhabditis elegans. Behavioral Neuroscience*, 106(2), 239-249.
5. Portions of Experiment 3 were adapted from H, McGray & S. Groff. (2009). Model organism laboratory handbook. *Caenorhabditis elegans* in the classroom: Using worms to teach biology.

Testing Score Sheet			**5 Trial Counts**	
Baseline Trials	Pre Response	Post Response	Baseline 1-5	Notes
1				
2				
3				
4				
5				
BLOCK 1 (30 training trials)				
Training Trials	Pre Tap Response	Post Tap Response	Trials 1-5	
1				
2				
3				
4				
5				
6			Trials 6-10	
7				
8				
9				
10				
11			Trials 11-15	
12				
13				
14				
15				
16			Trials 16-20	
17				
18				
19				
20				
21			Trials 21-25	
22				
23				
24				
25				
26			Trials 26-30	
27				
28				
29				
30				

10min Break				
BLOCK 2 (30 training trials)				
Training Trials	Pre Tap Response	Post Tap Response	Trials 31-35	
31				
32				
33				
34				
35				
36			Trials 36-40	
37				
38				
39				
40				
41			Trials 41-45	
42				
43				
44				
45				
46			Trials 46-50	
47				
48				
49				
50				
51			Trials 51-55	
52				
53				
54				
55				
56			Trials 56-60	
57				
58				
59				
60				
10min Break				
Testing Trials	Pre Tap Response	Post Tap Response	10min Test Trials 1-5	
1				
2				
3				
4				
5				
6			10min Test Trials 6-10	
7				
8				
9				
10				

Dishabituate!!!!				
Testing Trials	Pre Tap Response	Post Tap Response	Dishab Test Trials 1-5	
1				
2				
3				
4				
5				
6			Dishab Test Trials 6-10	
7				
8				
9				
10				



Lab 7 Writing Assignment: Laboratory Report

Your page limit is 6 pages of text (not including abstract, figures, legends or references), double-spaced in Times New Roman 12 pt font with 1" margins. ***I will not read or grade beyond 6 pages of text.***

Turnitin.com AND hard copy Due Date: _____

Hard copy turn-in location: _____

Compare course-wide data for the wild-type and _____ mutant only

Big Question: What role does the _____ receptor and its mutation play in non-associative learning?

The document should be separated into the following 7 Sections:

1) **Cover page**: Include your title, student ID#, due date, course name and number, TA and section. Each page thereafter should have your ID # and page # in the top right hand corner (heading).

2) **Abstract (max 250 words; on its own page):** Follow the format in the guidelines I handed out for your first 2 abstracts (still posted on class website).

3) **Introduction:** For this lab report, make sure you discuss the following (also feel free to include any other material you think is relevant). These points should be discussed in reference to both the material in your lab manual and at least TWO outside literature sources pertaining to the topic. However, I recommend using more as they may help to clarify certain topics:
   - Discuss the importance of non-associative learning. This can be on a larger scale- like the need for this learning for an organism's survival- and also on a small scale- like daily activities. Make sure to include a definition and description of non-associative learning.
   - How are we trying to measure memory in this experiment? Discuss the training protocols commonly used in the field.
   - Discuss *C. Elegans*, why they are good/bad organism to study learning and memory. What type of behavior are we measuring from them, and how does this correlate to memory? Might there be other reasons besides learning and memory for their behavior?
   - Discuss the limitations of trying to measure memory and the memory versus performance distinction.
   - Discuss how genetics can be used to help us understand more about the functions of different proteins in learning and memory.
   - End the introduction with your HYPOTHESIS.

4) **Materials and Methods:** Do not copy verbatim from the lab manual. Write in past tense the general set-up of equipment, animals etc. Then describe each experiment including the timing of the stimulus and what types of measurements were recorded. It may help to include a figure to describe the experimental paradigms.

5) **Results:** Report your results in the order of the experiments. Always start the paragraph for each experiment by telling what you were investigating, then go on to tell what you found. Include any formulas you used.

   If you state that any relationship was **significantly different** (i.e., $p<0.05$ as discussed in Lab 5), you must cite the P value in parentheses.

   Organize your data from the experiments into tables and figures that can be referenced here as "Table 1" or "Figure 4". These tables and figures need to be cited throughout the Results text as you refer to them.

   Include graphs/histograms that indicate the **means** and **standard error of the mean** for the reversal data from each experiment. Your table and figures belong in the Results Section with text as you would see in a journal article.

Analyze (statistics) and graph WT and _____ mutant data.
**** refer to Lab 5 Statistical Analysis (part 1-3, pages 5.8 – 5.9) for calculations****
All t-tests should be "paired" instead of "unpaired" as in Lab 5. This is because the same worms are re-tested throughout the experiment.
=TTEST(means for group A, means for group B, 2, 1)

Savings: A significant decrease of the average number of reversals between the first training set of each block.
Training Trials 1-5 vs. Training Trials 31-35 (t-test)
Compare the results to determine if the concept of savings was demonstrated during the second block of training.
Learning: Training Trails 1-5 vs. Training trials 56-60 (t-test)
Perform t-test to determine if learning occurred between the first and last blocks of training trials.
Dishabituation: Testing Trials 6-10 vs. Dishabituation Trials 1-5 (t-test)
Perform t-test to determine if the worm dishabituated following vortexing.

How to make graphs in Excel: http://www.youtube.com/watch?v=jLW1A7j7r3Y
How to make error bars on graphs in Excel: http://www.youtube.com/watch?v=RERSq5WbmRI

6) **Discussion:** Now you can begin to explain your results. Begin by restating your original hypothesis, and then discuss in detail each experiment and what your results mean. Talk about any **trends you observed**, **anomalous results** (and possible explanations as to why), **how your findings relate to other studies in the literature**, **whether the *C. Elegans* were a good model for studying non-associative memory**, and how mutations can be used to link genetics to behavior. Make sure to address any **performance problems**, in addition to memory deficits, that you observed in your mutants. You should also discuss how your experiments addressed the memory versus performance distinction. Also include **potential future studies** to further investigate your hypothesis/anomalous results, etc. Be sure to reference other literature here!

Do not reference tables or figures in this section.

7) **Literature Cited (max 1 page)**
- Cite at least 4 relevant sources.
  - **1 source** can be the lab manual, **2 sources** must be relevant journal articles that are NOT listed at the end of the lab. Find different sources to cite on Pubmed or Google Scholar.
  - You must cite articles referring to the _____ **mutants**!
  - **Use Pechenik Citation Style**:

    http://www.augustana.ualberta.ca/files/group/464/Pechenik%20Citation%20Style%20Quickguide.pdf

- The first page of each of your 4 sources must be printed and turned in with your hard copy!

Reminders:
1. Do not make unqualified statements- if you make a statement, make sure you measured it directly, or you back it up with a reference. All the inferences you make from the results belong in the discussion and should be presented as inference only.
2. Along those same lines, we are only providing data that SUPPORT/SUGGEST/INDICATE our hypothesis, but in science we rarely ever PROVE our hypothesis.
3. If you use a pronoun such as "this", "it", "they" etc, be sure that I can tell what the pronoun is referring to. If you think it is unclear, replace the pronoun with a qualifying descriptor such as "this increase in compound action potential amplitude indicates…."
4. Use spell check, have a peer read your report for errors.
5. **Be clear and concise. The easier it is for me to understand, the better.**

Laboratory Report Rubric Evaluation

	Points Earned
Title	**2**
Provides a specific indication of what the report is about	2
Abstract (200 words MAX!)	**10**
Background stated in 1 or 2 sentences	2
Clear statement of specific question addressed and/or hypothesis	2
Methods summarized in no more than 2 or 3 sentences	2
Major findings reported in no more than 3 or 4 sentences	2
Concluding sentence relates to statement of specific question addressed	2
Introduction	**30**
Includes relevant background information for an audience of your peers who have take the UCI Bio core	10
Follows a clear, logical progression	3
Contains an explicit statement of purpose or hypothesis	7
Explains the reasoning behind the hypothesis or significance of the purpose based on the published literature	10
Is not a materials and methods section	minus pts
Materials and Methods	**15**
Written in a descriptive style (not cookbook) in past tense	minus pts
Contains sufficient detail for the audience to validate the experiments	9
Includes brief description of how data were analyzed (equations, calculations, etc)	6
Results	**30**
Clearly states the purpose of each experiment	5
Clearly states the results and conclusions of each experiment	5
Describes important trends or patterns in the data	5
Addresses the hypothesis in relation to the results	2
Includes no in-depth analysis	minus pts
All general statements supported with data &/or stats	minus pts
Each figure and table makes an important and unique contribution	9
Figures and tables have titles and legends that are informative and complete (placed below each figure/table)	2
Figures and tables are presented to best allow reader to draw conclusions	minus pts
Numbers of individuals and numbers or replicates are clearly indicated (if applicable)	2

Discussion	**35**
Briefly restates the results within the context of the study	5
States whether the data support the hypothesis	5
If present, addresses unexpected or anomalous results with specific ideas (not speculation)	10
Compares results to class data and provides specific explanations for similarities or differences (if applicable)	
Proposes future directions that are relevant (related to the data) and specific (briefly describes the experiment)	10
Addresses the overall purpose of the report discussed in the introduction	5
Literature Cited	**14**
References are relevant and strengthen the ideas and arguments made in the report	5
References are listed in alphabetical order by the first author's last name	minus pts
References are formatted as described in your lab manual or class website (Pechenik, 2007)	minus pts
References are cited as directed by the lab manual or class website	2
All statements of fact or opinion (not already know by your audience) are supported with a citation/reference	5
Minimum 4 relevant primary research articles cited in both the introduction and discussion	minus pts
Paraphrases material from references (no quotes)	minus pts
The first page of each cited reference is included with the final report	2
Holistic	**14**
Report contains little to no inaccurate statements	2
All information is presented in the appropriate section of the report	5
Language is precise and possesses a scientific tone	minus pts
Writing is relatively free of grammar errors	2
Facts are carefully distinguished from speculation	5
Contains appropriate transitions between sections of the report and topics within each section	minus pts
Uses present and past tenses appropriately	minus pts
Extra Credit	2
TOTAL	**150**

Common Lab Report Grading Rubric

	Excellent	Average	Inadequate	No Effort
Title	Provides a specific, clear and concise indication of the report's contents.	Lacking one element from the excellent category.	A vague or irrelevant title from which the reader is unable to determine the contents of the report.	No title
Abstract	Demonstrates a command of the major concepts discussed in the report. Must clearly state the addressed question and hypothesis.	Clearly states the hypothesis and major findings but lacks information regarding the experimental background or methods.	Does not clearly state the hypothesis and major findings. Exhibits a rudimentary understanding of the background.	Contains unrelated material or no abstract is written.
Introduction	The purpose or hypothesis is explicitly stated. Includes relevant background information that shows depth and complexity supported by relevant references.	Similar content as the excellent category but lacks the complexity of thought. Statements are often vague and lack specifics.	Contains a weakly written purpose or hypothesis but no clear or logical progression of thought. The reasoning behind the purpose or hypothesis is little to nonexistent.	No obvious purpose given. Composed mostly of generic statements that do not touch on the experimental specifics.
Methods	Methods are consistent with the hypothesis or purpose of the experiment. Contains sufficient detail that can be used to validate the experiments and analyze the data.	Methods are consistent with the hypothesis or purpose of the experiment. Contains descriptions of the experimental procedure and data analysis conducted but is too vague for experiment validation.	Written in a "cook book" style narrative. Methods may not be consistent with the hypothesis or purpose of the experiment. May lack detail or proper instruction on how experiments are conducted or how data is analyzed.	Methods are essentially a copy of the protocol in the lab manual. No thought is given to the purpose or hypothesis.
Results	The narrative includes the purpose, results and conclusion of each experiment and all statements are supported by the data. All tables or figures are properly labeled and include a title and legend. Data is presented in the context of the main purpose or hypothesis of the report.	The narrative includes the purpose, results and conclusion of each experiment and is supported by the data, but no clear relationship between the data and the hypothesis is given. Figures are properly labeled but figure titles and legends are lacking.	Minimal narrative. Figures are present but the data is inadequately organized and the figure legends are lacking or non-existent.	No narrative, no figure legends, and incorrectly labeled figures.
Discussion	States the results within the context of the study, and describes in detail whether the data support the hypothesis. If present, unexpected results are explained with specific statements	Contains elements of the excellent category but fails to include sufficient statements that are relevant to the published literature for unexpected results or future directions.	Explanations for unexpected results and future directions are general and lack complex thought. Discussion is filled with pure speculation rather than concrete ideas supported	No attempt to address inaccuracies in a specific manner. Future directions beyond a version of "repeat the experiment" are

	that are supported by appropriate references. Future directions are specific and relevant to the results.		by the literature.	not included.
Literature Cited	Minimum number of relevant references are included which strengthen the arguments made in the report.	Minimum number of references are included but some are not relevant.	Minimum number of references are included but some are not relevant. References are not cited with the proper format.	Minimum number of references are not included.
Holistic		Contains all points addressed on rubric.	Lacking a few of the points addressed.	Lacking many of the points addressed.

Lab 7 – TA Information:

C. Elegans' Worm Preparation

Making Colony Plates (Large Plates)

- Use "expanded" colony plates (small plates that were shipped).
- Colony plates need to sit at room temperature for approximately 10 minutes prior to plating. Colony plates can be found in the refrigerator.
- To plate worms, do the chunking method and leave the piece of agar that was cut on top of the new agar plate, top (worm side) down. Cover the plates and seal using parafilm.

Label the mutant strain accordingly:

1. Glr-1 (KP4) → **LABEL "A"**
2. Eat-4 (MT6308) → **LABEL "B"**
3. NMR-1 (VM487) → **LABEL "C"**

Plating Days: (Colony plates are made 4 days prior to the lab section day)

Please have the small test plates out prior to each lab section.

TA Responsibilities:

1. **Plate Basics:** Large plates (colony plates) and Small plates (experimental plates)
   - Worms are kept in bins on the back lab bench. Each bin is labeled according to the day of the section. All sections falling on that day will use the same bin.
   - The large plates (referred to as colony plates) have 2 purposes: The first is for Experiment 1 of the exercises where students will view the different life cycles of the WT strain. The second is that some of those plates are allotted for you to use for plating onto the smaller, experimental plates.
   - The small plates are those that can fit snuggly into the tapping apparatus. If they are not already set out, they are found in the refrigerator. They need to sit for at least 10 minutes in room temperature before being used for plating. You will plate worms using the colony plates (large plates).
   - If you need additional plates, look in the bins that were used the day before your section (i.e., on Weds, look into the Tues bin).

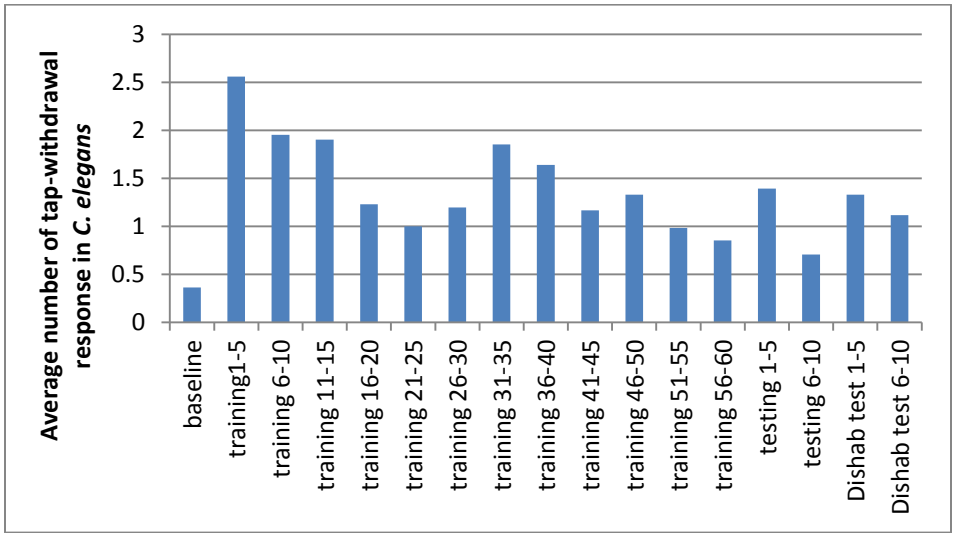
2. **Plating using the "Chunking Method"**
   - Wipe down microscope with ethanol prior to use
   - Spray gloves (hands) with ethanol. Do this each time you use a different strain of C. *elegans*
   - Clean spatula with alcohol pad and allow to dry (~1 minute). Clean spatula each time you use a different strain of *C. elegans*
   - Look through the dissecting microscope and find an area where you see a lot of ACTIVE worms. A good strategy for beginners is to plate more worms onto the plate to ensure students have worms to visualize!
   - Cut a small square "chunk" (approx. ½ x ½ centimeter) and place it face down onto the testing plate
   - Gently dab the "chunk" lightly on the agar, careful not to gauge the agar.
   - Remove the "chunk" and seal the test plate with parafilm.
   - Wipe down microscope and ethanol after use

3. **When do I plate my WT Strain?**
    1. You can plate during Experiment 1 while students are looking at the different stages of worms.
        - A good thing to do is make sure you have all your materials ready. This can be done before class.
            - Pre-cut Parafilm into strips
            - Label test plates (i.e., WT, Mutant A, B, C)
    2. If you are not confident in your plating, you can plate right before your lecture. Just make sure you time your lecture and do not go over 15 minutes!! (Students still need to do EXP 1)
    3. Just a note that the smaller plates do not have any food! This is why the worms are more active once you plate them. They're moving around looking for some grub! After 20 minutes, the worms travel to the end of the dish and crawl under the agar. With this, consider your lecture time and the amount of time you want students to spend on EXP 1.

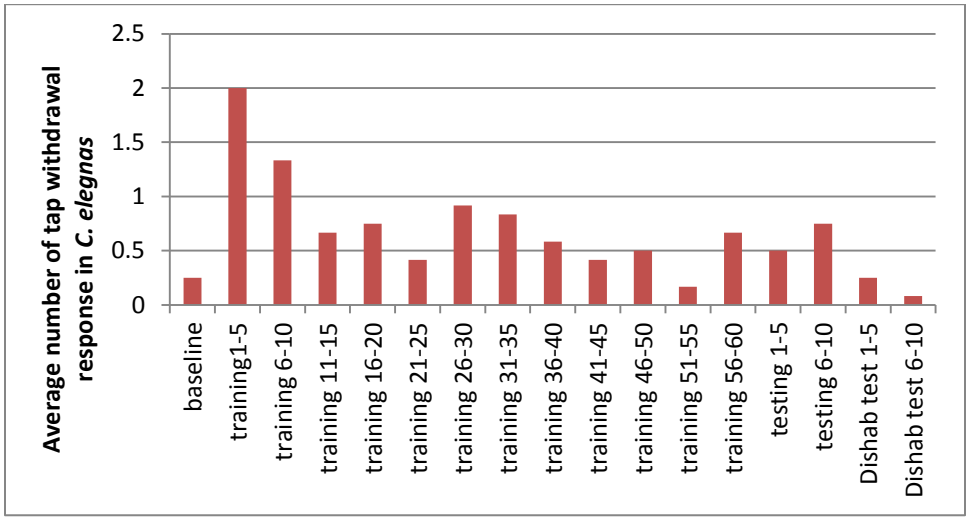
4. **When do I plate my Mutant Strains?**
    - You can start plating during the 2nd break of their WT experiment
    - When labeling the mutant plates, use A, B, and C, and keep note of which mutant corresponds to what letter. Afterward, you can tell students what strain they have when they are looking up the genotype. That way they can do a "blind" experiment when they conduct the experiment on their mutant strain. **See table for how many groups are assigned to each mutant
        - Glur-1 (KP4) → **LABEL "A"**
        - Eat-4 (MT6308) → **LABEL "B"**
        - NMR-1 (VM487) →**LABEL "C"**

5. **During Class**
    - Have your students clean their lab bench with ethanol BEFORE and AFTER use!
    - For Experiment 1, they just need to see that there are big worms and small ones. They do not need to classify the different worm stages!
    - For Experiment 2 and 3, have students observe an ACTIVE worm. If the worm students are focusing on moves out of their viewing range, have them observe another worm. All worms on the plate are receiving the same tapping stimulus.
    - Make sure to add your students' data on the Goggle document.
        - For the Google document, on the bottom left corner, there are different tabs for each section. Select your name.
        - Fill in the answers for your section.
    - At the end of the day, return the colony plates back to the bins. Throw away the testing plates into the biohazard trash bin (gray lidded trash bin)—no gloves allowed in that bin! At the end of the week, we can throw everything out.

Sample Data:



Graph 1. Wild type C. elegans average tap withdrawal response. Graphic representation of average number of tap-withdrawal responses in training, testing and dishabituation trials for wild type *C. elegans*. N=61



Graph 2. NMR-1 mutant C. elegans average tap-withdrawal response. Graphic representation of average number of tap-withdrawal responses in training, testing and dishabituation trials for mutant *C. elegans*.N=12

Table 1. Table of P-values calculated using two-tailed paired t-test for trials comparing savings, learning and dishabituation. P- values< .05 were considered significant.

	Wild-type	NMR-1 Mutant
Number of worms	61	12
Savings (training trials 1-5 vs 31-35)	.0038	.0086
Learning (training trials 1-5 vs 56-60)	9.56E-10	.0063
Dishabituation (testing trials 6-10 vs dishabituation trials 1-5)	.0054	.0819

Workshop 8

RABLE 2015

Battle of the Bacteria: Characterizing the Evolutionary Advantage of Stationary Phase Growth

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Abstract:

Providing students with authentic research opportunities has been shown to enhance learning and increase retention in STEM majors. This workshop centers on an investigative microbiology lab module, which focuses on the molecular mechanisms of evolution in *E. coli*, by examining the growth advantage in stationary phase (GASP) phenotype. The GASP phenotype is illustrated by growing cells into long-term stationary phase (LTSP), and competing them against unaged cells in a fresh culture. This module includes learning goals related to improving student understanding of evolution and strengthening practical laboratory skills. In addition, the students generate novel data regarding the effects of different types of media on GASP and the relationship between evolution, genotypic change, and cell stress. Pairs of students are provided with the experimental background, select a specific aspect of the growth medium to modify, and generate a hypothesis regarding how this alteration will impact GASP. Students explore not only the growth competition between aged and fresh cells, but also how the different types of media impact cell stress and mutation frequency. From this module, we have demonstrated that students are able to achieve the established learning goals and have produced data currently being explored by a research lab.

Workshop attendees will be introduced to the GASP phenotype and get hands on experience with the techniques utilized to gather data regarding this phenomenon. We will discuss how to incorporate this module into the lab curriculum at attendees' institutions, potential pitfalls for both students and instructs, and means to assess student learning.

Key words:

E. coli, growth advantage in stationary phase (GASP), evolution, hypothesis construction,

Introduction:

Cells grown into long term stationary phase (LTSP – greater than 10 days) are better adapted to the environment, and thus are able to outcompete un-aged cells. This is the GASP phenomenon. This module is hypothesis driven, and allows the students to alter the culture medium in a manner of their choosing to uncover the variable's impact on the GASP phenotype and its relationship with cell stress and frequency of genome mutation. In doing so, the students are capable of seeing evolution occur within this short module.

While essential to life, student understanding of evolution can be lacking. One common criticism is that species evolution happens on such a large time scale, and that an inability to observe it in real-time makes it somehow less credible. This is clearly not the case with microorganisms though, which reproduce much more rapidly. Bacterial evolution has been demonstrated in many scenarios, one of the most famous being Luria and Delbruck's fluctuation test with bacteriophages. This experiment demonstrated that mutations in a population can occur randomly and in the absence of a specific stress, as bacteriophage resistant cells arose whether or not the phages were present in the culture. Selection of these mutations and evolution of the population can then occur in the presence of the stress.

This module begins with this premise of mutations occurring naturally and then leads to the ability to characterize the surviving population's phenotype. Does the GASP population outcompete fresh cells only in the initial selective environment or does this apply to different types of media? Do we observe genetic or physiological changes in a major pathway responsible for survival in LTSP? Does the cell stress or mutation frequency change under various environments and does this impact the degree of the GASP phenotype?

The goals of this lab module are two-fold. First, students can increase our knowledge concerning the GASP phenotype through novel experiments of their design. Secondly, this exercise will help students achieve the following learning objectives.

1. Describe the basis of evolution/survival of the fittest on a molecular level.
2. Describe the importance of the ingredients in microbial growth media.
3. Design a testable hypothesis.
4. Define and describe the use of a transcriptional reporter gene.
5. Differentiate between bacterial species and bacterial strains.
6. Differentiate between genotypic and phenotypic changes.
7. Use pipettors with accuracy/confidence.
8. Perform dilution calculations.

The module requires four weeks of the curriculum, including time for students to select a variable and develop a testable hypothesis and perform four different protocols to study various aspects of the phenotype.

Based on a number of measures of assessment, students performing the lab module were able to achieve both goals. The class-generated experimental data agrees with published literature and contributes novel information, and the students attained the established learning outcomes.

Questions, comments, want to do this experiment in your class? – Feel free to contact us!

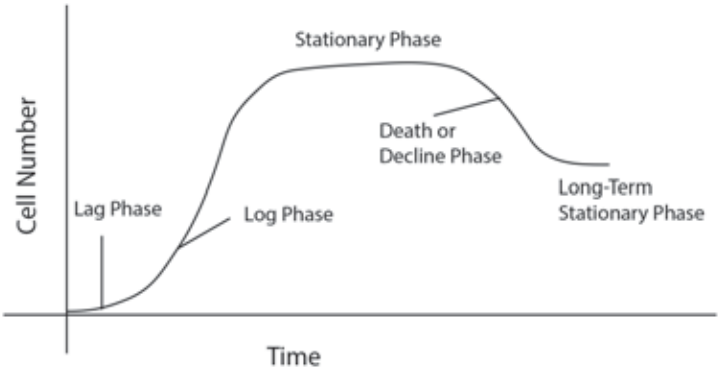
Selected Student Handouts for Module

Evolution of populations occurs in two steps. First, organisms within those populations gain mutations as part of normal biological processes. Second, conditions in the environment allow a few organisms with beneficial mutations to survive longer than their non-mutated counterparts. Those organisms with beneficial mutations are more fit, and will be selected for over time.

Experiments conducted by scientists have shown that mutations arise in a population regardless of the environment. This is because there is a constant mutation rate simply due to mistakes made in DNA replication and repair. One example is the famous fluctuation test done by Luria and Delbruck (1943). The scientists demonstrated that mutations, which confer resistance to bacteriophage attack, occur randomly during growth of bacterial cultures, even when the bacteriophages are not present. This shows that mutations occur during growth with or without the selective environment. If, however, we place the bacteria into selective conditions, we can show that those mutations can be selected out of the population, which leads to evolution. These selective environments generate a number of interesting questions regarding the surviving mutant population. Are the mutations beneficial in other environments, what characteristics of the environment enhance this phenotype, etc?

In this experiment, we will be growing bacteria in a selective condition, and showing that selection causes the bacteria to be better adapted to their environment after merely a few days of exposure. The selective environment will be long-term stationary phase (LTSP), during which populations of bacteria must survive harsh conditions including low nutrients, basic pH, and high waste product levels.

Bacteria experience five phases of growth during their life cycle: lag phase, exponential phase, stationary phase, death phase, and LTSP.



Most often when we grow bacteria in the lab, we examine them during log phase or early stationary phase growth. However, in the natural environment, and in certain laboratory conditions, bacteria more often experience LTSP. Some strains of *Escherichia coli* can survive in LTSP without the addition of nutrients for years.

We can take bacterial populations that have been grown for over 10 days into LTSP, and compete them with un-aged bacteria in fresh media. The aged bacteria will be better adapted to the environment, and will therefore grow faster than the un-aged cells. We call this phenomenon growth advantage in stationary phase (GASP). Further, we will age our cells in a slightly different environment, which will change the conditions to which it will adapt, and determine whether or not those adaptations also help them in the original environment. Some conditions may select mutations that give the bacteria an advantage in a wide variety of environments, while other

conditions may select for mutations that only give an advantage to the bacteria in that specific environment. Using data from this experiment, we can determine what factors influence adaptation.

We will also characterize the GASP phenotype displayed by your cells. Are cells exhibiting the GASP phenotype surviving due to physiological adaptations or genetic mutations? Does increased environmental stress assist or hinder the growth advantage? Do GASP populations also experience a higher mutation frequency? With these experiments, we will illustrate that our aged strain is genetically different strain from the strain that began the experiment. We will see evolution occurring in real-time! Keep in mind that this is also novel work you will be conducting in collaboration with Dr. Steven Finkel's lab at USC. You have a shot of identifying something that nobody has ever seen!

Below is an outline of each experiment, and the specific questions that experiment will be answering. In addition, you will be examining how cells grow in LTSP when the media is altered. You and your group can choose one of the below variations of LB:

LB + 0.5% NaCl
LB + 50mM CaCl or MgCl
LB + 0.5% tryptone
LB + 0.5% yeast extract
LB + 0.2% glucose or fructose or sucrose

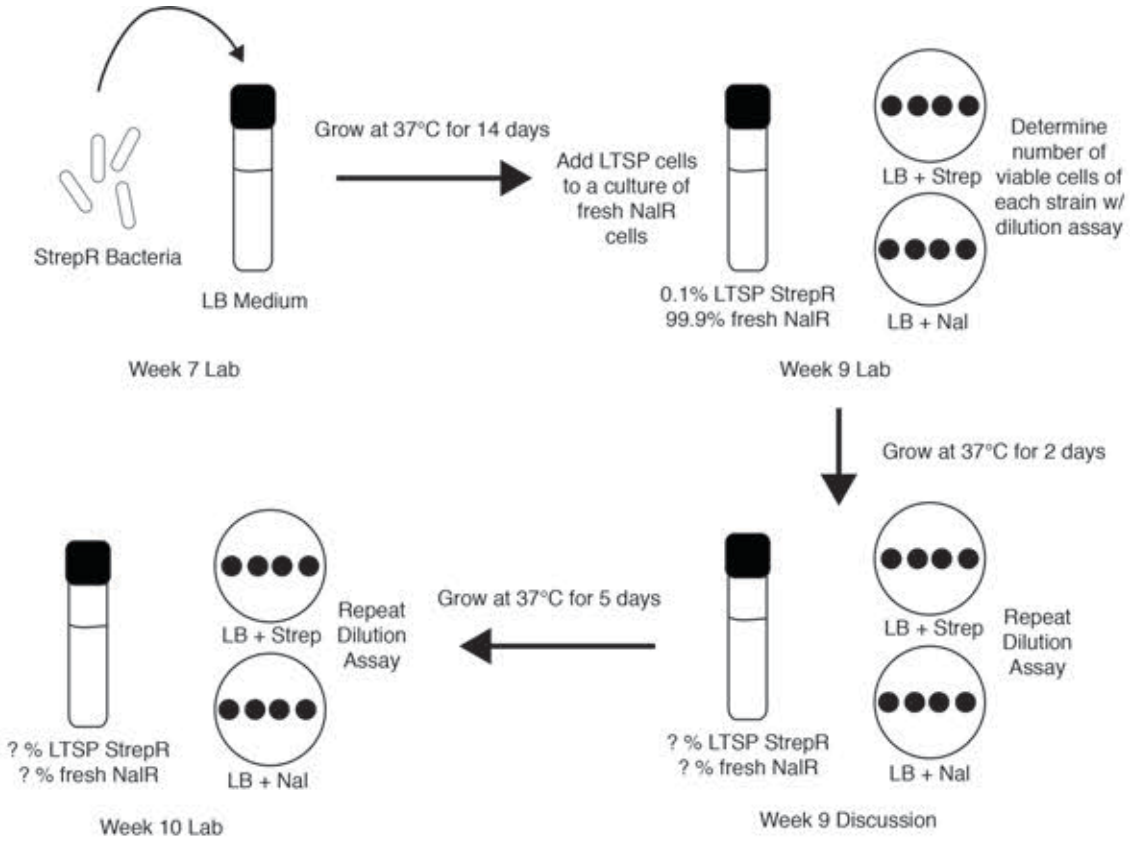
Assay I: Bacteria Growth Advantage in Stationary Phase (GASP) Assay

We need to select for cells that exhibit the GASP phenotype, by first growing a culture of *E. coli* into long-term stationary phase and then adding the survivors to a fresh bacteria culture. We will then compare survival of the aged versus the un-aged cells using different antibiotic resistance markers to distinguish each (see diagram below).

In addition, you will be modifying the culture media in a specific manner (by adding Variable X) to determine how it impacts the GASP phenotype. For this aspect of the experiment, we will need to make slight modifications to the general experimental protocol outlined below.

Experimental Questions:

1. Does growth into long-term stationary phase select for certain mutations (existing or spontaneous) producing a culture with a growth advantage?
2. Does the addition of Variable X to the LB alter the growth advantage for long-term stationary phase cells?
3. Is the growth advantage observed in LB + Variable X specific for growth in LB + X or evident in regular LB as well?



Rough Protocol

Start cultures for LTSP growth (Week 1)

Materials: 1 test tube1, StrepR *E. coli* culture, LB medium

1. Set up 1 tube.
2. Add 5ml of LB and then 5μl of the overnight culture (the StrepR *E. coli*).
3. Place in your section's rack for incubation at 37°C.

Start GASP assay (Week 3)

Materials: 1 test tube, aged StrepR *E. coli* cultures, fresh NalR *E. coli* cultures, 2 LB + Strep plates, 2 LB + Nal plates

You will now compete the StrepR *E. coli* against another strain of *E. coli*, which is resistant to nalidixic acid, but is otherwise identical to the strains you aged. To see whether the LTSP culture possesses the GASP phenotype, you will survey the mixed culture today, in discussion and next week in lab.

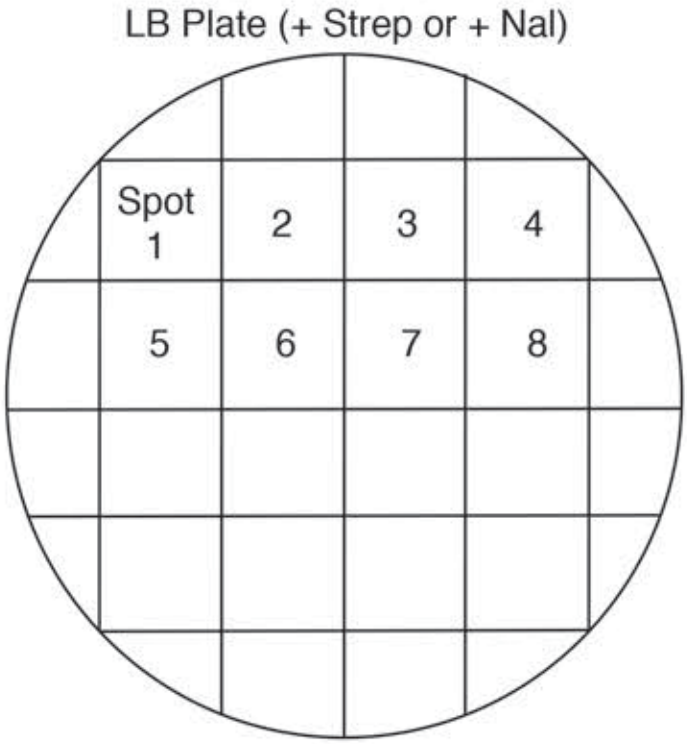
1. Set up 1 tube. Add 5ml of the NalR overnight culture.
2. Shake the tube to mix.
3. Now you need to add the aged StrepR bacteria. You will add only 5μl of the LTSP culture.
4. Place in your section's rack for incubation at 37°C.

Remember, the goal is to monitor how the LTSP StrepR bacteria grow compared to the fresh NalR bacteria. This will be determined by looking at growth on an LB + streptomycin plate (where only the StrepR bacteria will survive) versus growth on an LB + nalidixic acid plate.

Each 1:10 dilution will involve adding 90μl media and 10μl cells. These dilutions will be performed using an empty petri dish (either the lid or the dish), where you add eight spots of 90μl LB. You will then transfer 10μl of your culture to the first spot, pipette up and down a few times and mix the drop with the pipette tip. The next spot will be made with 10μl of the first spot and 90μl LB, mixing by pipetting up and down and using the pipette tip. This will be repeated for all eight spots. Your TA will demonstrate this procedure for you.

1. Mix the tubes thoroughly before removing any liquid. You should plate one strain in its entirety before moving on to the next.
2. In an empty petri dish, you will make eight spots with 90μl each. Do not let the drops merge.
3. Take 10μl of your culture and add it into the first drop. Pipette up and down and stir the drop with the pipette tip to mix the dilution.
4. Using the same tip, pipette 10μl of the first drop onto your LB + Strep plate. You will use the grid below to keep track of your spots. Then pipette 10μl of the same drop onto your LB + Nal plate.
5. Take a new pipette tip and remove 10μl of the first drop and pipette into the second drop. Mix and pipette 10μl onto both plates.
6. Repeat for drops three through eight.

7. Let the plate dry face up. Then flip upside down and incubate at room temperature. If it does not dry in time, leave face up on the room temperature shelf.



Determination of CFU/ml for culture (1 to 2 days later)

You will be analyzing the plates you inoculated in lab (at day 0) and setting up a new set to see the ratio of StrepR and NalR bacteria today.

First, examine the plates from lab. You want to determine the ratio of StrepR CFU/NalR CFU. Find the highest dilution where single colonies are easily distinguished.

LB + Strep Colonies _____ Spot # _____ LB + Nal Colonies: _____ Spot # _____

Now we need to convert this to CFU/ml for each condition/plate.

LB + Strep CFU/ml: _____ LB + Nal CFU/ml: _____

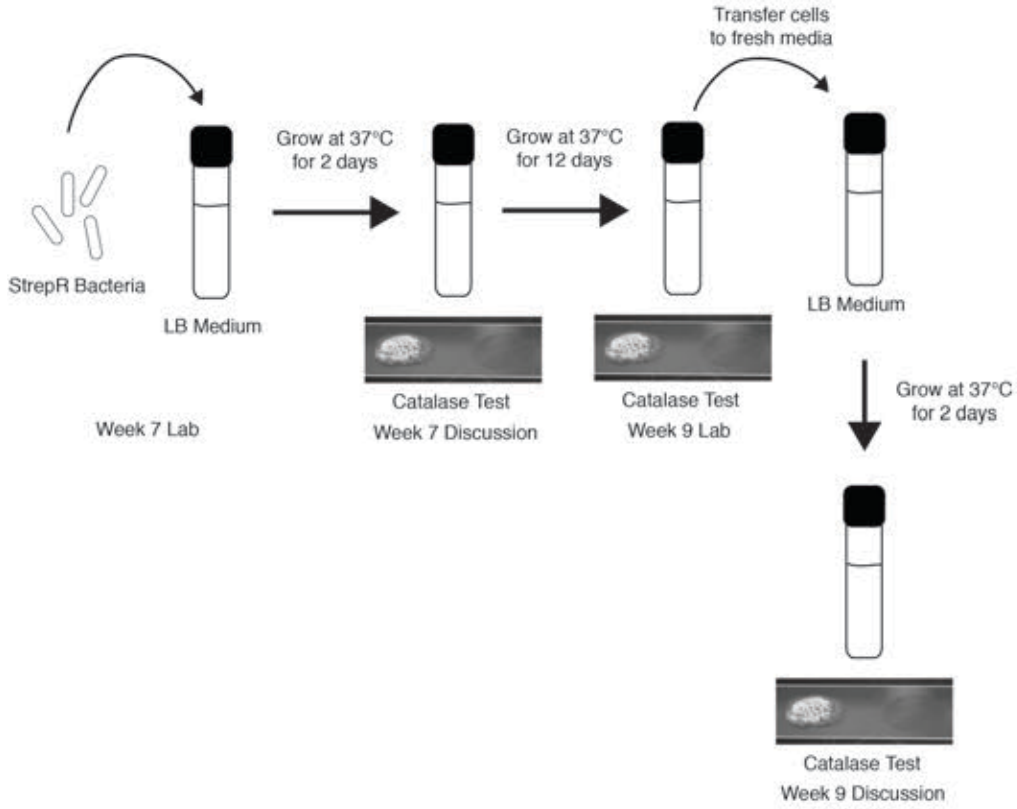
Repeat the week 3 procedure roughly 2 days and 7 days after setting up the mixed culture to determine whether the GASP phenotype is occurring with your LTSP cells.

Assay II: Examination of *rpoS* Activity – Genotypic vs. Phenotypic for LTSP survival

rpoS is a transcription factor that activates genes responsible for fighting cell stress and allowing cells to enter stationary phase. Surprisingly, *rpoS* activity appears to be detrimental to cell survival in long-term stationary phase, and many cells will downregulate *rpoS* in LTSP. In this part of the experiment, you will determine whether your LTSP cells have decreased *rpoS* activity. If so, this may be accomplished through one of two mechanisms, cellular downregulation of *rpoS* (through decreased transcription or increased protein degradation, for example) or through mutation, which results in chronically decreased *rpoS* levels.

One of the targets of *rpoS* is catalase, and you will measure *rpoS* activity by examining hydrogen peroxide breakdown by your cells. By conducting this test at various intervals throughout the experiment, you will answer the following questions:

1. Does *rpoS* activity change in cultures that are grown to LTSP?
2. If *rpoS* is being downregulated in LTSP, is this due to a phenotypic or genotypic modification of *rpoS*?
3. Do the answers to these questions differ for cells grown in LB + Variable X?



There are a few potential results you may see:

1. If catalase expression is similar on day 2, 14 and the following day 2, *rpoS* activity is unaltered by LTSP.
2. If catalase expression decreases on day 14, but is restored when the cells are added back to fresh media, *rpoS* activity is altered by LTSP conditions. This signifies a physiological, but not genomic change.
3. If catalase expression decreases on day 14 and remains decreased in fresh media, this signifies a genomic change to *rpoS*.

Rough protocol

Examine rpoS activity in fresh cultures (week 1, roughly 2 days after starting cultures)

Materials: Bacterial culture, microscope slide, H_2O_2

We want to measure catalase activity (and thus *rpoS* activity) before the cells reach LTSP.

1. Agitate tube if a pellet is at the bottom of the tube.
2. Remove 5μl of your cultures and add them to a glass slide.
3. Add one drop of H_2O_2 to each aliquot.
4. Note whether bubbles form (large/small, many/few, form immediately/after time, etc).

Repeat once cultures in long term stationary phase (roughly 2 weeks after starting culture) and again once the LTSP cells are added to fresh media (roughly 2 days after starting the second set of cultures).

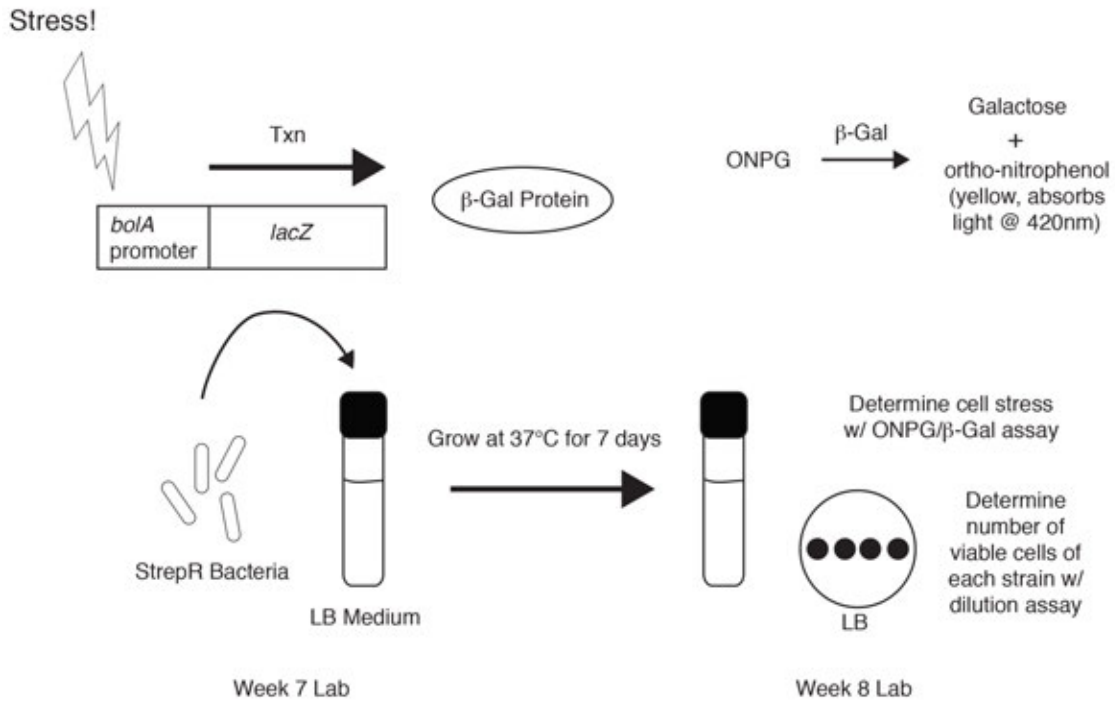
Assay III: Cell Stress Measurement using a β-Galactosidase Assay

Environmental stress can be a major factor in driving the evolution of organisms. Bacteria are equipped with cellular mechanisms to handle a variety of stresses, including elevated temperatures, oxidative stress, and low nutrient environments, among others. But in extreme scenarios, it is likely that the stress will overwhelm these pathways and the cells will be unable to survive. In rare cases, it is also possible that cells contain or acquire beneficial mutations that allow them to survive to a greater extent in these environments compared to the rest of the population.

We will measure cell stress using a *lacZ* fusion protein, driven by the *bolA* promoter. *bolA* is part of the *E. coli* stress response and is upregulated under carbon starvation, increased osmolarity and oxidative stress. When stressed, the cell will transcribe from the *bolA* promoter, turning on the lacZ gene resulting in the production of β-galactosidase protein. We will measure β-galactosidase activity with the addition of its substrate, ortho-nitrophenyl-β-galactoside (ONPG), which is broken down to produce ortho-nitrophenol, a molecule with a yellow color. We can measure the amount of this molecule using the spectrophotometer.

With this reporter protein, we can ask the following questions:

1. Does the amount of stress experienced by the cells correlate with the intensity of the GASP phenotype?
2. Does the addition of Variable X to the media alter cell stress?

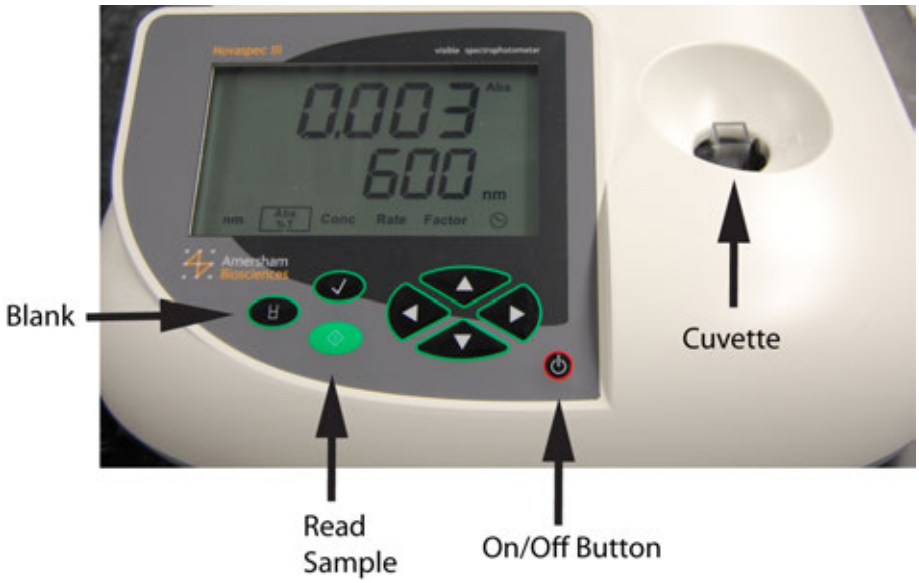


Spectrophotometry allows one to measure the components in a solution. Different molecules are capable of absorption of specific wavelengths of light. This absorption can be measured with a spectrophotometer. A solution is added to a cuvette that is placed into the spectrophotometer. Light of a specific wavelength is directed at the cuvette and may be absorbed by specific molecules. The amount of light, which is not absorbed and passes through the cuvette, is read resulting in the calculation of an absorbance. More of that specific molecule results in more light absorbed and a higher absorbance value, known as the optical density (O.D.).

Since we want to know the light absorbed by a specific molecule (in this case ortho-nitrophenol), we need to eliminate any light absorbed by the buffer. To eliminate the absorbance by any components in the buffer, we first "blank" the spectrophotometer with a cuvette containing only buffer. Thus, when we add the cuvette with ONPG and β-galactosidase, the resulting absorbance will be due only to ortho-nitrophenol.

Below is the protocol for the spectrophotometer based β-galactosidase assay. The purpose of the experiment is to lyse the cells to expose the β-galactosidase protein to the ONPG substrate. In addition, you will need to determine the viable cell count in the population similarly to what you did the past week with the rifampicin assay. This is necessary because higher β-galactosidase activity could merely be due to a greater number of viable cells, and not necessarily greater *bolA* activation in each cell.

Before you begin, familiarize yourself with the spectrophotometer.



1. On the screen, confirm that the wavelength is set at 550nm (not 600nm as above).
2. Next, add your blank cuvette (Z buffer only) to the spectrophotometer and hit "blank".
3. After this is complete, you will add your cuvette containing your β-galactosidase assay.
4. Hit the green read sample button. This will generate an absorbance (OD) value.

Protocol (roughly 2-7 days after starting week 1 culture)

Materials: Z buffer (60mM Na_2HPO_4, 40mM NaH_2PO_4, 10mM KCl, 1mM $MgSO_4$, 50mM BME), phosphate buffer (60mM Na_2HPO_4, 40mM NaH_2PO_4), 0.1% SDS, 4mg/ml ONPG, 1M Na_2CO_3, spectrophotometer

*For the RABLE workshop, you will be performing this protocol twice, once for a culture in LB and once for a culture in LB + NaCl.

1. Remove 0.5ml of your culture to a 1.5ml eppendorf tubes.
2. Centrifuge on full speed for 1 minute to pellet the cells. Pipette off the liquid but do not disturb the pellet. This removes the media, which may contain molecules that impair β-galactosidase activity or light absorbance by the spectrophotometer.
3. Resuspend the pellet in 0.5ml chilled Z buffer.
4. In a new 2.0ml tube, add 900μl Z buffer and 100μl of cells (resuspended in Z buffer).
5. Add 50μl SDS and mix by inverting the tube a few times. SDS is a detergent, which will lyse the cells.
6. Let the tubes sit on your bench for 5 minutes at room temperature.
7. Add 200μl ONPG to the tube and mix by inverting a few times.
8. Incubate the tube in the 28°C water bath until you see color (usually around 15 minutes).
9. Once you see yellow color in both tubes, stop the reaction by adding 0.5ml Na_2CO_3 and mix by inverting a few times. The sodium carbonate raises the pH to 11, which inactivates the β-galactosidase protein.
10. Transfer 1ml of the solution to a new 1.5ml tube and centrifuge for 5 minutes at max speed.
11. Instructions for using the spectrophotometer are on the following page. Blank the spectrophotometer with 1ml Z buffer in a cuvette (at OD_{420}).
12. Remove the supernatant from the centrifuged tubes to cuvettes.

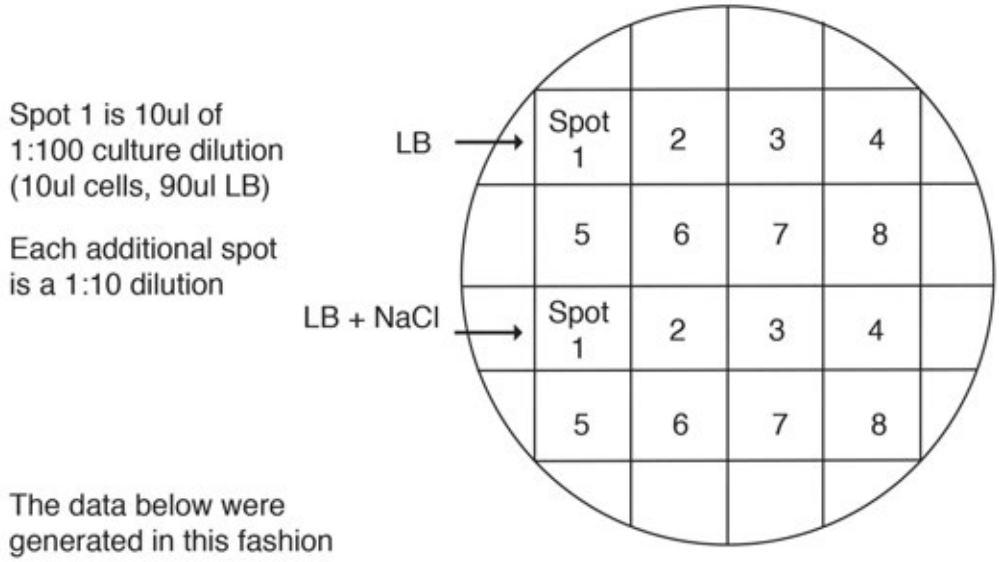
13. Determine the OD of the tubes. LB only OD_{420}: _____ LB + X OD_{420}: _____

Determine CFU count for cells

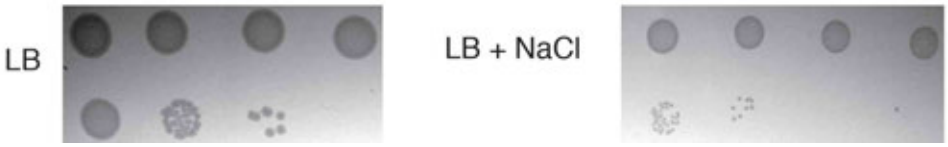
As mentioned above, to accurately measure the β-galactosidase activity, it is necessary to determine the viable cell count in the population. This will be accomplished by performing a dilution assay using LB plates.

This would be performed similarly to the dilution plating described in the GASP assay.

Mock results for this workshop are on the following page:



The data below were generated in this fashion



To determine the CFU/ml for viable cells, you will look at your dilution spots. The more concentrated spots are likely completely covered with growth and it is no longer possible to count individual colonies. Instead, select the spot with individual colonies.

Colonies from LB only: _____ Spot # _____ Colonies from LB + NaCl: _____ Spot # _____

Now you need to determine the CFU/ml using the example from the previous class. Imagine you had 2 colonies on spot 5. The very first spot was created by adding 10μl of cells to 90μl LB and plating 10μl ($1/10^{th}$) of this dilution. Since you plated $1/10^{th}$ of that original 10μl (from the culture), you actually only added 1μl of the original culture. This means the growth in the first spot signifies the CFU/0.001ml or CFU/10^{-3} ml. Spot number two is a 1:10 dilution of the first, so growth in this spot signifies the CFU/10^{-4} ml.

This means the 2 colonies on spot 5 signify a value of 2 x 10⁻⁻/ml or _____CFU/ml.

Now, based on this little tutorial, calculate your CFU/ml for all viable cells using the values you noted from your LB plates.

LB only CFU/ml: _____ LB + X CFU/ml:_____

And finally, you can calculate your cell stress:

$$\frac{OD_{420}}{CFU/ml}$$

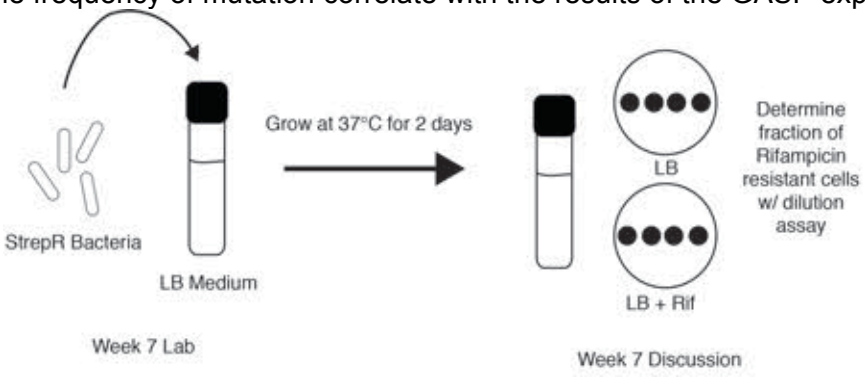
LB only OD_{420}: _____ LB + X OD_{420}: _____ (values from last week)

LB only: _____ LB + X: _____

Assay IV. Examination of Mutation Frequency by Rifampicin Survival

Environmental stresses can promote the formation of mutations, for example through increased DNA alterations caused by base damage or decreased activity of DNA repair pathways. While most are likely detrimental or have no effect on the cell, it is also possible a cell will acquire a beneficial mutation. Normally your cells are sensitive to rifampicin, but it is possible for them to become antibiotic resistant. In this experiment, you will be measuring the mutation frequency in your bacterial culture to answer the following questions:

1. What is the frequency of mutations in the bacteria population?
2. Does this frequency change in LB + Variable X media?
3. Does the frequency of mutation correlate with the results of the GASP experiment?



Normally your bacteria are rifampicin sensitive. It is possible that they acquire a mutation that renders them rifampicin resistant. You will examine this by plating your cultures on an LB + Rif plate. In addition, you will plate them on an LB plate to determine the total viable cells in the population to calculate the percentage of the culture that is rifampicin resistant.

Rough Protocol (roughly 2-7 days after starting week 1 culture)

Materials: 1 LB plate, 1 LB + Rif plate, 1 empty petri dish, liquid LB

To determine the frequency of rifampicin resistant cells in the population, you must identify how many cells are rifampicin resistant (by plating on LB + Rif) and comparing this to how many viable cells there are total (by plating on LB alone). We anticipate the frequency of rifampicin resistant cells to be low, so there will be no need to dilute the culture plated on LB + Rif.

1. Take 1 LB plate and 1 LB + Rif plate. Label the bottom of these as either LB or LB + Rif, along with your identifying information.
2. Prepare your LB plate first as described in the GASP assay dilution plate protocol.
3. Now prepare the LB + Rif plate. Because the number of rifampicin resistant cells will be low, you will not need to do the above procedure. Instead, plate 100μl of your first culture (well-mixed) on an LB + Rif plate.
4. Spread the 100μl using the ethanol spreader (dip in ethanol, briefly flame, let cool by placing on a region on the plate where the cells are not).
5. Repeat this with your second culture using another LB + Rif plate.

Your mutation frequency will be based on counting colony forming units (CFU). CFU estimates the number of viable cells by assuming each viable cell is capable of cell division resulting in the formation of a colony. So CFU is determined by counting colony number on a plate.

The equation for the mutation frequency will be: $\frac{\text{CFU/ml Rif Res Bacteria (on LB + Rif plate)}}{\text{CFU/ml Total Cells (on LB plate)}}$

1. To determine the CFU/ml for Rif resistant bacteria you will count the colonies on the LB + Rif

   plate. Note that number here: _____ (LB only) _____(LB + X media)
   Remember, you plated 100μl of your original cultures onto the LB + Rif plate. So rather than identifying the CFU/ml, your values above represent the CFU/0.1ml. This means you need to multiply the numbers above by a factor of _____.

   Your CFU/ml values for Rif resistance cells are: _____ (LB) _____ (LB + X)

2. To determine the CFU/ml for all viable cells (plated on the LB media), you will look at your dilution spots. This is to be performed as described in the cell stress assay.

Materials

-Micropipettors (P20, P200, P1000) – one set per group of four
-Bench top centrifuge – one per section of 20 students
-Glass tubes (20mm x 150mm)
-Spectrophotometer

E. coli – both strains express the bolA-lacZ fusion protein
-Naladixic acid resistant – SF2054 (W3110 deltalacU169 tna2 Nalr lambda-bolA::lacZ-Kan-r)
-Streptomycin resistant – SF2055 (W3110 deltalacU169 tna2 Strr lambda-bolA::lacZ-Kan-r)
More information can be found in Farrell and Finkel, 2003 .

Lysogeny broth (LB) – for bacteria growth
LB + Drug plates
-Rifampicin - 100μg/ml (100mg/ml Rifampicin stock in DMSO)
-Naladixic acid - 20μg/ml (20mg/ml Naladixic acid stock in water, add NaOH to get in solution)
-Streptomycin - 25μg/ml (25mg/ml Streptomycin stock in water)

Reagents per experiment (per group of 4)
-Experiment is performed in pairs, although 2 pairs (group of 4) will perform the identical experiment for repeatability purposes

25X Variable solutions (others can be used as well) – make in water, sterile filter before use
-12.5% NaCl, 1.25M $CaCl_2$,12.5% tryptone, 12.5% yeast extract, 5% glucose, 5% fructose

Starting cultures to generate LTSP conditions
-4 glass tubes, 25ml LB

GASP Assay
-Variable stock solutions, 10 glass tubes, 25ml LB
-8 LB + Nal plates, 8 LB + Strep plates, and 8 empty petri dishes (4 for day 0, 4 for day 2)
-5ml sterile water

rpoS downregulation (catalase test)
-a few drops of 3% H_2O_2, microscope slides

Mutation frequency (Rifimpicin resistance)
-2 LB plates, 4 LB + Rif plates, 2 empty plastic petri dishes, 5ml sterile water

Cell Stress (β-galactosidase Assay)
-Z buffer (must be made within a few days of use) – 8ml
To make 150ml - 2.4g $Na_2HPO_4•7H_2O$, 0.84g $NaH_2PO_4•H_2O$, 1.5ml 1M KCl, 0.15ml 1M $MgSO_4$, 0.405ml β-mercaptoethanol
adjust pH to 7.0, then bring to 150ml with water, keep at 4°C (12 tubes per class, on ice)
-ONPG solution (must be made within a few days of use) – 4mg/ml in water
-0.1% SDS solution - 300μl
-1M Na_2CO_3 – 2.5ml
-2 LB plates, 2 empty plastic petri dishes
-5ml sterile water

Example Student Data

Comparison of GASP phenotype in LB versus LB + 50mM $CaCl_2$

Figure 1. Competition assay comparing growth of aged versus un-aged cells grown in LB or LB + $CaCl_2$. *E. coli* were aged in either LB or LB + $CaCl_2$ and then diluted 1:1000 in a culture of fresh cells growing in either LB or LB + $CaCl_2$. Aged cells grown in LB media for both conditions outcompeted the fresh cells. Cells aged in LB + $CaCl_2$ were incapable of overtaking the fresh cells in the given time frame.

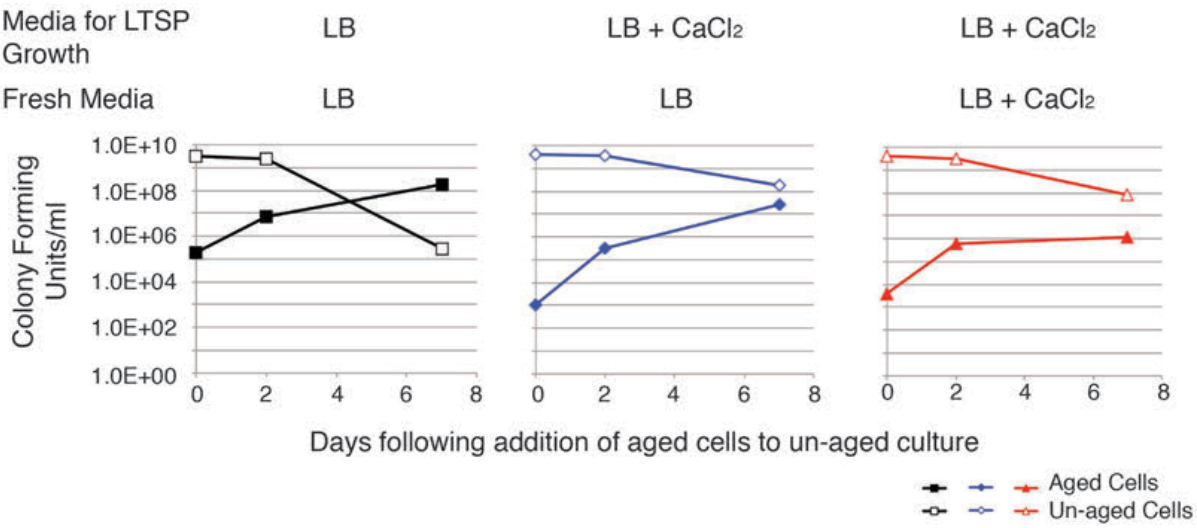


Figure 2. Example plates displaying results from competition assays comparing growth of aged versus un-aged cells grown in LB. Cells were plated at the indicated days following the addition of aged cells to the un-aged cultures on the appropriate media at ten-fold dilutions. Plates were grown at room temperature for 48 hours and photographed.

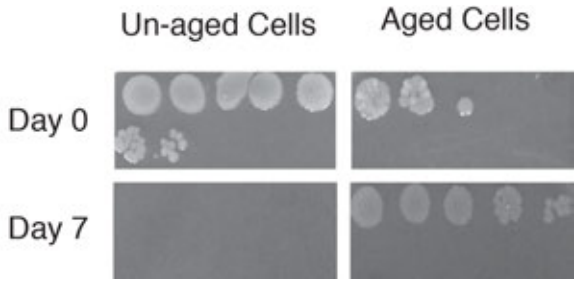


Figure 3. Measuring catalase activity of cells grown in either LB or LB + $CaCl_2$. *E. coli* were aged in either LB or LB + $CaCl_2$ and then diluted 1:1000 in fresh cells growing in either LB or LB + $CaCl_2$ (at day 14). Cells grown in $CaCl_2$ experienced increased catalase activity both early on (day 2) and in LTSP (day 14). Upon re-addition to fresh media, the LTSP catalase phenotype is still present, illustrating that the lower *rpoS* activity is due to a genetic change in the cells.

	Growth in LB	Growth in LB + $CaCl_2$
Day 2	Few bubbles, appear after a couple seconds	Faster reaction, many bubbles, maintained for a longer time
Day 14 (LTSP culture)	Took a very long time, very few small bubbles	Took a couple seconds, formed many bubbles
Day 16 (following re-addition to fresh media)	Took a very long time, very few small bubbles	Took a couple seconds, formed many bubbles

Figure 4. Measuring rifampicin resistance to determine the mutation frequency for cells grown in either LB or LB + $CaCl_2$. Cells from day 2 cultures were plated on either LB (to determine CFU/ml) or LB + Rifampicin (to determine number of rifampicin resistant cells). Cells growing in LB + $CaCl_2$ experienced a lower frequency of mutation relative to cells grown in LB.

	Growth in LB	Growth in LB + $CaCl_2$
CFU/ml	2.70E+09	1.90E+09
Rifamipcin resistant colonies	1010	290
Mutation Frequency	3.74E-07	1.53E-07

Figure 5. Measuring *bolA* activity as a gauge of cell stress for cells grown in either LB or LB + $CaCl_2$. *E. coli* expressing β-galactosidase driven by the *bolA* promoter are lysed at day 7 of growth in the indicated media. CFU/ml are determined by growing cells on LB to normalize for the cell population. Cells grown in LB + $CaCl_2$ exhibited higher stress levels compared to those grown in LB.

	Growth in LB	Growth in LB + $CaCl_2$
CFU/ml	2.50E+07	4.80E+08
OD_{420} (*bolA* expression)	0.14	0.32
β-gal units (CFU/ml / OD)	5.60E-09	6.67E-10

Battle of the Bacteria: Characterizing the Evolutionary Advantage of Stationary Phase Growth

Brian Sato, University of California, Irvine

Karin Kram, University of Southern California

USC
UNIVERSITY
OF SOUTHERN
CALIFORNIA

Welcome!

- Name
- Institution
- What labs do you teach?

Workshop Outline

- Evolution in the classroom
- What is the GASP phenotype?
- Description of Lab Module
- Hands-on time with Module
- Implementation at UC Irvine
- Adopting the module to your classroom

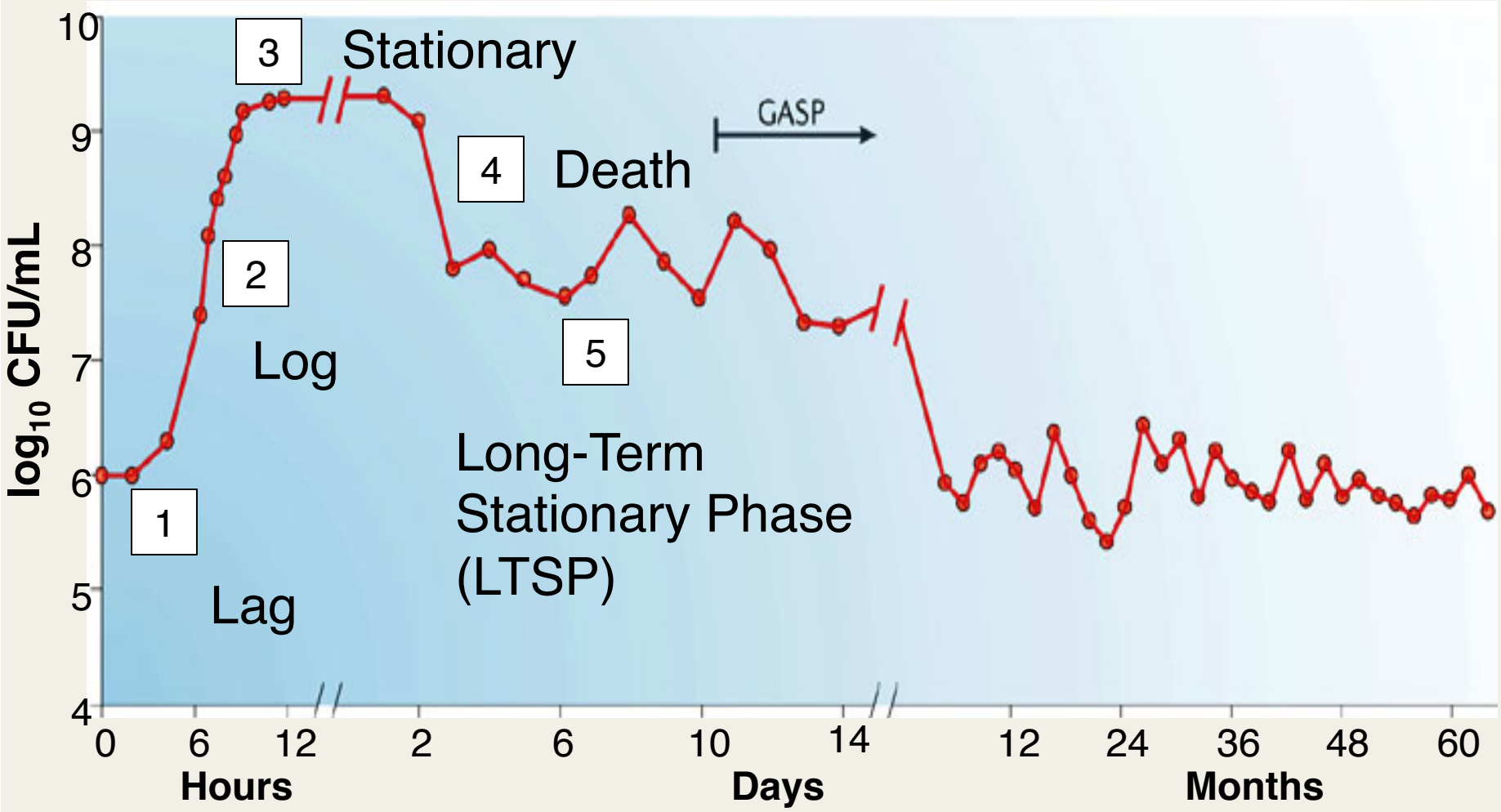
Discuss: Why is evolution hard to teach?

What is the GASP phenotype?

Long-term survival during stationary phase: evolution and the GASP phenotype

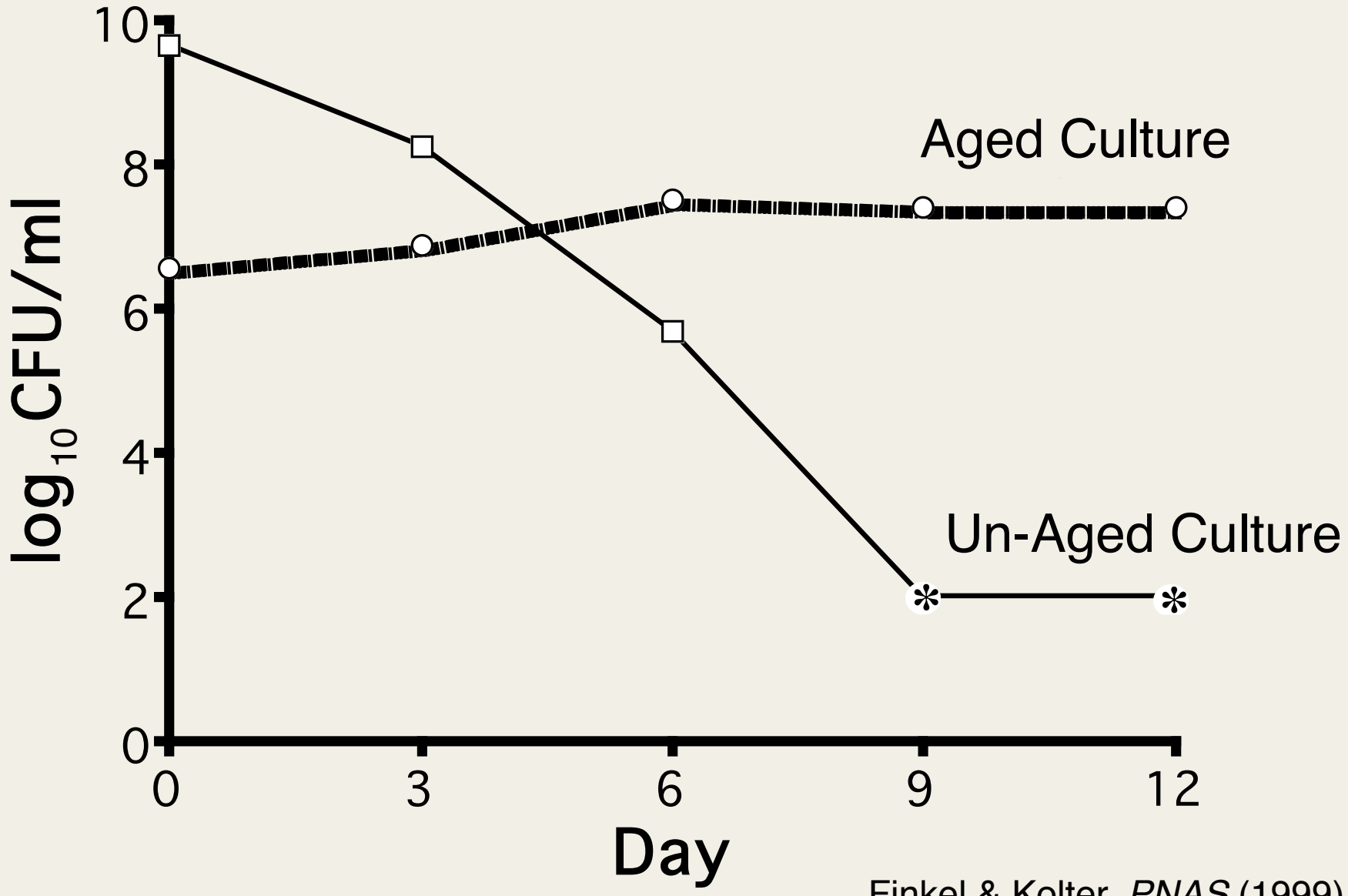
Steven E. Finkel

Phases of Bacterial Growth in Batch Culture



Finkel SE. *Nature Reviews Microbiology*. 2006 Feb;4(2):113-20.

Growth Advantage in Stationary Phase (GASP)



Finkel & Kolter, *PNAS* (1999)

GASP is due to a:

A. Phenotypic change
B. Genotypic change
C. It depends

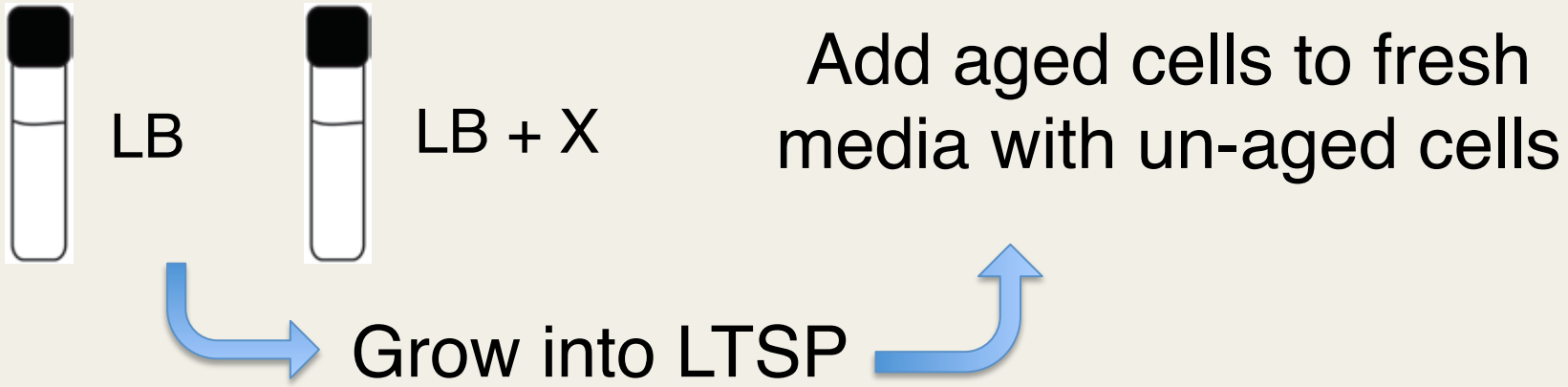
Genotypic – In the absence of stress the phenotype remains

Module Components

GASP

Do we observe the GASP phenotype?

Is the GASP phenotype environment specific?

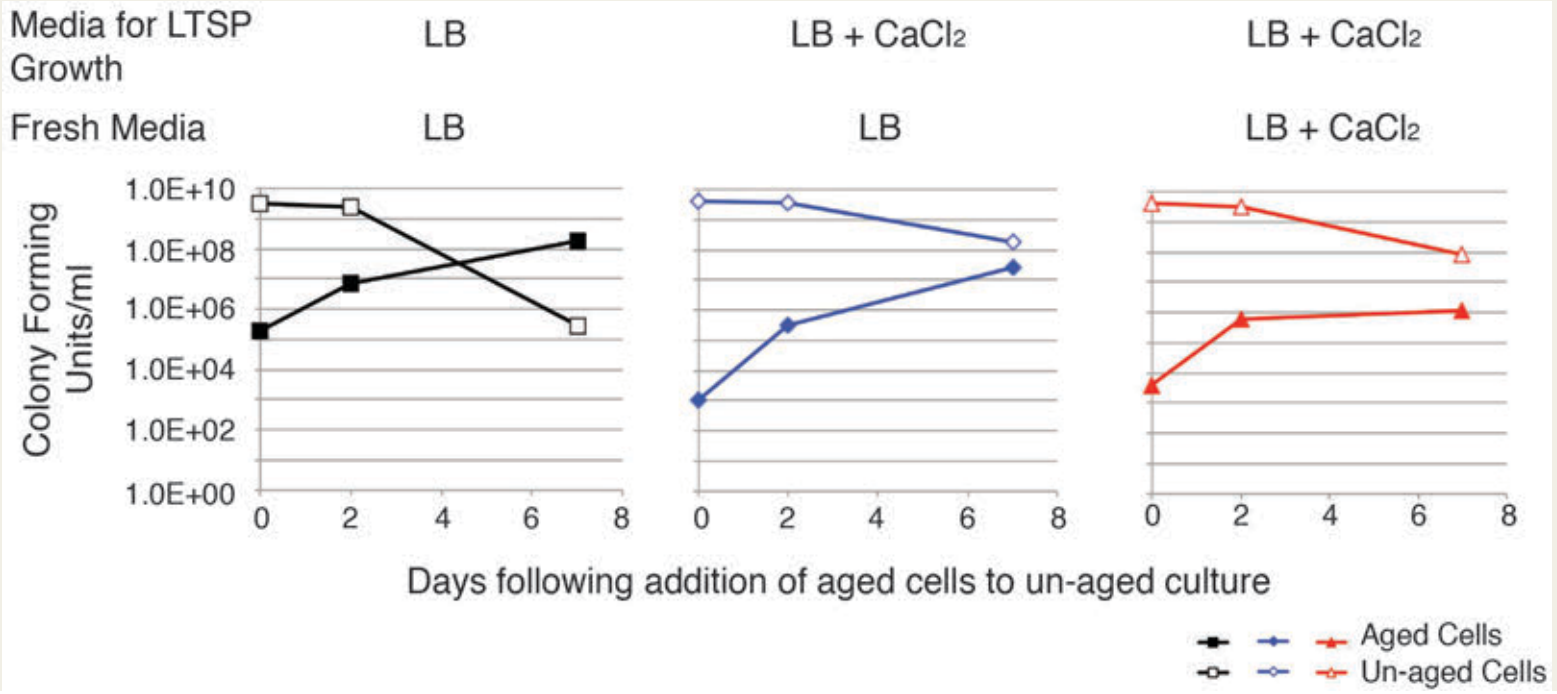


Module Components

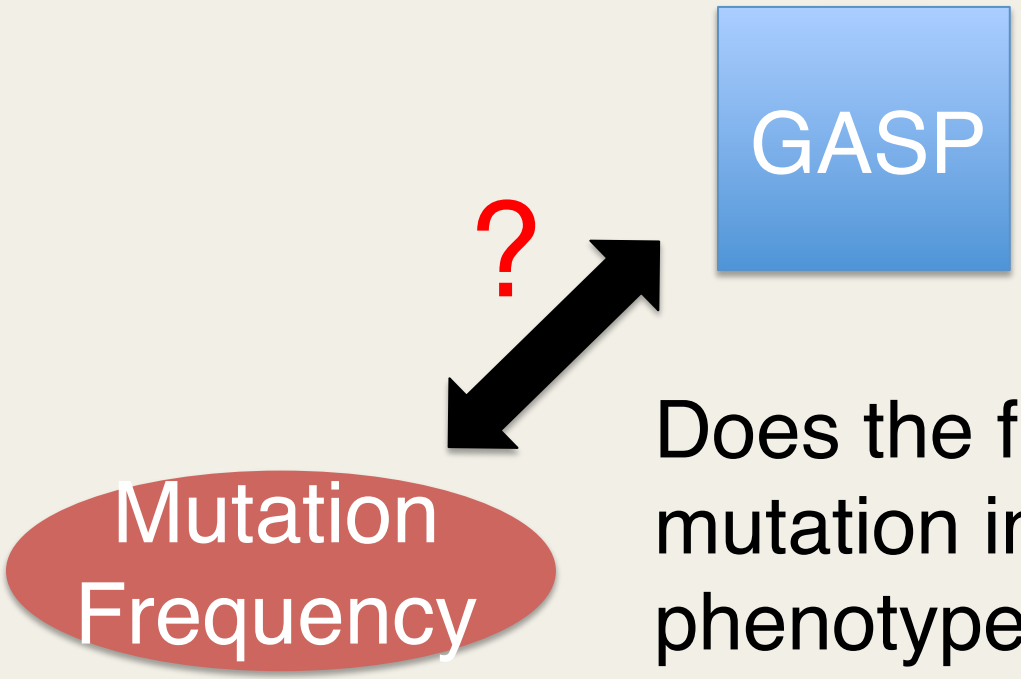
Real, novel data!

GASP

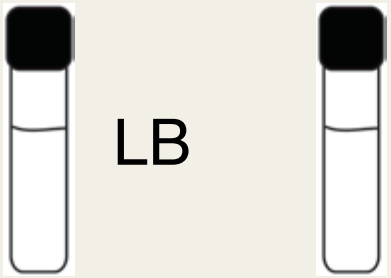
Is the GASP phenotype environment specific?



Module Components

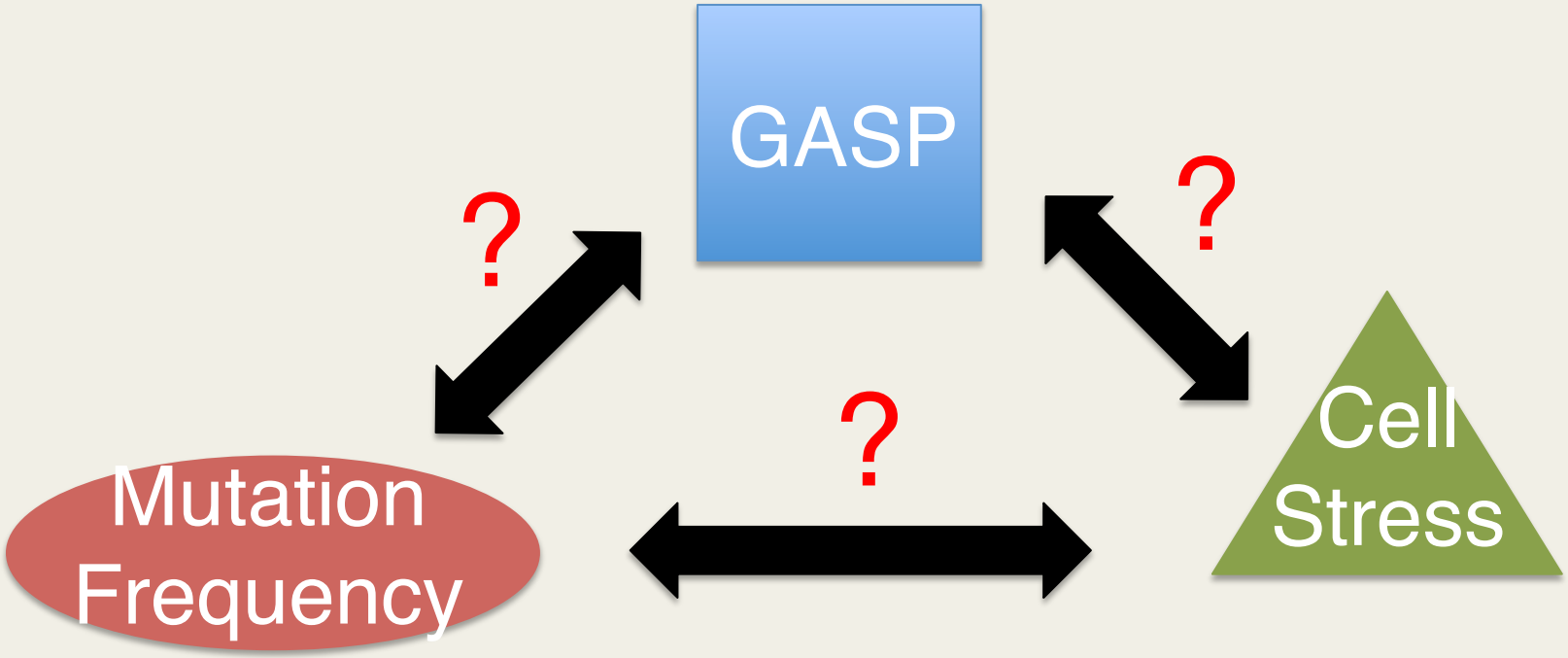


Does the frequency of bacterial mutation impact the GASP phenotype?



Measure frequency of antibiotic resistance

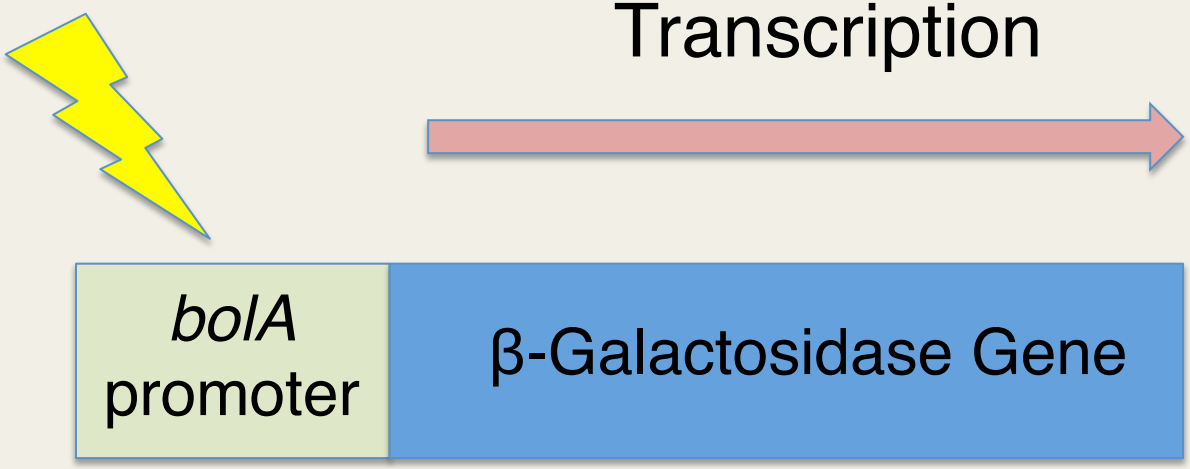
Module Components



Does the degree of cell stress correlate with mutation frequency and the strength of the GASP phenotype?

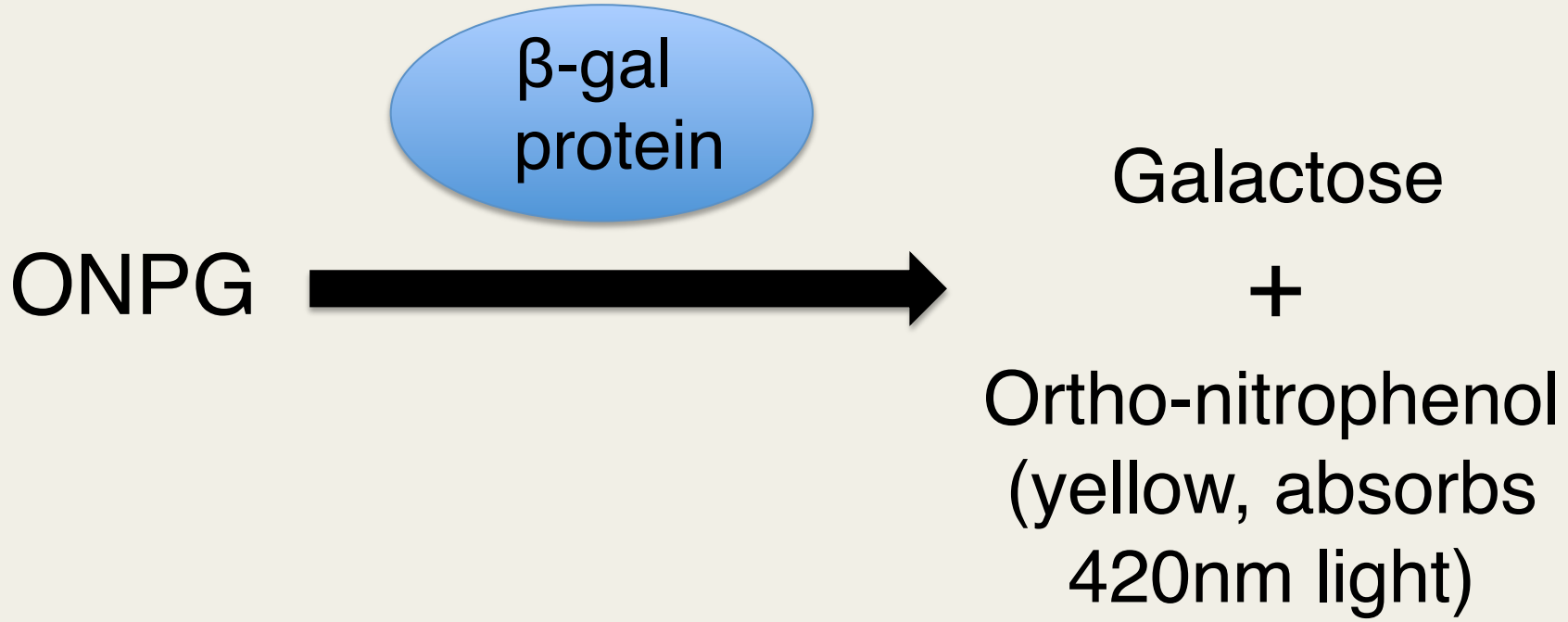
How to measure cell stress

- *bolA* reporter gene

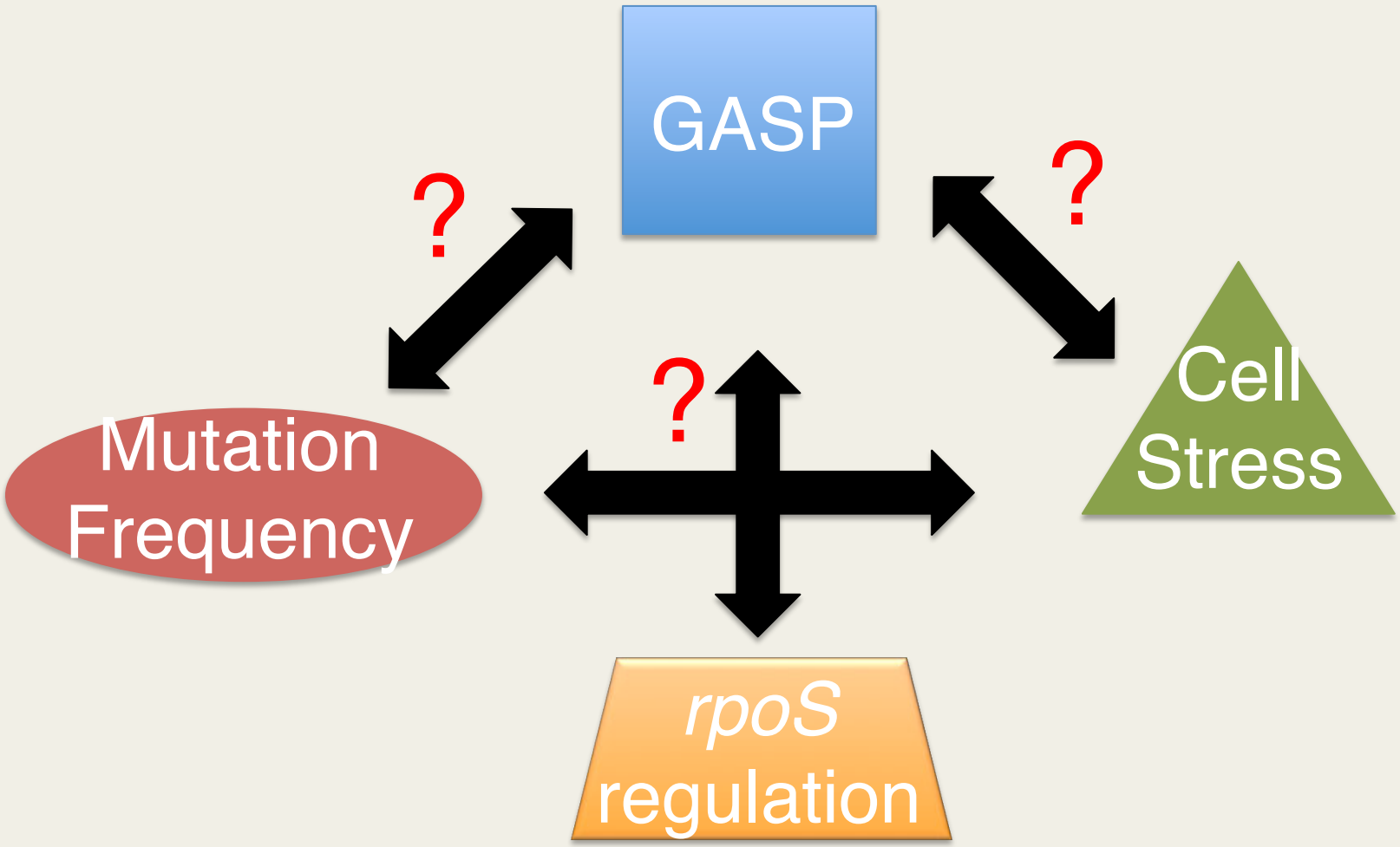


How to measure cell stress

- *bolA* reporter gene

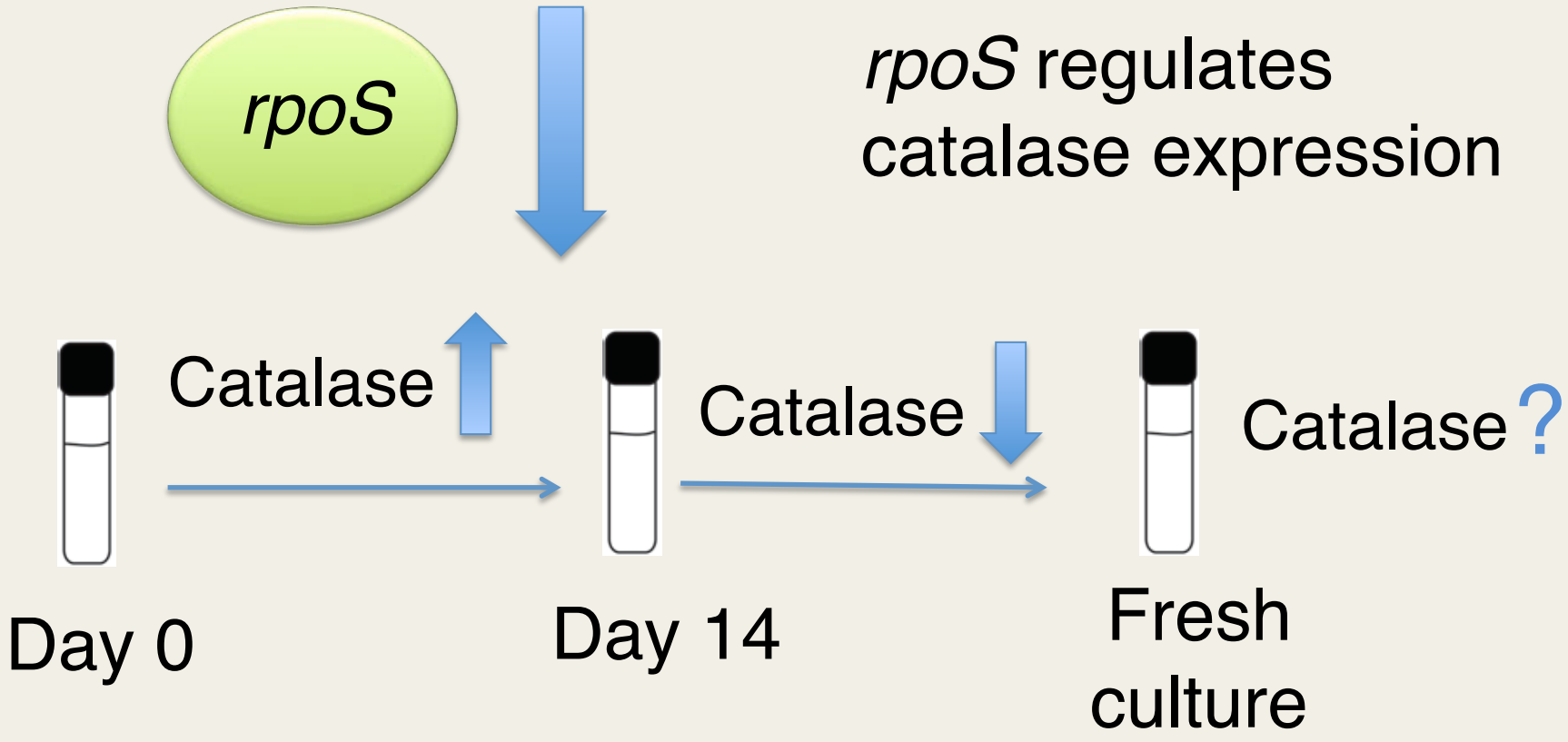


Module Components

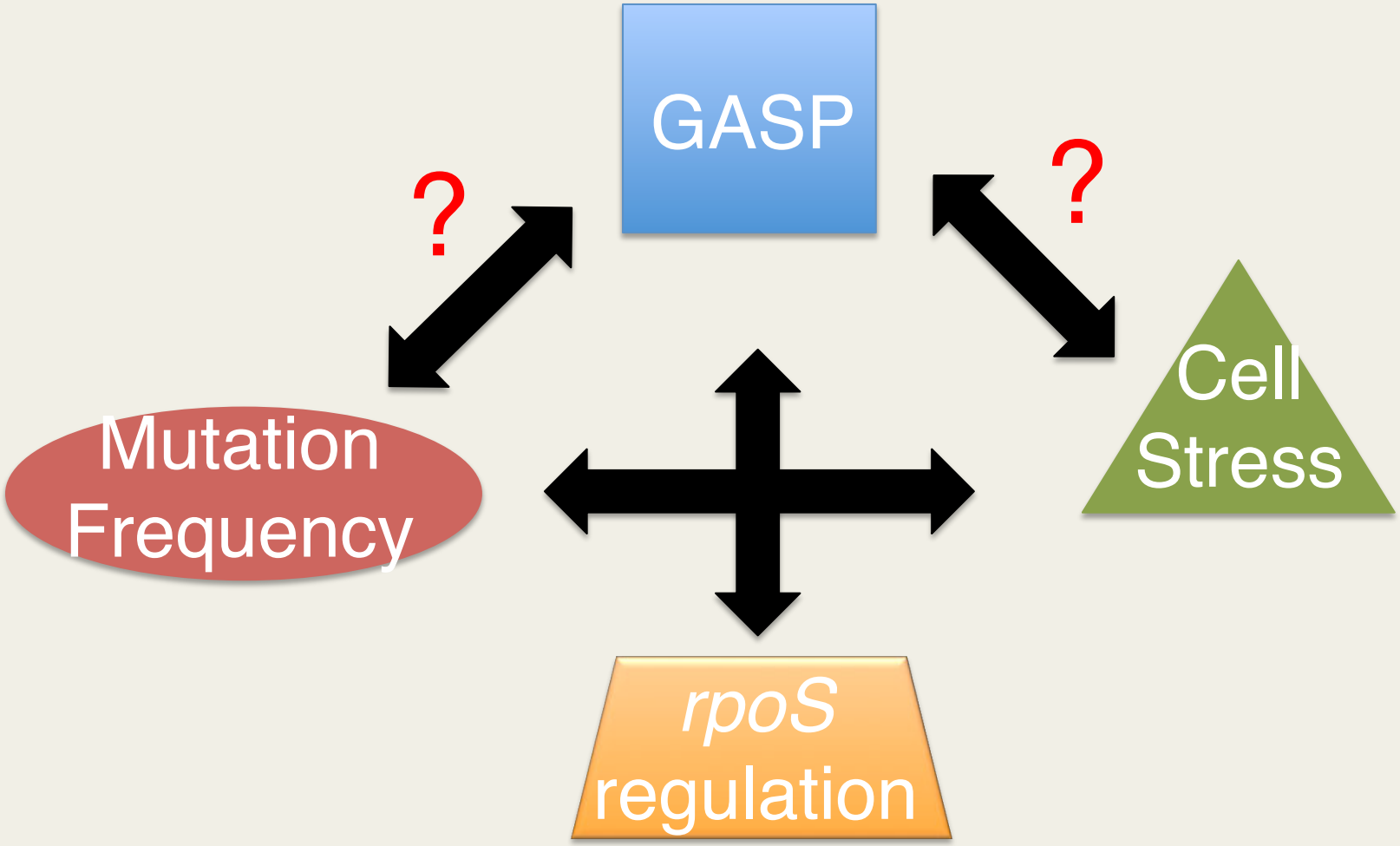


GASP is due to genotypic changes in the bacteria

But what about survival in LTSP?



Module Components



Can choose to include any or all of these modules

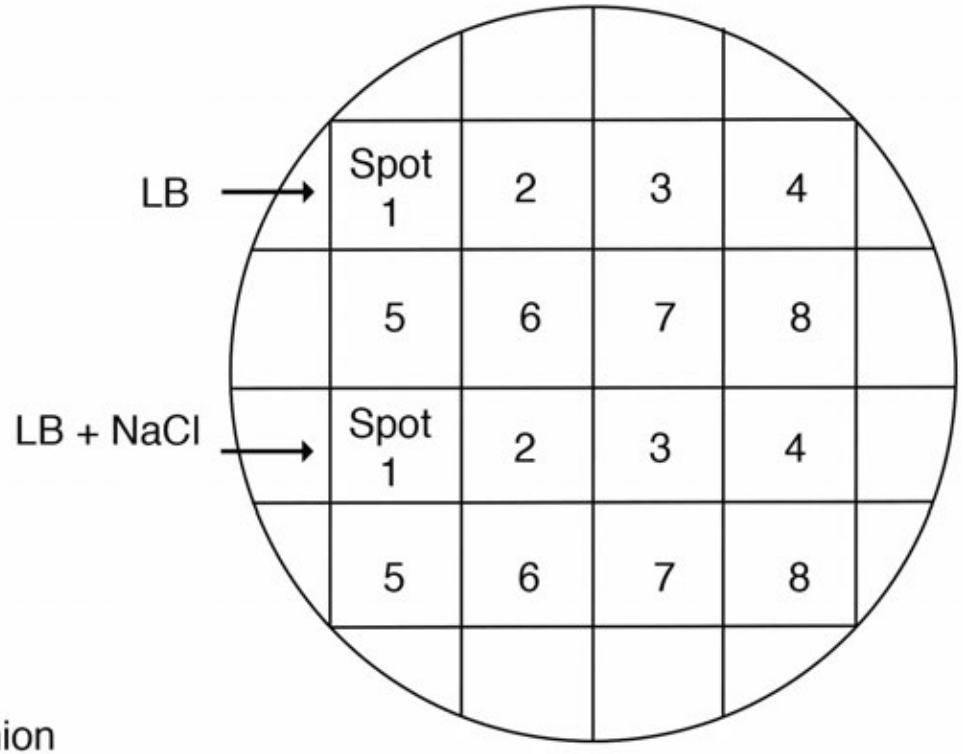
Hands-On Time w/Module

- (1) Catalase test (page 8 in handout)
  - Compare old versus new cultures
- (2) β-gal assay (page 10 in handout)
  - Compare cells grown in 2 different environments
  - Which environment (LB or LB + NaCl) leads to more cell stress?
  - Analyze data on page 13

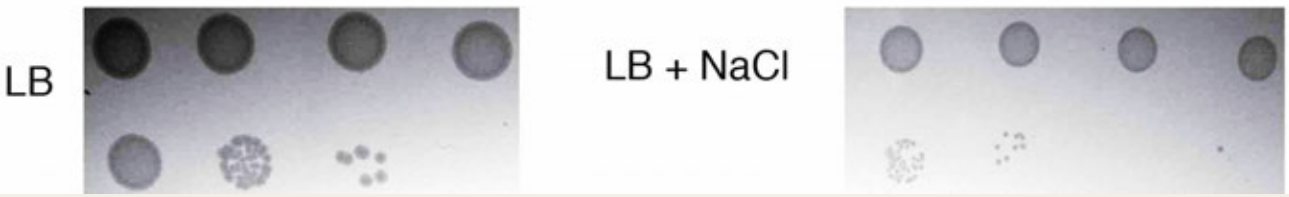
Hands-On Time w/Module

Spot 1 is 10ul of 1:100 culture dilution (10ul cells, 90ul LB)

Each additional spot is a 1:10 dilution



The data below were generated in this fashion



2A

2B

Page 13 in handout

What do we conclude?

- *rpoS* is downregulated in culture 1A or 1B

- Media 2A or 2B results in more cell stress

Implementation at UCI

- Fall 2014, Winter 2015
  - Students select a variable to modify the media
  - Hypothesize how this impacts GASP, mutation frequency, cell stress, *rpoS* regulation

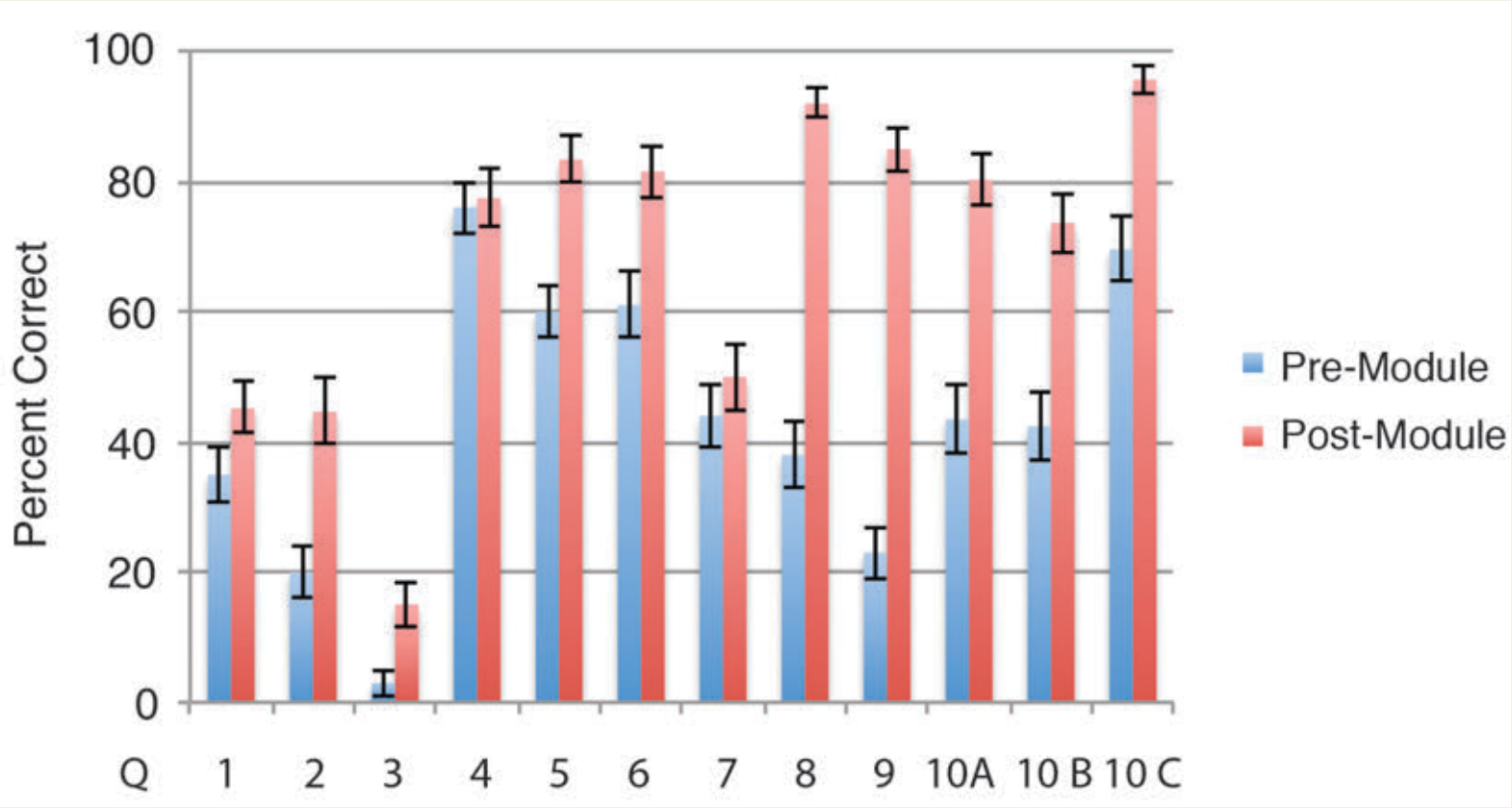
Implementation at UCI

Learning Goals

1. Describe the basis of evolution/survival of the fittest on a molecular level.
2. Describe the importance of the ingredients in microbial growth media.
3. Design a testable hypothesis.
4. Define and describe the use of a transcriptional reporter gene.
5. Differentiate between bacterial species and bacterial strains.
6. Differentiate between genotypic and phenotypic changes.
7. Use pipettors with accuracy/confidence.

Implementation at UCI

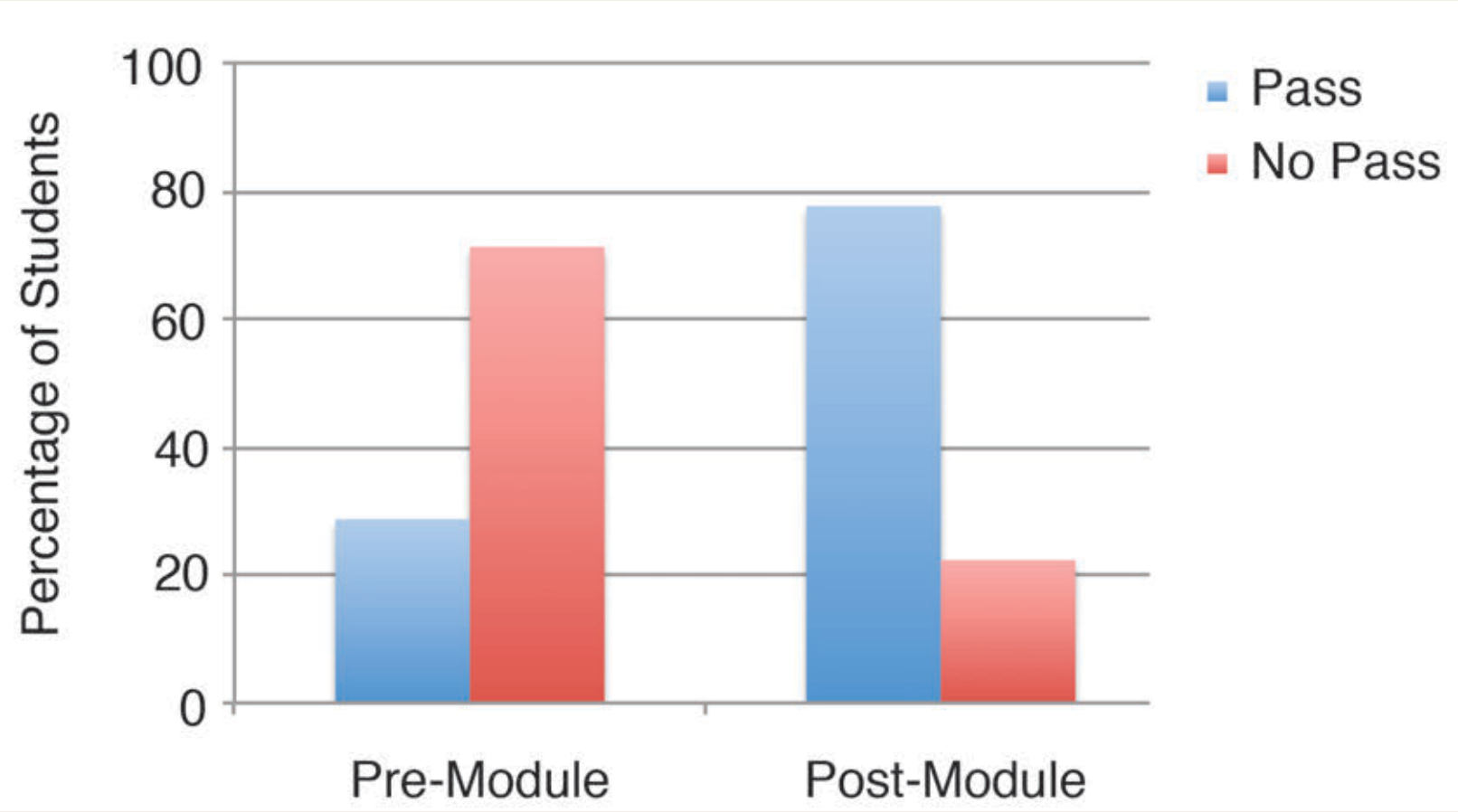
- Assessment Pre/Post Test



All $p < 0.05$ except for Q4

Implementation at UCI

- Pipetting Test

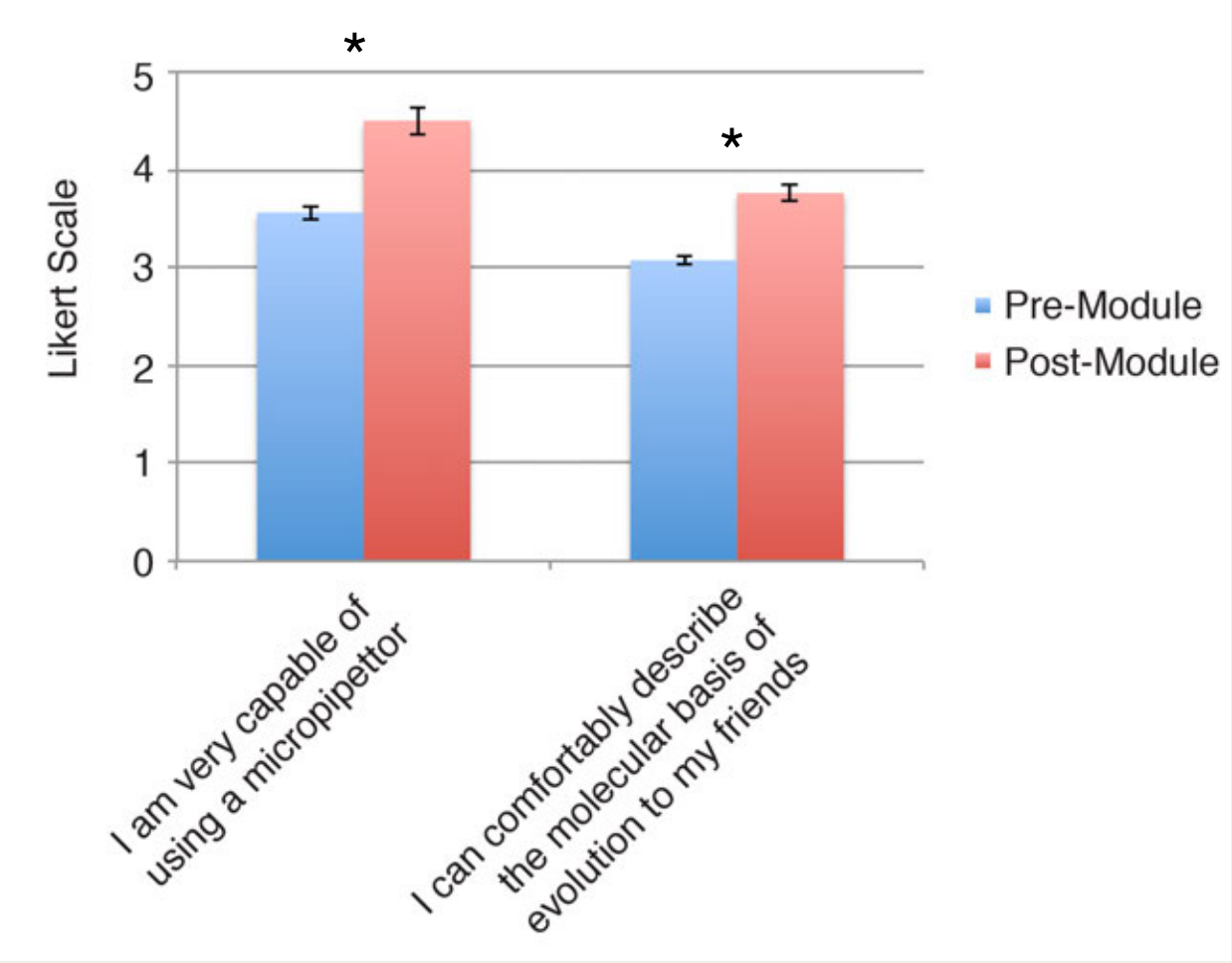


Implementation at UCI

- Student Self-Assessment

5 = strongly agree

1 = strongly disagree



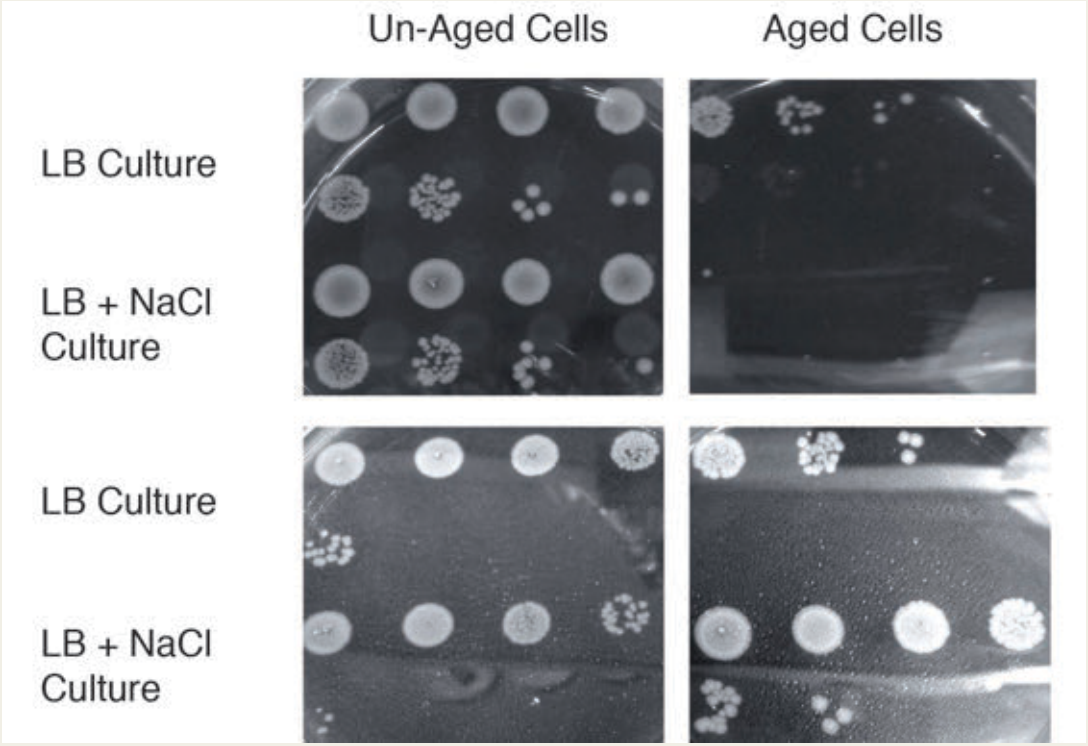
* $p < 0.05$

Bringing this to your institution

- What are the impediments?

Questions?

Data analysis



- Using this data:
  - Plot cell survival over time (page x)
  - Is the GASP phenotype occurring?
  - Is GASP impacted by environment?

Microbiology Lab Course: Environmental Unknown Project Name: _____

Question/Hypothesis:

Exercise 3

Sample:

Whole Colony Description	Margin	Elevation

Exercise 11

Streak plate unknown

Whole Colony Description	Margin	Elevation

Complete a subculture to nutrient agar plate.

Exercise 5

Simple staining result

Morphology	Arrangement

Complete a culture transfer to a nutrient broth.

Exercise 7

Gram staining result

Gram result	Morphology	Arrangement

Complete a culture transfer from broth to a slant.

Exercise 12

Special Media results

	EMB	MSA
Growth		
Color		

Exercise 14

Fermentation results

	Glucose
Growth	
Color	
Gas	

Complete a culture transfer from the slant to broth.

Exercise 25

Antibiotic resistance results

Antibiotic	Disk code	Diameter (mm)	R, I, or S
1.			
2.			
3.			
4.			
5.			

Complete a culture transfer to a nutrient agar plate.

For Gram-negative sample: complete an Enterotube II

Enterotube II results

Test	**Negative or Positive (±)**	**Value**
Glucose		
Gas		
Lysine		
Ornithine		
H_2S		
Indole		
Adonitol		
Lactose		
Arabinose		
Sorbitol		
VP		
Dulcitol		
PA		
Urea		
Citrate		

Notes: Fermentation test (blue text)

ID code: _____

For Gram-positive sample

Catalase test:

Coagulase test:

Hemolysin test:

Identification of your unknown environmental sample: _____

If a patient had an infection from the environmental microbe that you identified above, then what antibiotic(s) could be used: _____

Workshop 9

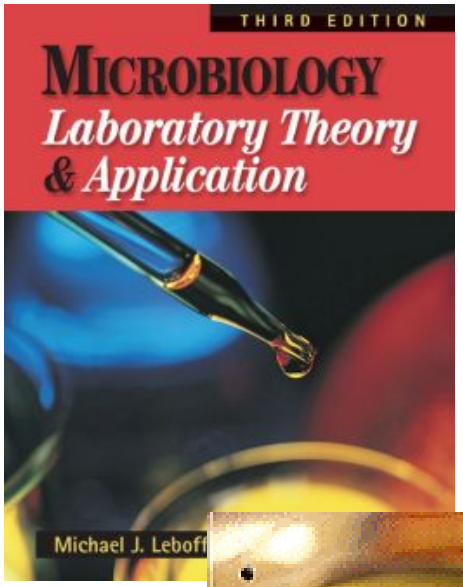
RABLE 2015

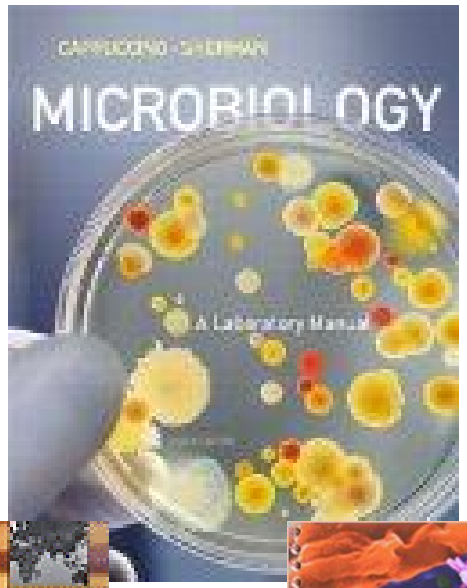
Welcome to Implementing Inquiry-based Microbial Project

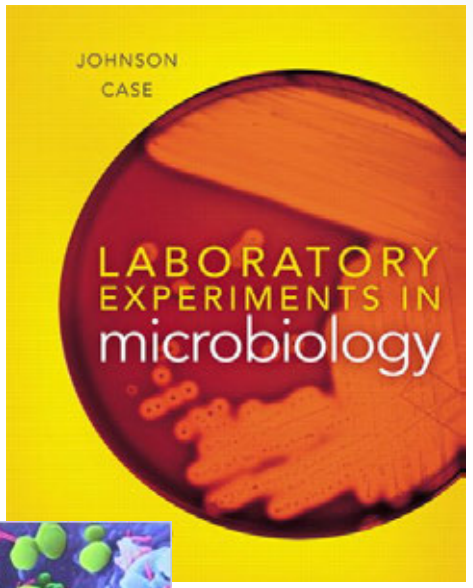
Veronica Ardi, PhD

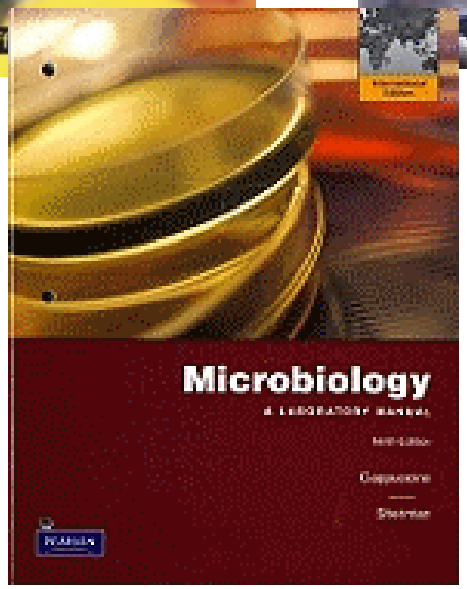
NATIONAL UNIVERSITY

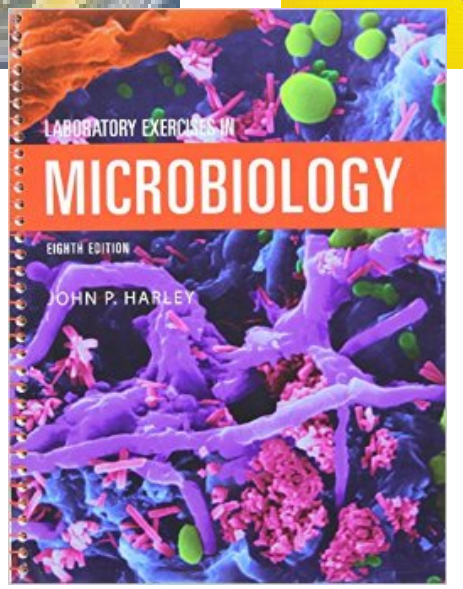
Microbiology Laboratory Courses











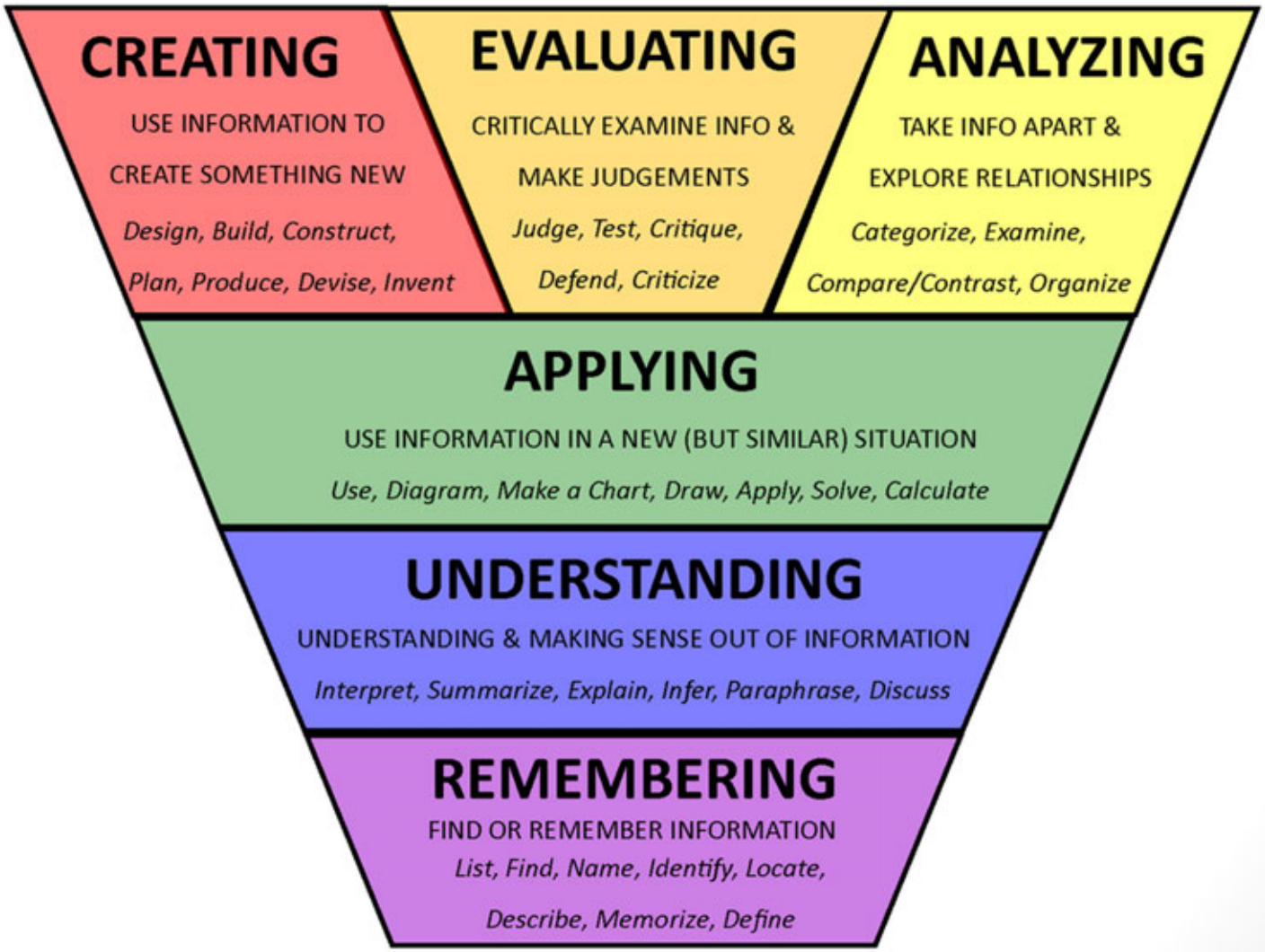
CourseSmart: ebook resources – http://instructors.coursesmart.com/

Microbiology Laboratory Courses

- Traditional Lab Manual
  - Individual experiments
  - Defined results
  - Post-lab questions with right answers

- Hypothesis-driven or Inquiry-based lab class
  - Creative
  - Critical thinking
  - Frustrating?

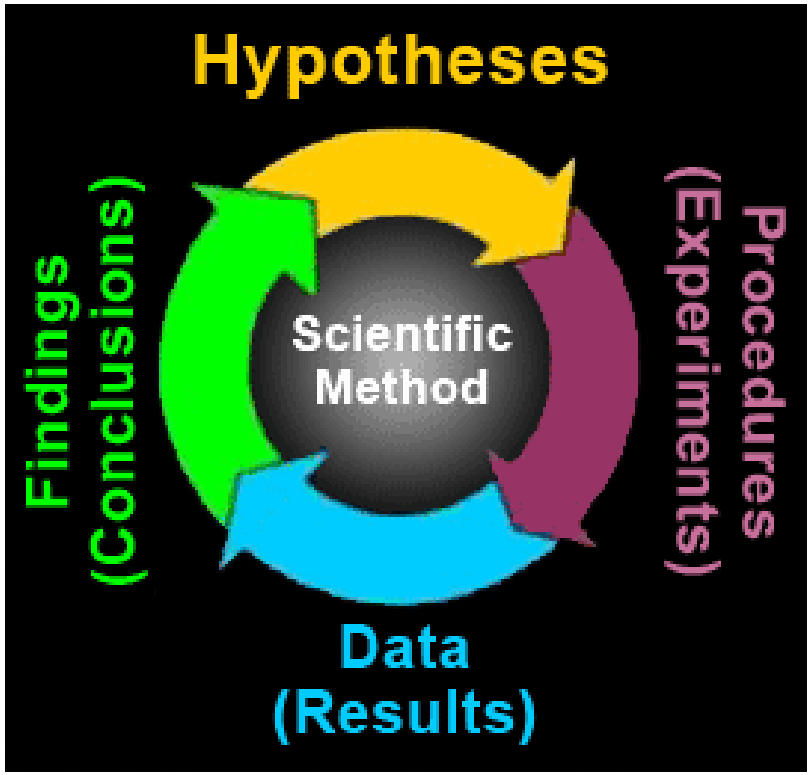
Microbiology Laboratory Courses



M. Hos-McGrane. 2014.

Building on previous courses

- General Biology, Genetics, Molecular biology, etc.
- Scientific Method



NHS. 2009. http://web2.newtown-h.schools.nsw.edu.au/Science/CommonFolder/scienceindex.html

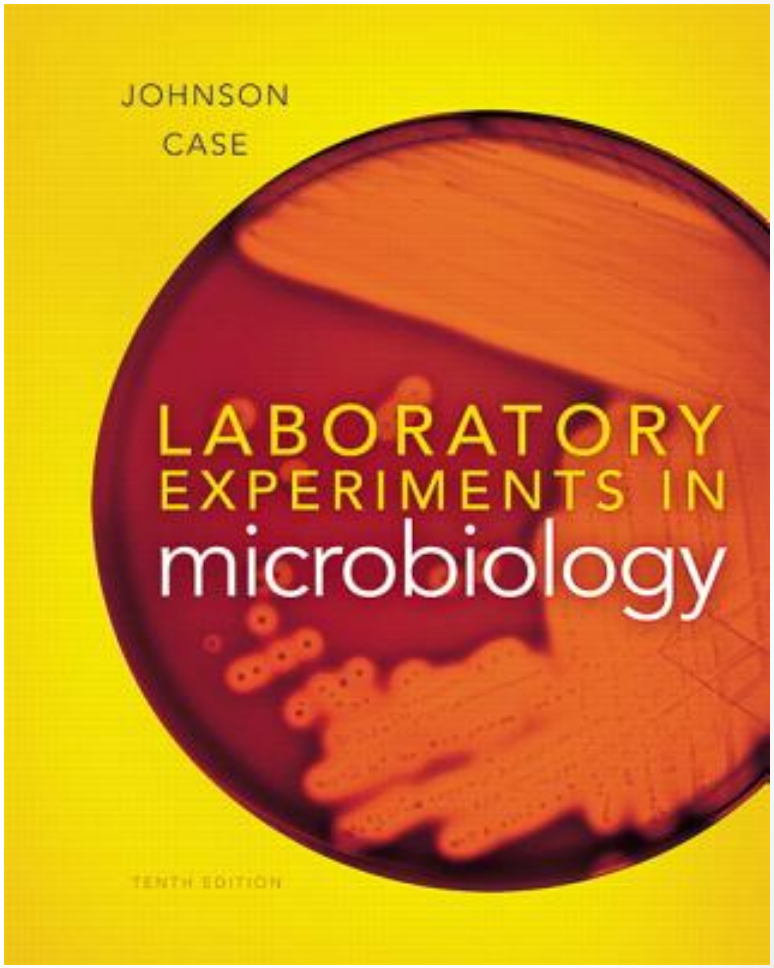
Microbial Techniques

_____ Acid-fast stain
_____ Aseptic Technique
_____ Biochemical tests
_____ Capsule stain
_____ DNA isolation & sequencing
_____ Gram stain
_____ Growth curve / Spectrophotometer
_____ Kirby-Bauer test
_____ Microscope
_____ Most probable number
_____ Motility
_____ Negative stain
_____ PCR
_____ Pure culture isolation
_____ Serial Dilution
_____ Selective Media
_____ Simple stain
_____ Spread plating
_____ Streak plating
_____ Wet mount
_____ Other: _____

Sample Timeline

- Does your order of techniques aid in inquiry-based project?

- Johnson, T. and Case, C. Laboratory Experiments in Microbiology, 10th edition, 2012; Pearson, Benjamin Cummings, San Francisco, CA



Sample Timeline

Date/ 2015	Laboratory Topics
	General procedures, Lab Safety, Microscope Video, Turn in Safety Contract. Ex. 1: Parts of the Microscope & Oil immersion
	Ex. 3: Microbes in the Environment, Morphological unknown Ex. 26: Effectiveness of Hand washing
	Continue Ex. 3 & Isolate environmental sample Aseptic technique Ex. 11: Isolation of bacteria (& unknown environmental sample)
	Continue Ex. 11 & 26 (& unknown environmental sample)
	Ex. 5: Smears & Simple Stains (& unknown environmental sample)
	Ex. 7: Gram Staining (& unknown environmental sample)
	Ex. 12: Special Media (& unknown environmental sample) Ex. 20: Bacterial Growth Curve
	Continue Ex.12 Ex. 19: Oxygen & the growth of bacteria
	Lab MIDTERM

Sample Timeline

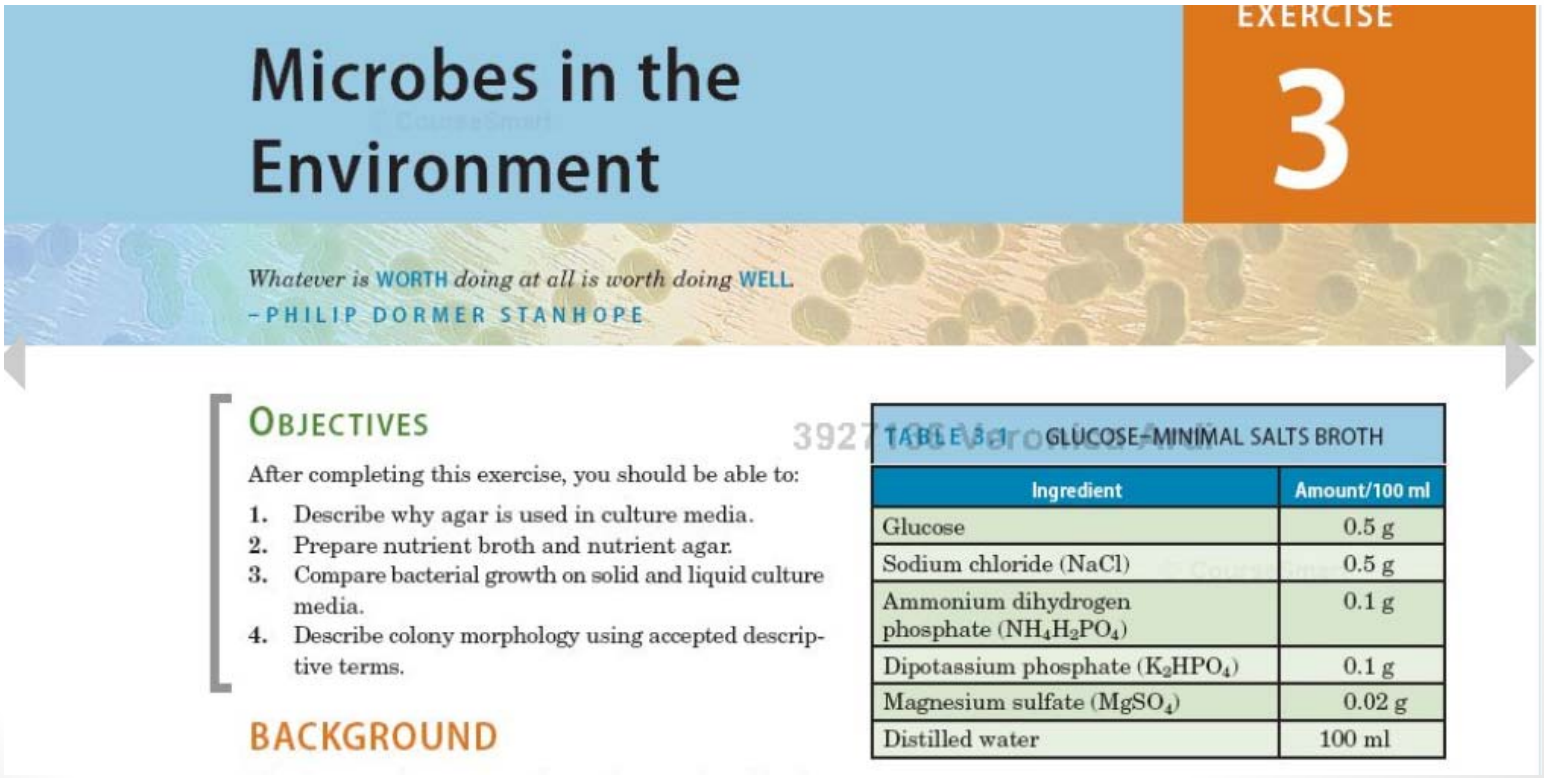
Date/ 2015	Laboratory Topics
	Continue Ex.19 **Ex. 14: Fermentation (& unknown environmental sample)**
	Continue Ex. 14 **Ex. 24: Disinfectants & Antiseptics**
	Continue Ex. 24 **Ex. 25: Antimicrobial drugs (& unknown environmental sample)** **Documentary: Nightmare Bacteria**
	Continue Ex. 25 **Ex. 45: Bacteria of the Skin** **Unknown environmental sample / project**
	Continue Ex. 45 **Ex. 46: Bacteria of the Respiratory Tract** **Unknown environmental sample / project**
	Continue Ex. 46 **Ex. 51: Enterotube – ID unknown environmental sample**
	Continue Ex. 51 – Unknown environmental sample / project
	Final Lab Exam (Cumulative) **Turn in Environmental Unknown Project**

General Equipment for Microbiology Lab

- Antibiotic disks (at least 5 different classes): ampicillin, penicillin, streptomycin, tetracycline, & vancomycin [typically used in lab] or any that is typically used in clinics
- BD Enterotubes II
- API 20E
- Blood agar, EMB, Mannitol salt agar (MSA), and Mueller-Hinton agar plates
- Bunsen burner
- Wire loops
- Catalase test: H_2O_2
- Coagulase test: rabbit plasma
- Gram stain reagents
- Glucose fermentation tube
- Nutrient agar plates & slants
- Personal protective equipment
- Sterile cotton swabs & sterile saline solution (or broth)

Microorganisms in our world

- In pairs, come up with hypothesis or inquiry.
- How will you sample? [Design your method.]
- Procedure (Exp. 3): inoculate 1 plate from the environment using cotton swab (dip in saline first).



EXERCISE 3

Microbes in the Environment

Whatever is WORTH doing at all is worth doing WELL.
– PHILIP DORMER STANHOPE

OBJECTIVES

After completing this exercise, you should be able to:

1. Describe why agar is used in culture media.
2. Prepare nutrient broth and nutrient agar.
3. Compare bacterial growth on solid and liquid culture media.
4. Describe colony morphology using accepted descriptive terms.

BACKGROUND

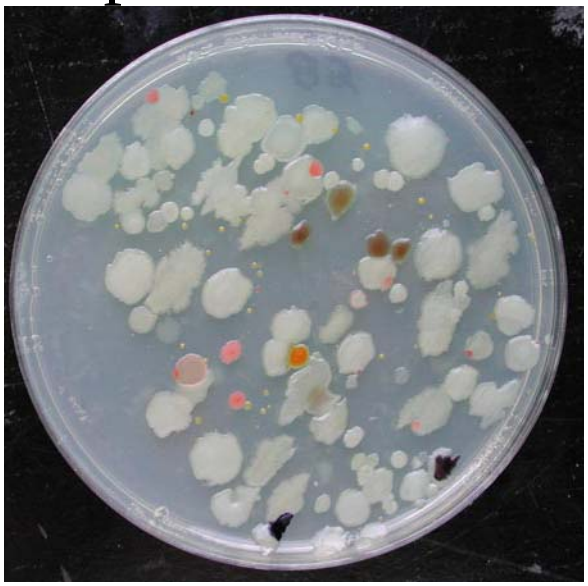
TABLE 3.1 GLUCOSE–MINIMAL SALTS BROTH

Ingredient	Amount/100 ml
Glucose	0.5 g
Sodium chloride (NaCl)	0.5 g
Ammonium dihydrogen phosphate ($NH_4H_2PO_4$)	0.1 g
Dipotassium phosphate (K_2HPO_4)	0.1 g
Magnesium sulfate ($MgSO_4$)	0.02 g
Distilled water	100 ml

Microorganisms in our world

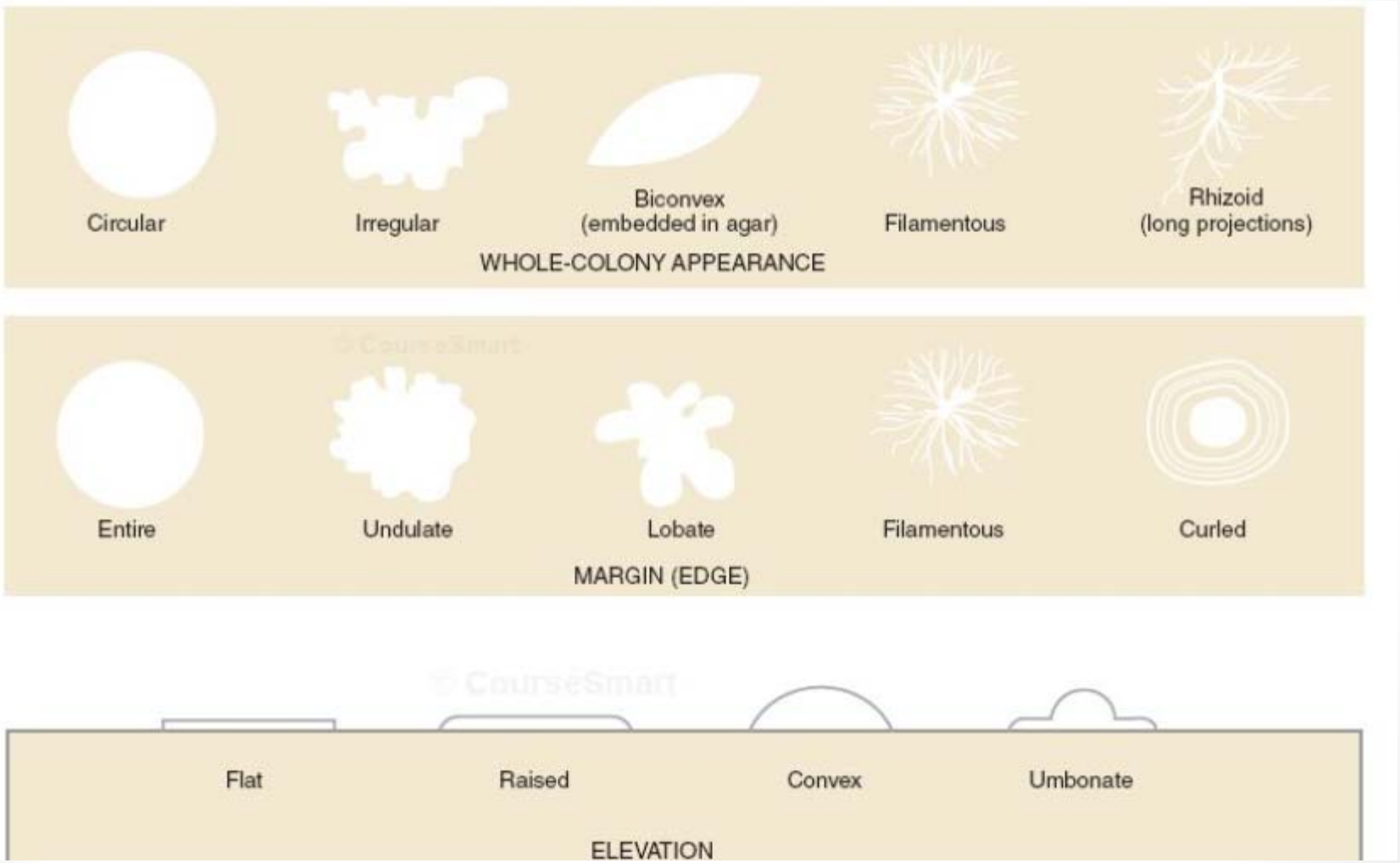
- Go sampling!
- Any problems during sampling?

- Incubate
- Environmental samples



NOAA-OE. 2007.

Results: variety of microbes



Results: Picking a colony

- Students would describe all the colonies from the sampling.
- Guide students to select 1 colony

	Colony Description					
	Estimated Diameter	Whole-Colony Appearance	Margin	Elevation	Pigment	Number of This Type
Area sampled:						
_____						
Incubated at						
_____°C for						
_____ days						

Alternatives in sampling

- If no growth or mostly fungus …
- Instructors should complete an initial environmental sample (i.e. sample the bottom of your shoes).
- Instructor's can have pure cultures labeled as environmental unknown.
  - Try not to use common bacteria (*E. coli*, *S. aureus*, etc.)

Timeline

Date/ 2015	Laboratory Topics
	General procedures, Lab Safety, Microscope Video, Turn in Safety Contract. Ex. 1: Parts of the Microscope & Oil immersion
	Ex. 3: Microbes in the Environment, Morphological unknown Ex. 26: Effectiveness of Hand washing
	Continue Ex. 3 & Isolate environmental sample Aseptic technique Ex. 11: Isolation of bacteria (& unknown environmental sample)
	Continue Ex. 11 & 26 (& unknown environmental sample)
	Isolate unknown environmental sample – pure culture
	Ex. 5: Smears & Simple Stains (& unknown environmental sample)
	Ex. 7: Gram Staining (& unknown environmental sample)

Collecting Data

Question/Hypothesis:

Exercise 3

Sample:

Whole Colony Description	Margin	Elevation

Exercise 11

Streak plate unknown

Whole Colony Description	Margin	Elevation

Complete a subculture to nutrient agar plate.

Exercise 5

Simple staining result

Morphology	Arrangement

Complete a culture transfer to a nutrient broth.

Exercise 7

Gram staining result

Gram result	Morphology	Arrangement

Complete a culture transfer from broth to a slant.

Collecting Data

Exercise 12

Special Media results

	EMB	MSA
Growth		
Color		

Exercise 14

Fermentation results

	Glucose
Growth	
Color	
Gas	

Complete a culture transfer from the slant to broth.

Exercise 25

Antibiotic resistance results

Antibiotic	Disk code	Diameter (mm)	R, I, or S
1.			
2.			
3.			
4.			
5.			

Complete a culture transfer to a nutrient agar plate.

Collecting Data

For Gram-negative sample: complete an Enterotube II

Enterotube II results

Test	Negative or Positive (±)	Value
Glucose		
Gas		
Lysine		
Ornithine		
H_2S		
Indole		
Adonitol		
Lactose		
Arabinose		
Sorbitol		
VP		
Dulcitol		
PA		
Urea		
Citrate		

Notes: Fermentation test (blue text)

ID code: _____

Collecting Data

- Enterotube II or API 20E

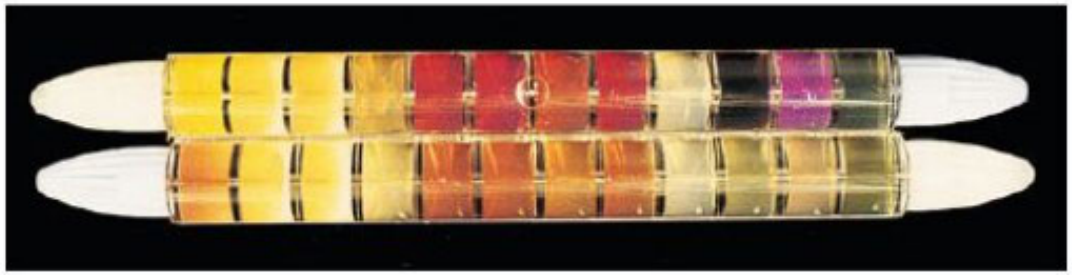


FIGURE 51.1 **Enterotube II.** Fifteen biochemical tests are performed in Enterotube II. The bottom tube is uninoculated.

APIWeb: https://apiweb.biomerieux.com/servlet/Authenticate?action=prepareLogin

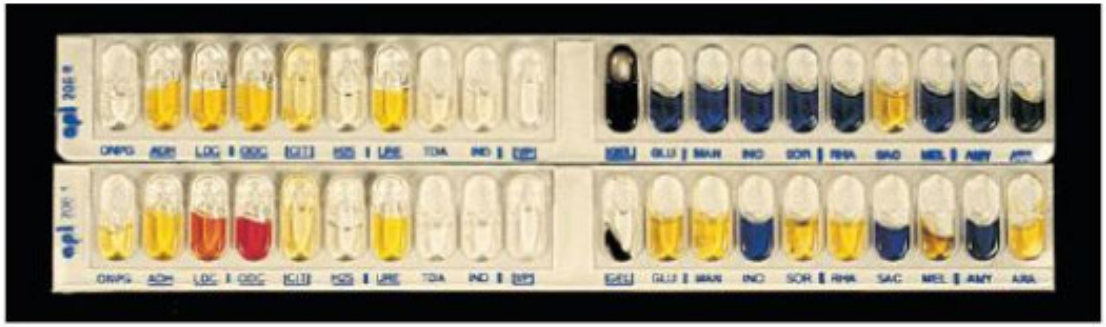


FIGURE 51.2 **API 20E strip.** Twenty biochemical tests are performed in an API 20E strip. The top strip is inoculated with *Proteus vulgaris* and the bottom strip with *Escherichia coli*.

Collecting Data

For Gram-positive sample

Catalase test:

Coagulase test:

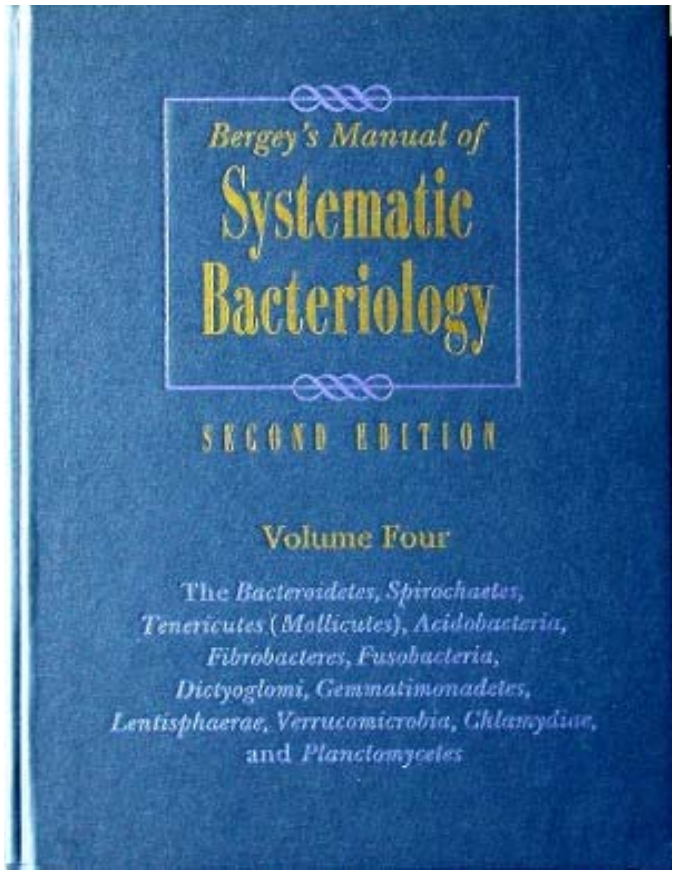
Hemolysin test:

Identification of your unknown environmental sample:

If a patient had an infection from the environmental microbe that you identified above, then what antibiotic(s) could be used:

Analyses

- Identification of unknown – applying Bergey's Manual



Bergey's Manual:
https://archive.org/details/bergeysmanualofd1957amer
http://www.uiweb.uidaho.edu/micro_biology/250/IDFlowcharts.pdf

Analyses

- Identification of unknown – applying dichotomous key

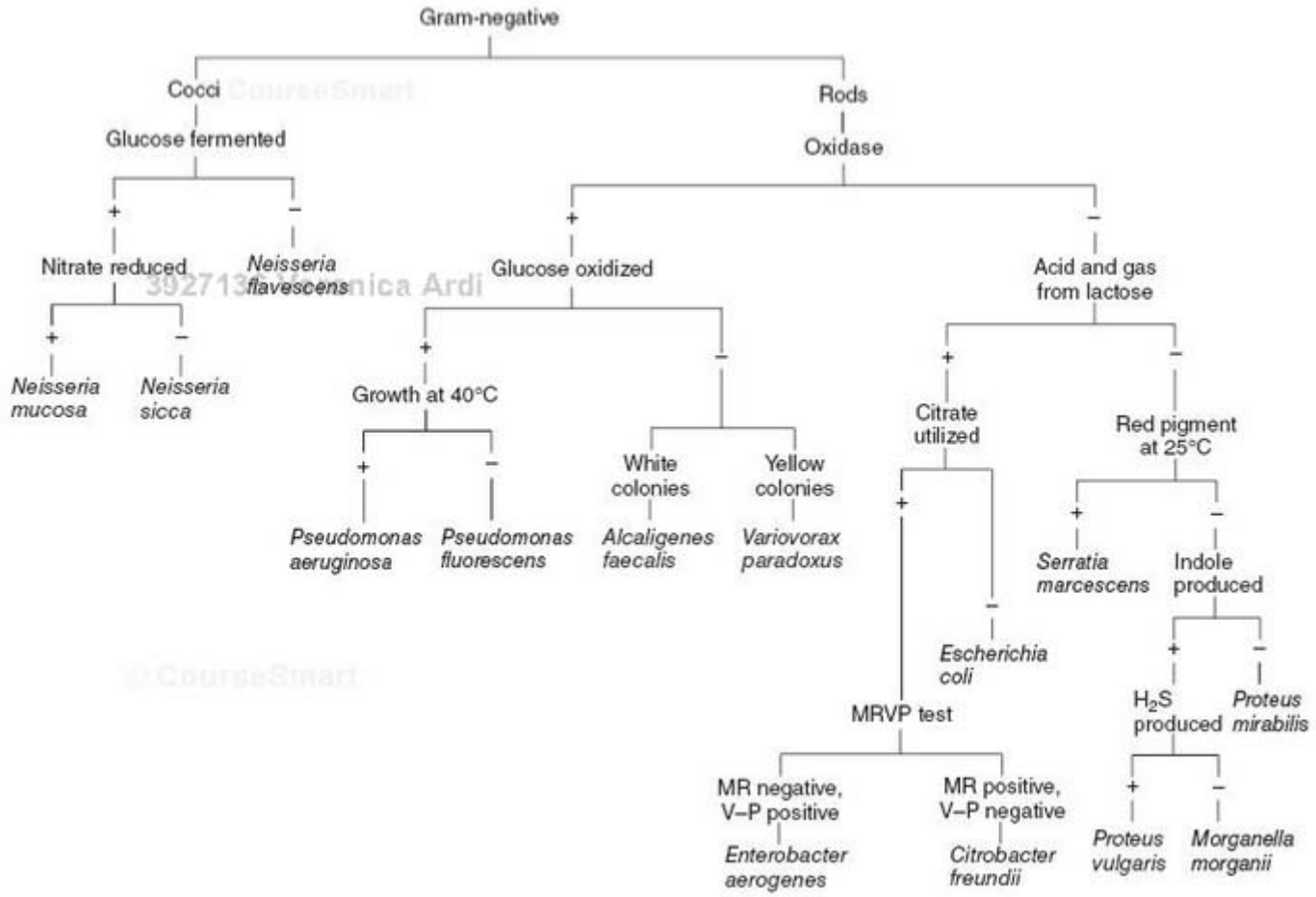


FIGURE H.1 Key to selected gram-negative heterotrophic bacteria.

Analyses

- Identification of unknown – applying dichotomous key

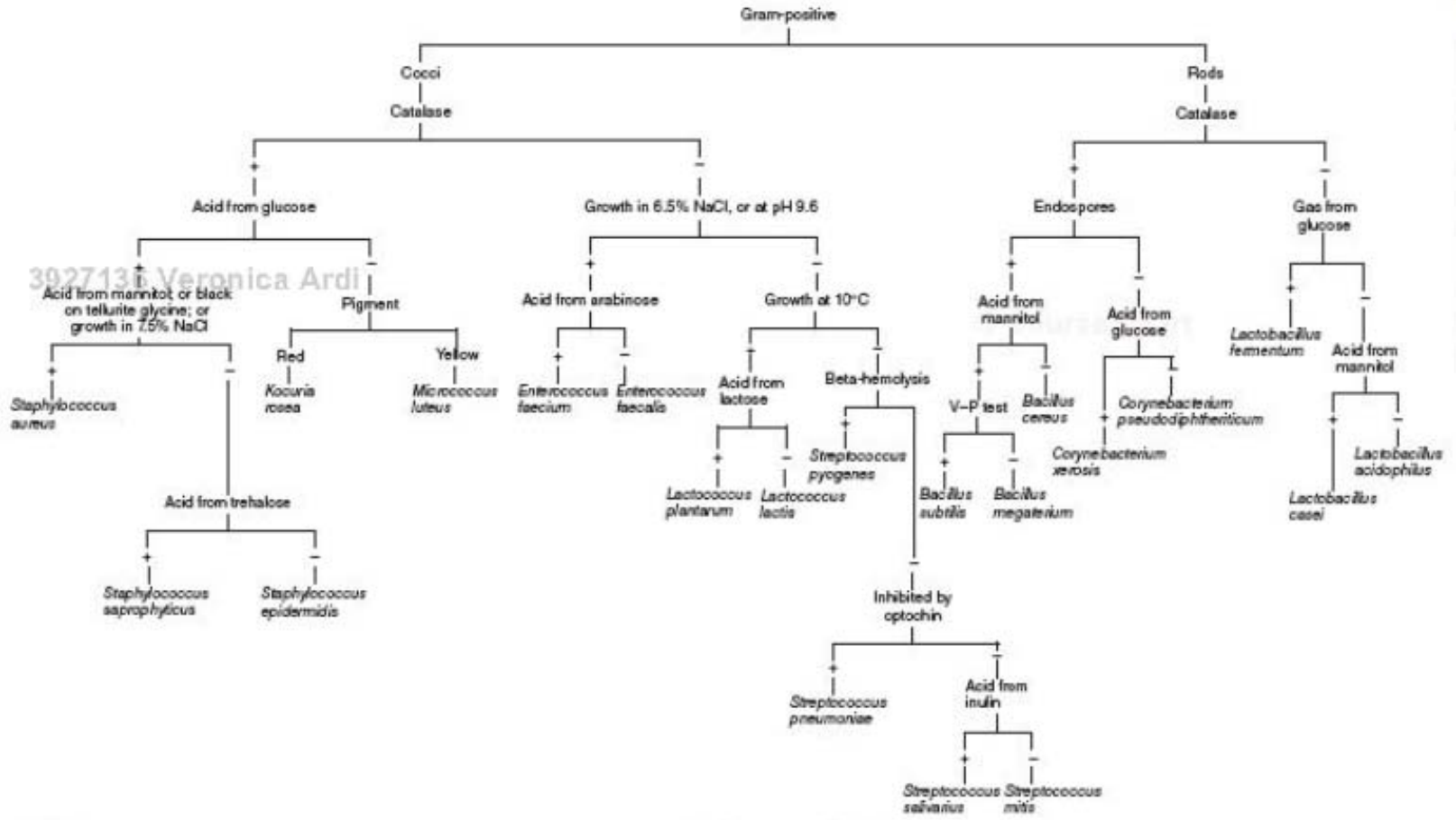


FIGURE H.2 Key to selected gram-positive heterotrophic bacteria.

Actual Student Results

- What are the issues in identification of unknown?
- What happens when student's are absent?

- What if there is no identification?

- Motivation
- Timing

Rubric

- Establish a rubric to grade the project
- Unknown Environmental Project: 5-15% of the total grade
- Example of rubric found in packet

	Excellent	Satisfactory	Needs Improvement	Inadequate

Additional Assignments

- Paper: write a 500-700 word paper about the bacterial organism was identified.
- Student presentation
- What should the paper / presentation include?
- A clinical case study, if applicable, should be required in the writing or presentation assignment.
- Ecological significance of the bacterium should be part of the presentation or paper.

Top Student Interest

- Experiments that they have vested interest
- Experiments exploring themselves

EXERCISE

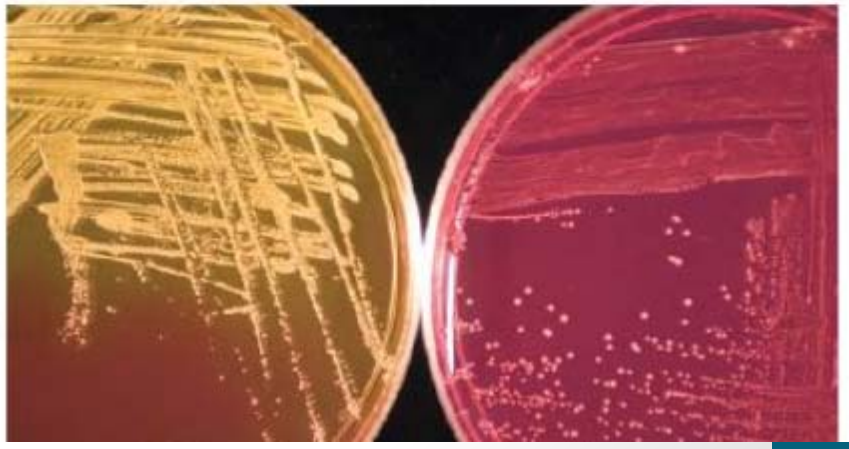
45

Bacteria of the Skin

OBJECTIVES

After completing this exercise, you should be able to:

1. Isolate and identify bacteria from the human skin.
2. Provide an example of normal skin microbiota.
3. List characteristics used to identify the staphylococci.
4. Explain why many bacteria are unable to grow on human skin.



Top Student Interest

- Experiments that they have vested interest
- Experiments exploring themselves

EXERCISE

Bacteria of the Respiratory Tract

46

OBJECTIVES

After completing this exercise, you should be able to:

1. List representative normal microbiota of the respiratory tract.
2. Differentiate the pathogenic streptococci based on biochemical testing.

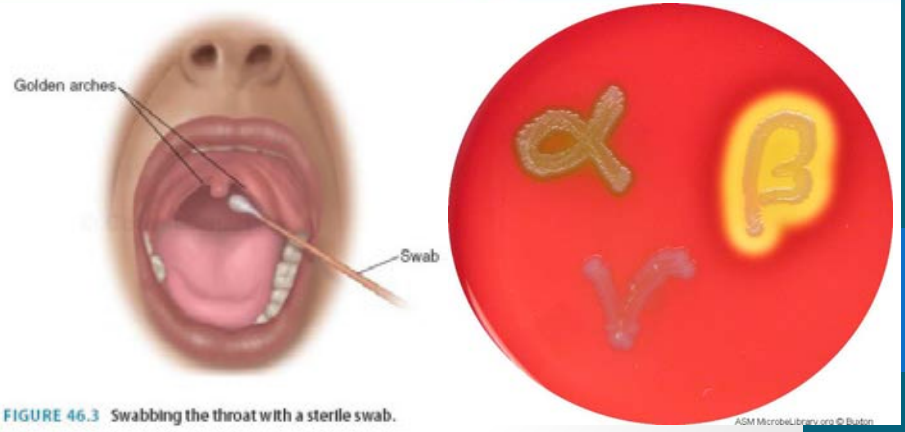


FIGURE 46.3 Swabbing the throat with a sterile swab.

Limitations in Inquiry-based project

- This workshop is limited to bacterial samples.
- What if the students do not understand the technique, how can they perform it?
- How can they interpret the test?
- Master the purpose of the test is key.
  - How can they apply it to a project?

Sex in the Garden: Pollen Germination Basic Protocols

Basic Background Information on Pollen Germination

Pollen germination occurs when a pollen grain develops a pollen tube. In nature, pollen germination occurs on the stigma of a flower and the pollen tube elongates towards the flower's ovule. Sperm will travel through the pollen tube to fertilize the ovule. The evolution of pollen eliminated the dependence on standing water for fertilization in seed plants.

Pollen develops in the anthers of the stamen of the flower. The anther has four pollen sacs held together by connective tissue. Each pollen sac has microsporocytes (microscope mother cells) which divide by meiosis to produce microspores. Each microspore divides by mitosis to produce either a 2 cell microspore or a binucleated microspore. Each microspore differentiates into a pollen grain.

Pollen grains range from 6 to 100 micrometers and have species specific morphology. The wall of the pollen has three layers: the inner intine wall, the outer exine wall and the pollen coat. The pollen walls have modifications to allow for shrinking and swelling of the grain and thinner areas (apertures) where the pollen tube can break though the pollen wall.

Pollen germination and elongation is a dynamic process during which pollen must respond to different signals and develop a complex structure to deliver sperm cells for fertilization. Pollen germination can be visualized easily. This system is a simple model to study cell to cell interactions and environmental effects on development.

Pollen Germination Assays

Hanging Drop Method

Materials

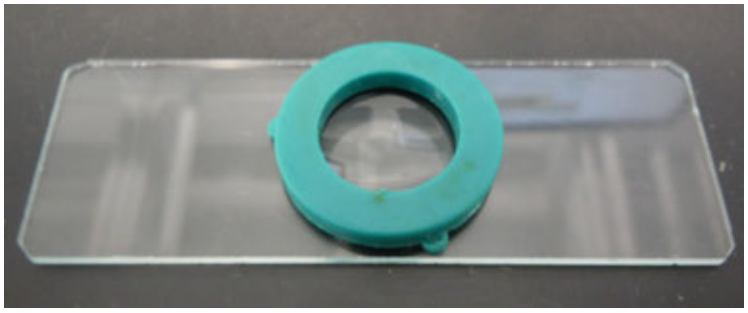
Basic Germination Media (1mM KCl, 0.1 mM $CaCl_2$, 1.6 mM H_3B0_3, 10% Glucose)

Regular glass slides
Cover slips
Petri dish
Filter paper (to fit into Petri dish)
Pipette tips

Procedure

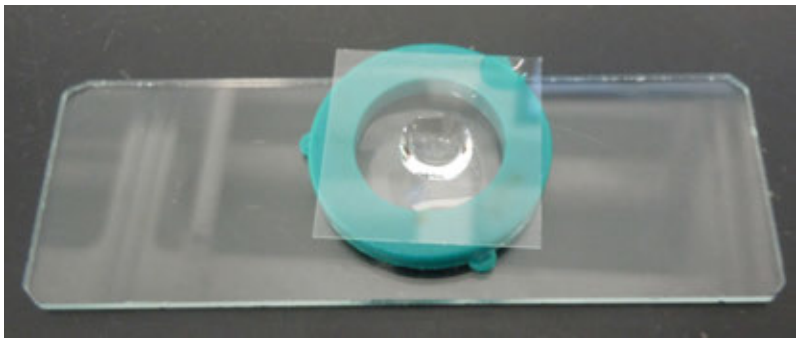
A. Hanging Drop Procedure
The hanging drop procedure is used to observe living organisms over time where desiccation could be an issue. The surface tension of the drop concentrates the cells to the edge of the drop. Oil immersion cannot be used with a hanging drop preparation.

1. Prepare humidity chamber by placing filter paper in Petri dish.
2. Moisten paper with water.
3. Clean microscope slides and cover slides using ethanol. Rinse with water and dry using a Kimwipe.
4. Prepare a slide with a slide chamber where the hanging drop can be inverted onto.
5. Lightly grease the bottom of a gasket. Place the gasket onto a regular microscope slide or a depression slide.



6. Determine the amount of media you will need to add to the cover slip to get a hanging drop (20 to 100 micrometers). The larger the drop, the less likely the drop will evaporate.

7. Flip the coverslip over and place onto the gasket.



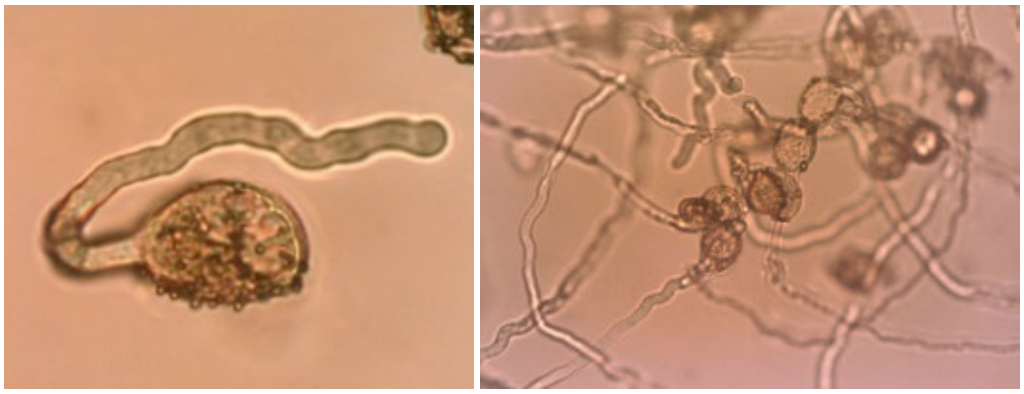
8. Observe the drop under the low power objective.
9. Use the edge of the drop to focus the microscope.
10. Change the objective lens to 10X and refocus. You cannot use the 40X objective lens with this procedure.

B. Obtaining Pollen

1. Observe the flower.
2. Identify the different parts of the flower.
3. Find the anthers.
4. The best pollen to collect for germination comes from anthers that have burst or split open. The process is referred to as dehisce. You can quickly tell if the pollen is mature by gently brushing your finger across the anther. If you see a residue of pollen on your finger, the pollen is most likely mature.
5. Remove a pipette tip using the P2-- micropipettor.
6. Eject the tip into your hand.
7. **Gently** rub the anther with the pipette tip until a small amount of pollen is transferred to the needle.
8. Transfer the pollen to the drop of media on the cover slip by tapping the tip with your finger.
9. Invert the cover slip over your slide.
10. Record the time.

C. Observing Pollen Tube Growth

1. Observe the pollen under 100 x magnification
   (**10 x objective lens** and 10x ocular lens)
2. Record the shape, size and any unique features of the pollen grains. You may draw the pollen shape on a piece of paper and then take a photo to upload to your lab notebook.
3. Check the pollen every 30 minutes until the pollen tubes begin to grow. Keep the slide in the humidity chamber between observations. You may search through the fields of views until you find a field of pollen with a good density of pollen.
4. Once the pollen tubes have begun to germinate, record the time, percentage of pollen that has germinated and the length of five pollen tubes every 10 minutes for 30 minutes.



Narcissus sp. (Daffodil) Pollen Germination

Agarose Method

Agarose gels are transparent, easy to make and can be made with a variety of buffers.

1. 1% molten agarose can be found at the back of the room in tubes in a hot plate.
2. Clean a microscope slide.
3. Warm the microscope slides by placing the slides on the edge of the hot plate.
4. Using a glass pipette, add 1 drop of agarose to the warm slide.
5. Allow the agarose to spread over the warm slide for 15 seconds.
6. Remove the slide from the hot plate and allow the slide to cool.
7. Prepare humidity chamber by placing filter paper in Petri dish.
8. Store your agarose slide in the humidity chamber when not in use.

C. Observing Pollen Tube Growth

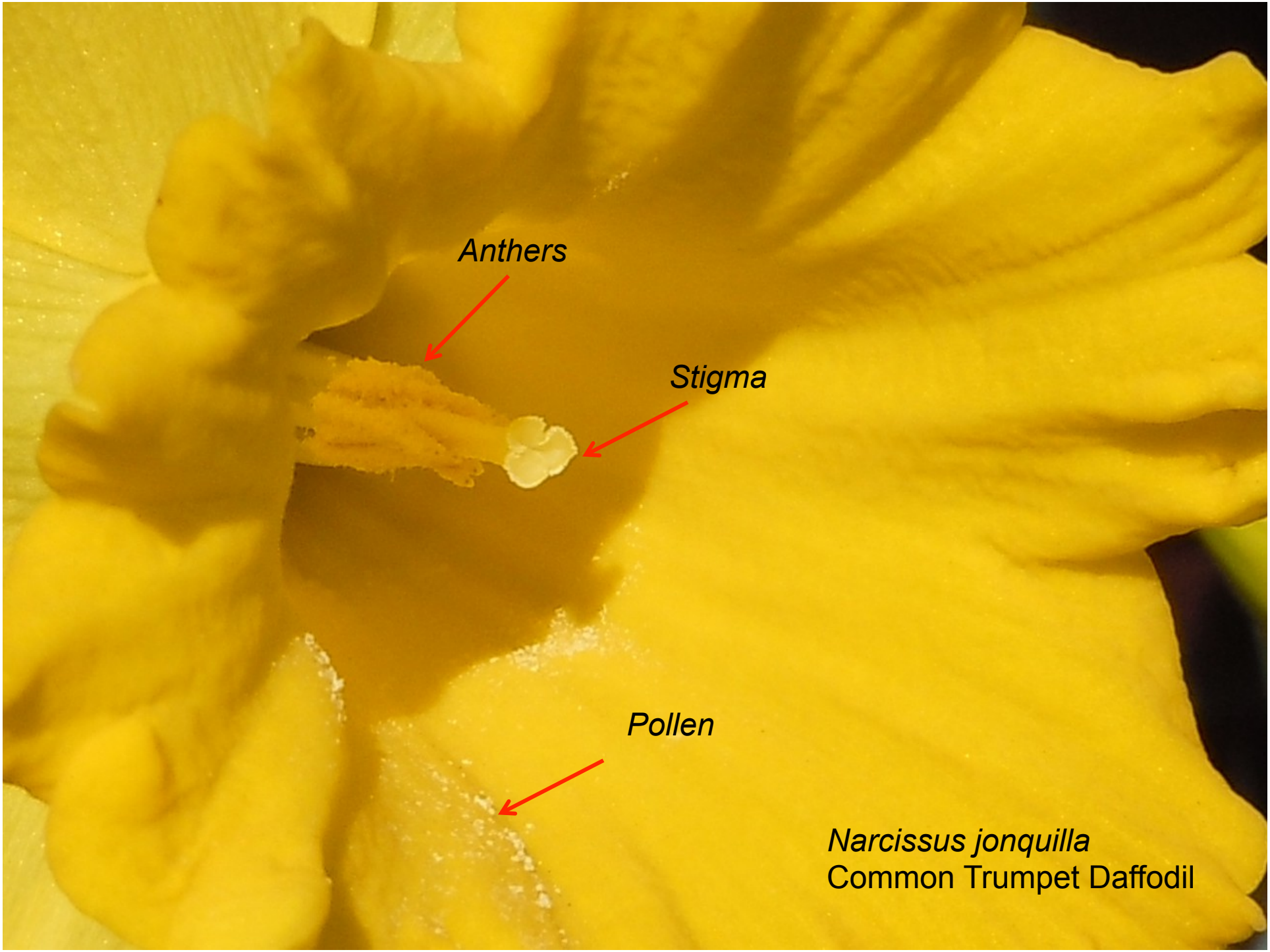
1. Remove the slide from the humidity chamber.
2. Dry the bottom of the slide using a Kimwipe.
3. Observe the pollen under 100X and 400X magnification
4. Record the shape, size and any unique features of the pollen grains. You may draw the pollen shape on a piece of paper and then take a photo.
5. Continue to check the pollen.

Workshop 10

RABLE 2015

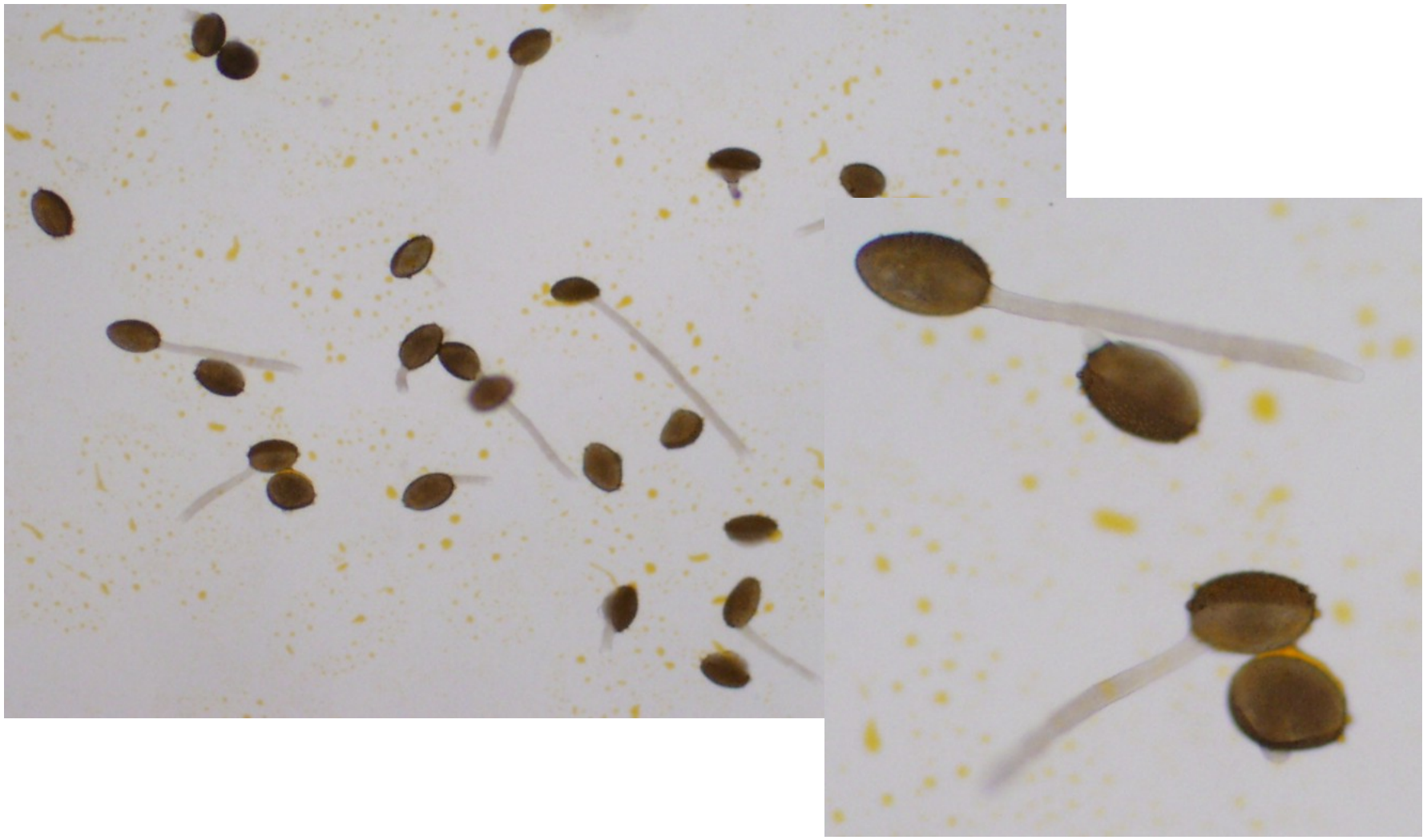
RABLE 2015


Sex in the Garden
Medium Workshop 2 (3:30-4:50)
Pollen Germination

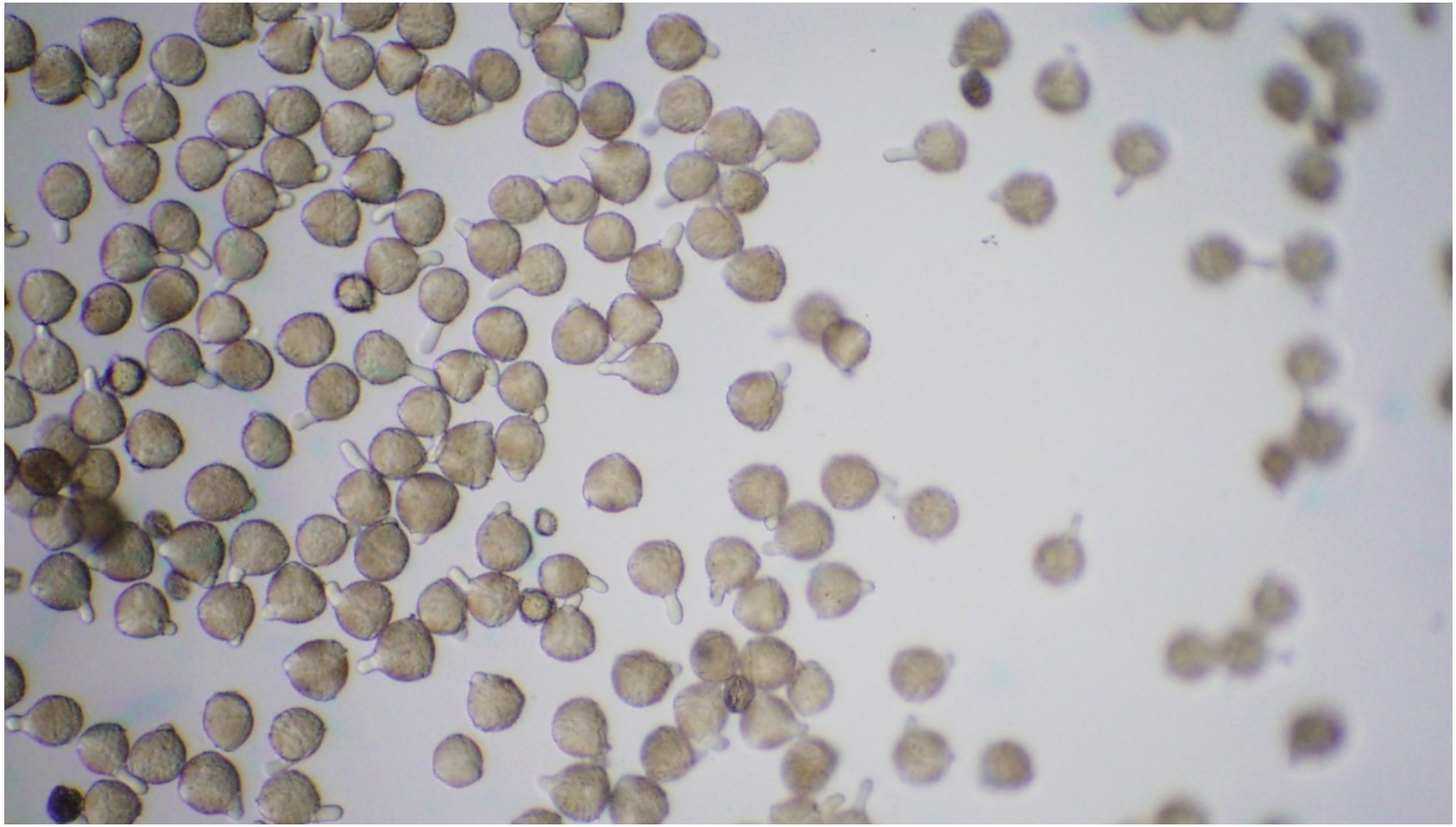

Anthers
Stigma
Pollen
Narcissus jonquilla
Common Trumpet Daffodil

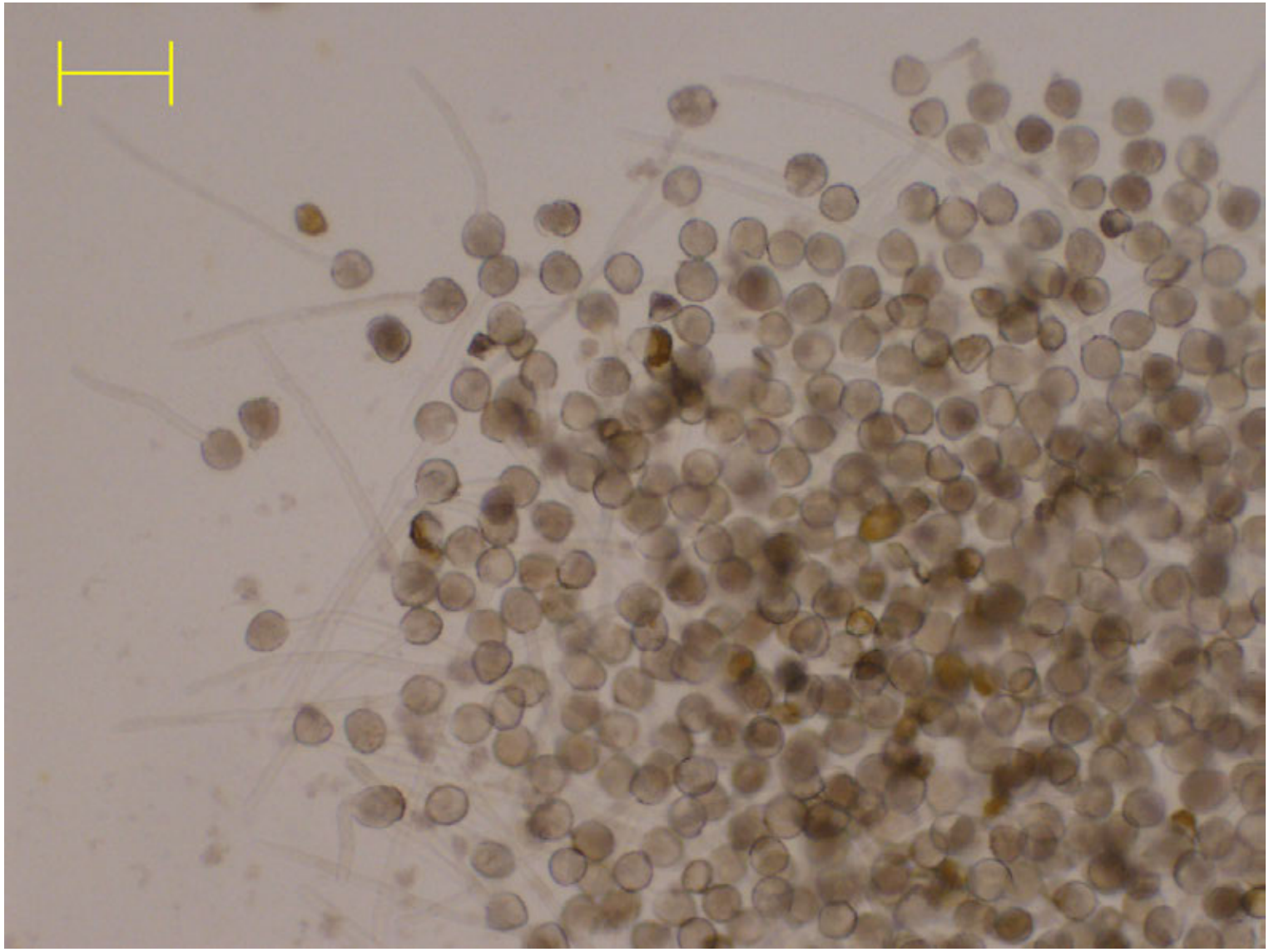

Tip: Pollen
Make sure you can “brush off” some pollen with your finger

Hemerocallis sp.
40 min hanging drop



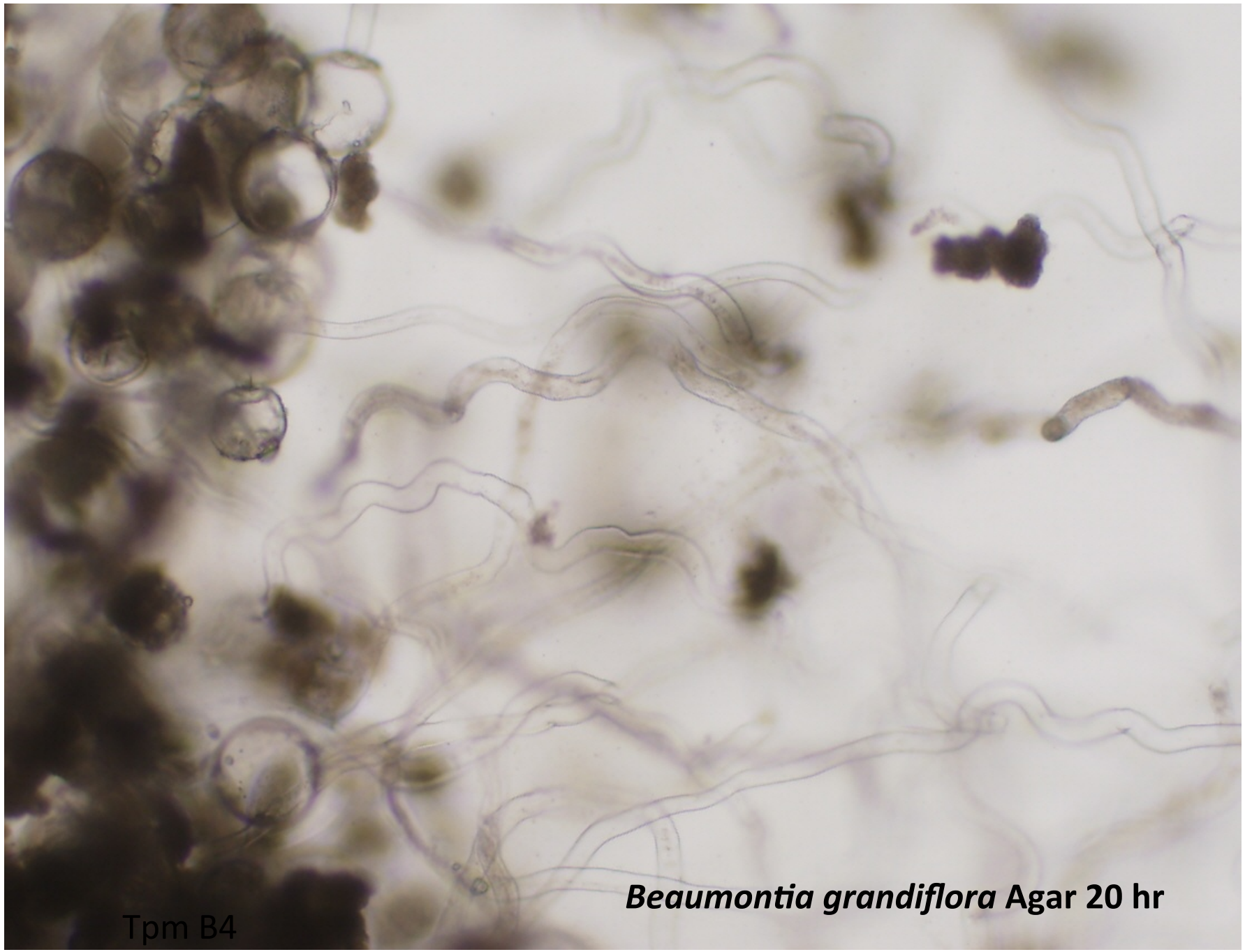
Petunia exserta
30 min hanging drop

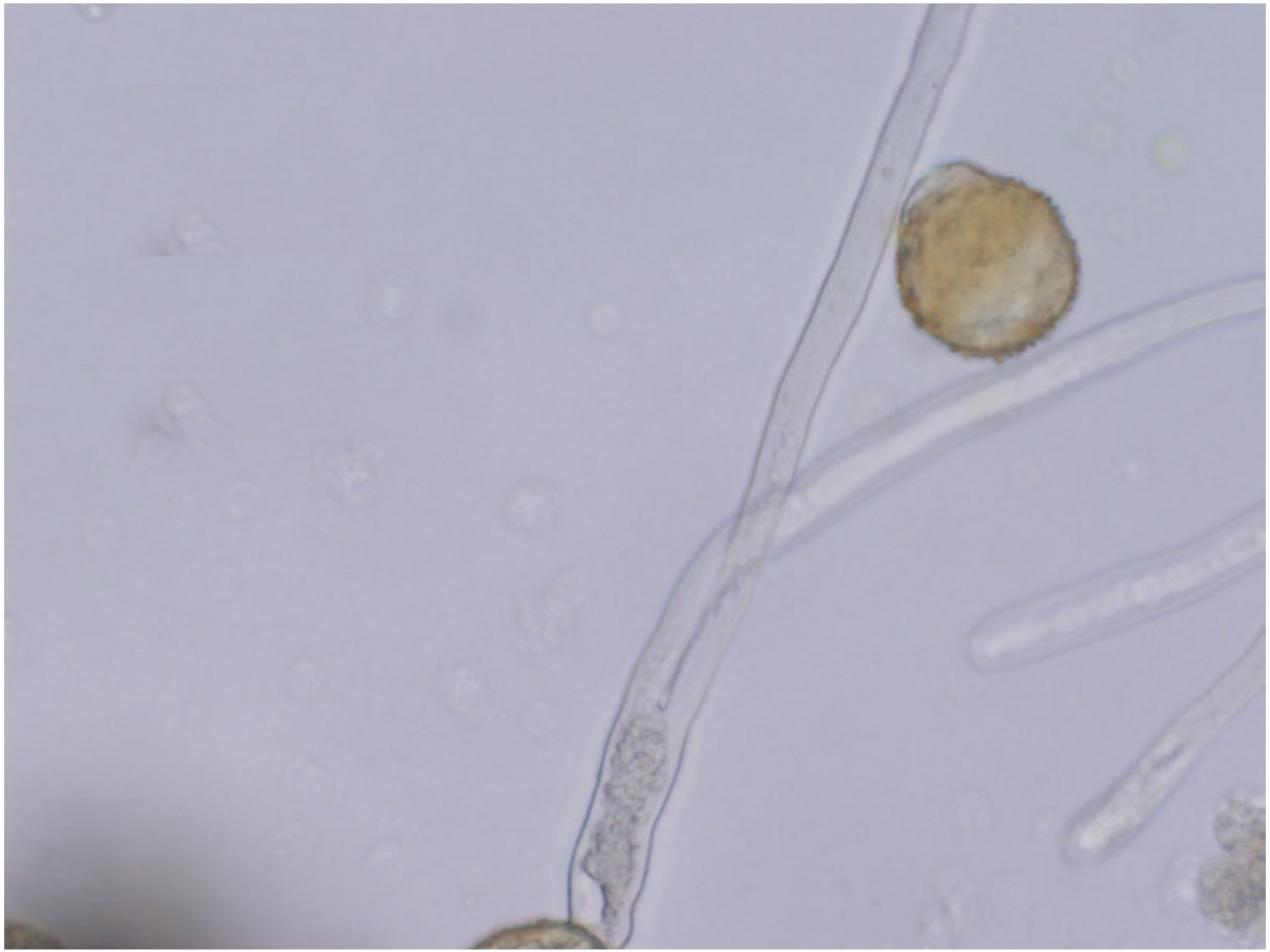




***Tulaghia violacea* 75 min**

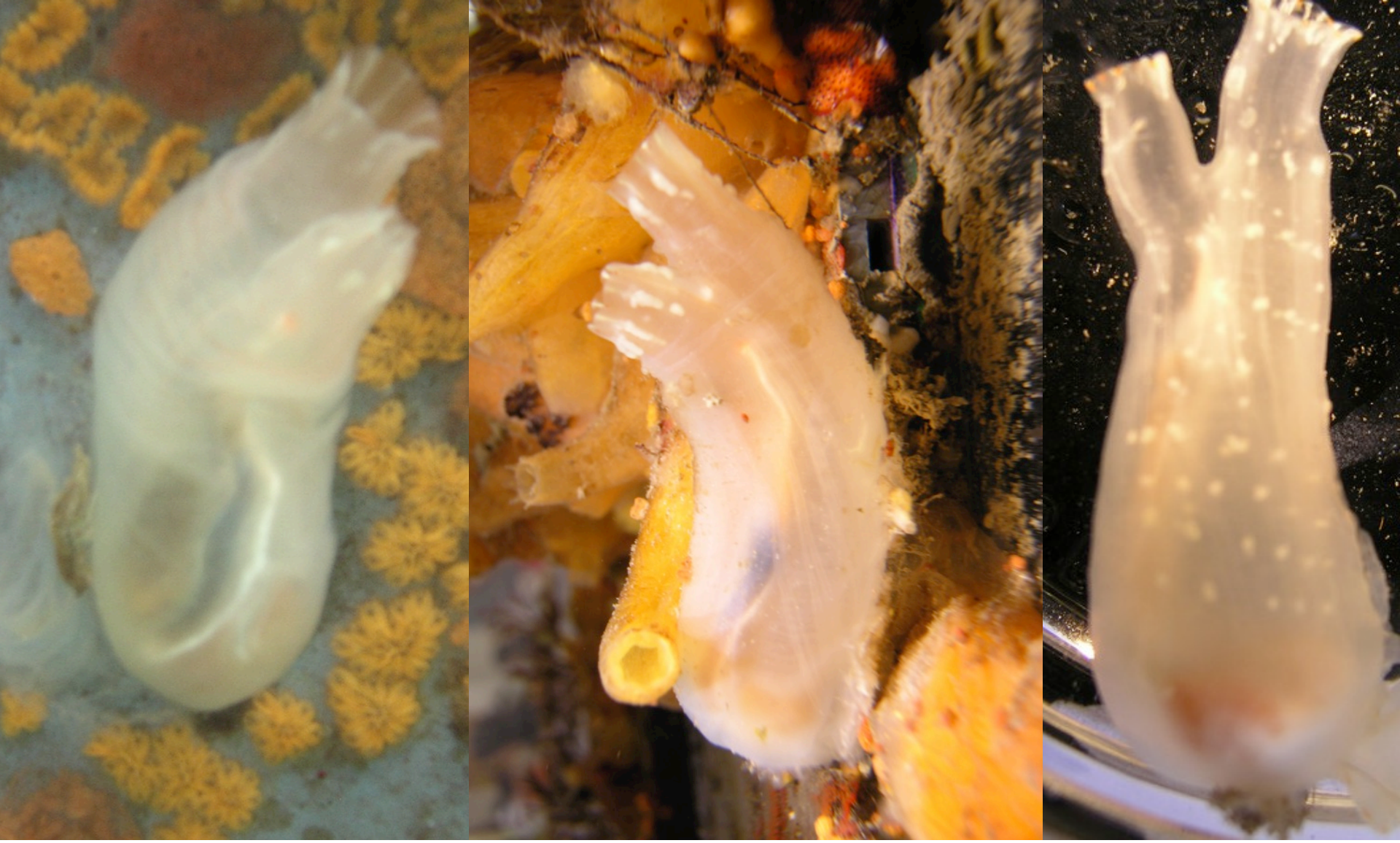

Clivia sp. Agar 24 hr


Beaumontia grandiflora Agar 20 hr
Tpm B4



***Rosa* 90 min**


Aloe agar 60 min



Ciona spp.

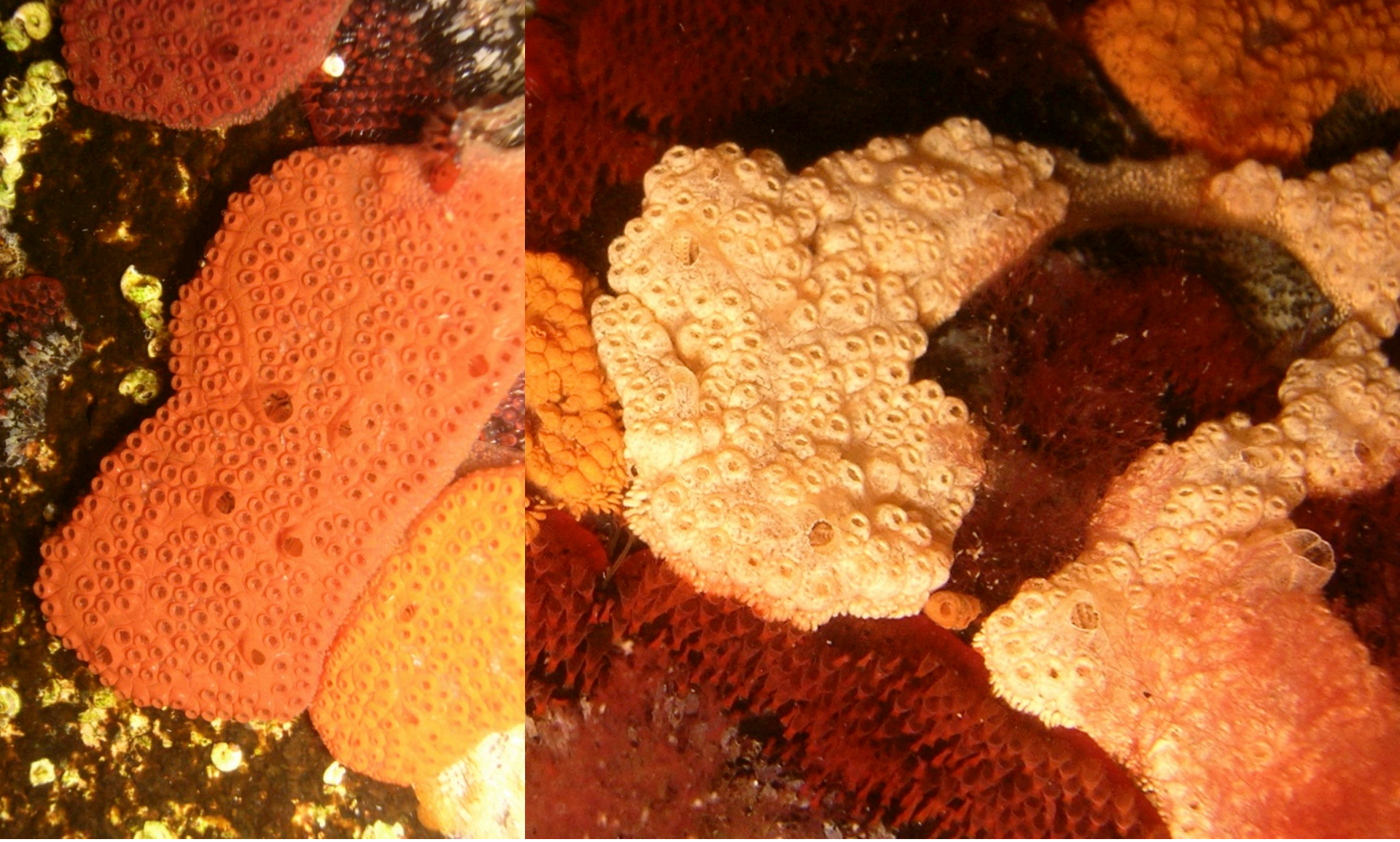
- Translucent or yellowish solitary tunicate
- Siphons are close together

Ascidia ceratodes

- Yellow to orange solitary tunicate
- Siphons are far apart and perpendicular to each other

Styela spp.

- Brownish solitary tunicate (not common on our plates)
- Some have a shorter stalk than others

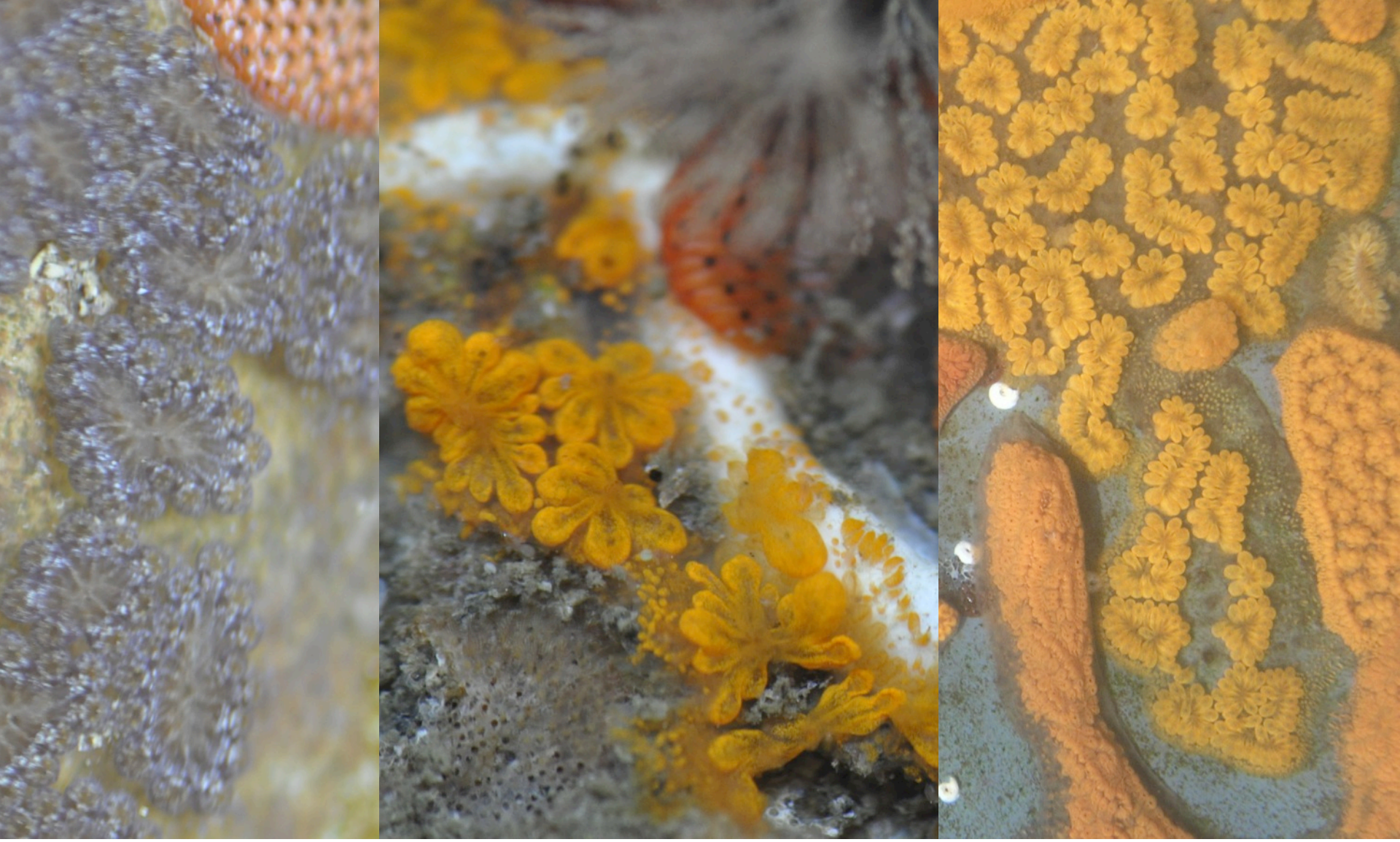


Botrylloides violaceous

- A colonial tunicate with irregularly arranged zooids
- Often entirely, orange, pink purple or red

Botrylloides diegensis

- A colonial tunicate with irregularly arranged zooids
- Always bi-color, often with orange zooids



Botryllus schlosseri

- Colonial tunicate with circular or flower-shaped patterns
- Usually orange, but can be variable

Distaplia occidentalis

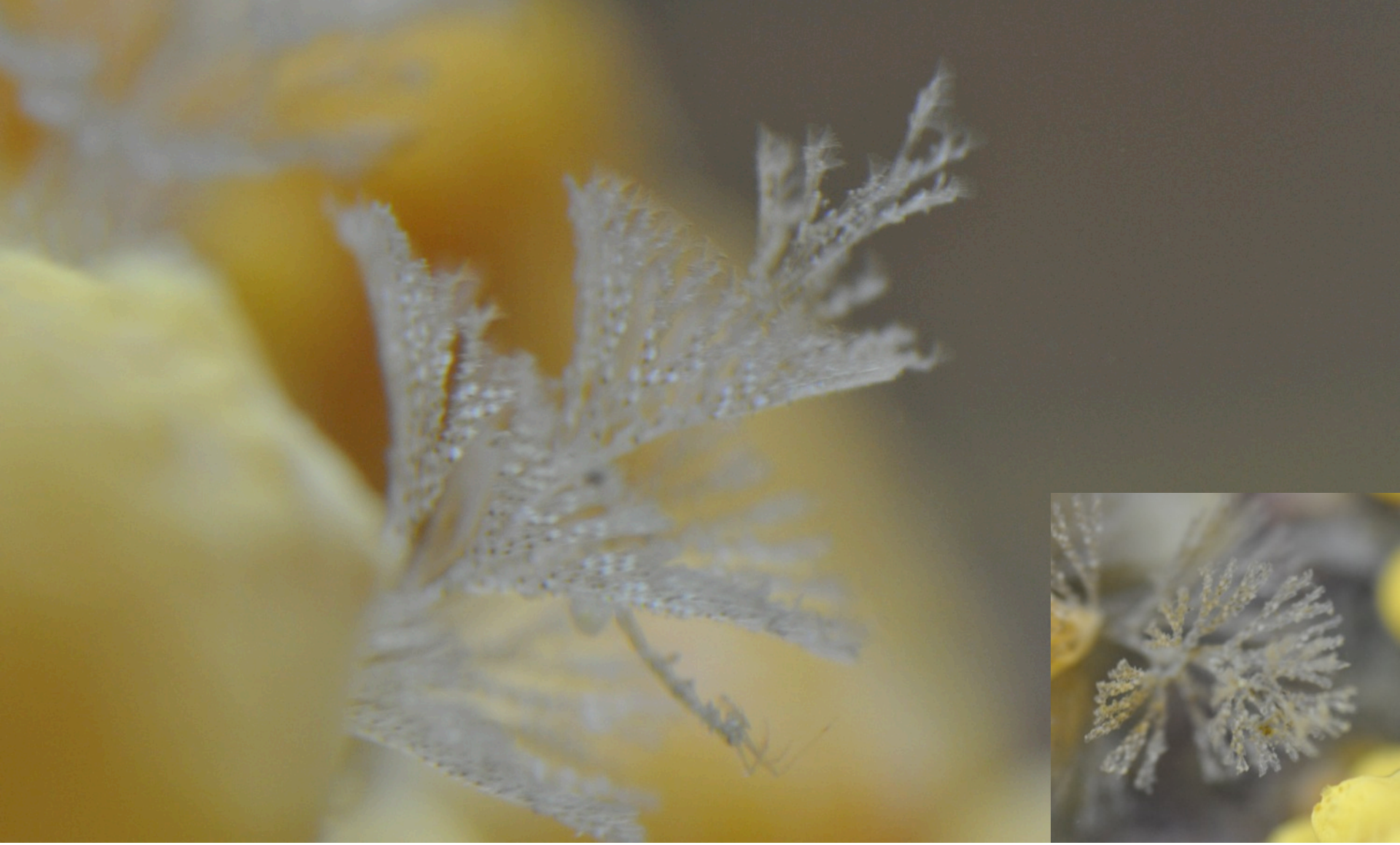
- Mushroom shaped colonial tunicate
- Usually yellow or white

Didemnum sp.

- Yellowish to cream colored colonial tunicate with irregularly arranged zooids
- Larger colonies often have lobes that do not sit flat on the substrate

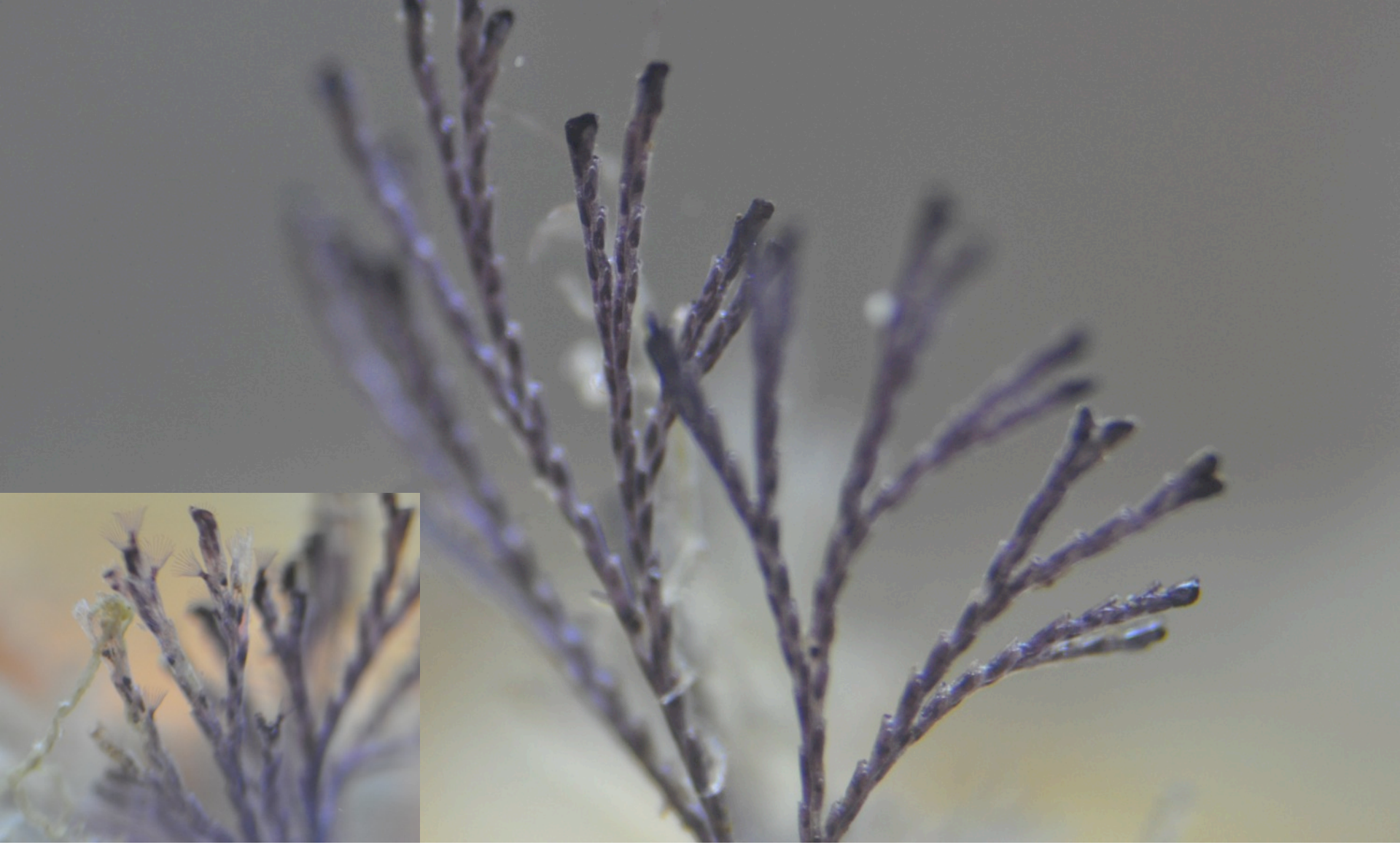
Diplosoma listerianum

- Olive green to translucent colonial tunicate
- Very thin and encrusting with tiny zooids



Bugula sp.

- White arborescent bryozoan
- Spiral branching polyps



Bugula neritina

- Purple to black arborescent bryozoan
- Does not have spiral branches

Watersipora subtorquata

- Bright encrusting, reddish orange bryozoan with black dots
- Often rises up three dimensionally and is very brittle

Schizoporella unicornis

- Bright orange encrusting bryozoan
- Always lays flat

Metridium senile

- Small white or orange anemone
- Tentacles can be white, orange or brown

Obelia sp.

- Branching colonial hydroid with tiny white polyps at the tips of branches
- Polyps are often retracted

Spirorbid polychaete worms

- Small spiral shaped calcareous tubes
- Worms are often retracted when disturbed

Serpulid polychaete worms

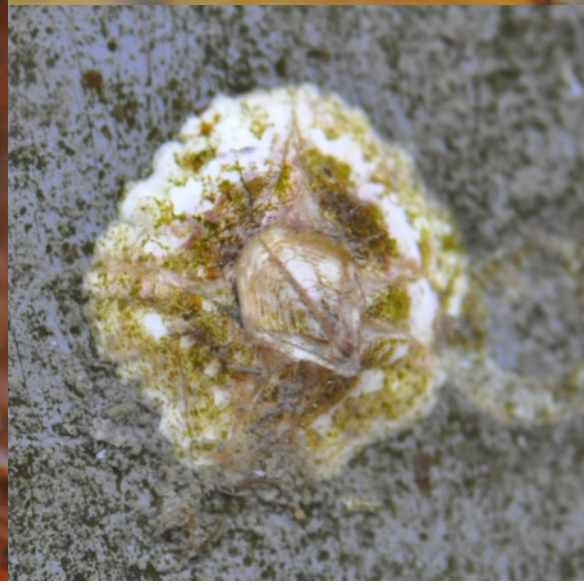
- Larger than spirorbid worms with irregular shaped calcareous tubes

Sabellid polychaete worms

- Feather duster worms
- Frilly tentacles are often closed when disturbed

Terrebellid polychaete worms

- Commonly called spaghetti worms
- Has a mud tube with small strands of tentacles searching for food.



Acorn barnacles

- Small sessile crustacean that cements plates to a surface
- Uses appendages (cirri) as a net to catch food

Mytilus spp.

- A common black or grey bivalve that attaches using byssal threads
- It usually has other organisms growing on its shell.

Workshop 11

RABLE 2015

Lab 6
Succession in a Marine Fouling Community

Goals and Objectives

At the end of this laboratory you should be able to:

1. Estimate the distribution and abundance of species within a community.
2. Explain what resources are shared among community members.
3. Predict the possible outcomes of competition for shared resources.
4. Describe the mechanisms by which resources are renewed.
5. Analyze the change in community constituents over time.
6. Propose mechanisms to account for the observed changes.
7. Explain the process of ecological succession and suggest mechanisms underlying observed changes.
8. Describe the extent to which scientific statements are limited by data.

Pre-lab Introduction for Lab 6

Read and complete the pre-lab exercise before Monday 9 a.m. of lab week. Read the rest of the lab to get a general idea of what you will be doing.

A community is a collection of different organisms living in a particular place. Recall that the communities you examined in the diversity lab differed from each other in species richness and abundance. Community richness and composition changes over time in a process called **succession**. Biologists have long wondered why communities differ in diversity and composition, so there are many hypothesized explanations, each of which places different amounts of emphasis on the arrival of colonists, availability of resources, and interactions among residents. Some of these hypotheses are listed below:

Community age—older communities have had more time to be colonized by species from surrounding areas (increasing diversity) and for interactions among species to have effects (increasing or decreasing diversity).

Energy inputs—all food webs are based on primary productivity (carbon fixation), so productive areas, such as equatorial regions with abundant light and warm temperatures, should support more species.

Resource availability—communities with a range of resources (heterogeneous habitats) should contain more species.

Competition—given sufficient time, a superior competitor may dominate a community, monopolize resources, and exclude other species (reduces diversity).

Predation—large or ecologically important predators reduce prey population sizes; this may reduce competition, permitting many different species to persist.

Facilitation or habitat modification—the activities or mere presence of some species modify the physical environment in a way that enhances the success of other species, leading to changes in species composition.

Access to colonists—isolated communities receive fewer colonists than less isolated communities, so isolated communities may have lower diversity.

Rates of disturbance —frequent disturbance removes both individuals and species, continually providing open space and preventing dominance by superior competitors. This may lead to an increase in diversity.

The potential explanations for differences in diversity just presented are not mutually exclusive, meaning that more than one can be accepted as true. To distinguish among potential explanations, one must have unique testable predictions for each. Tests of a hypothesis can be made using experimental or comparative methods.

Comparative methods are often used when experiments are very difficult to do. For example, if you wanted to know whether plant diversity declined as redwood forests aged, it would be difficult to create replicate forests and follow species over time because redwood trees live for so long. However, it would be possible to compare the tree species present in natural redwood forests of different ages to look at patterns and causes of succession.

Natural experiments are another useful tool for studies of community development and species succession. As stated earlier, **succession** describes the pattern of change in species composition in a community. Succession often proceeds through "stages" characterized by different types of species. Sometimes these stages are quite distinct, but more often they grade into one another. Ecologists study succession as a way of predicting the trajectory of community recovery after a human-caused or natural disturbance that creates free space. A natural experiment presents biologists with the opportunity to study the results of an event that could not be experimentally induced. For example, the volcanic eruption on Mount St. Helens created empty habitats, resulting in the perfect opportunity for a natural succession experiment. Biologists wondered whether plants would colonize the slopes of the mountain in a particular order. They began to study the site immediately. Lupines were among the first plants to colonize, and subsequent study revealed that lupines modify the soil by adding nitrogen to it. A symbiotic association with an α-proteobacterium (*Rhizobium*) permits lupines to fix atmospheric nitrogen; at first nitrogen is bound in plant tissues, but it later enters the soil when dead plant matter falls to the ground and decomposes. This nitrogen addition is vital to later colonists without similar symbiotic associations. By combining observations from a large natural experiment with results from detailed experimental studies, ecologists were able to show why certain plants were among the first colonists and how these plants modified the environment for species that arrived later.

Figure 6-1

In communities with long-lived organisms like trees, succession is often studied using comparative approaches in combination with natural experiments. For example, ecologists have developed an understanding of how forests regenerate by comparing the species composition of communities that currently exist in areas that were once farmed but have since been abandoned. These sites are usually called "old fields." Ecologists use the time since old-field abandonment as a surrogate for the age of the community and arrange communities in order of age to infer the likely sequence of succession. Abandoned fields are shown in Figure 6-1 on the previous page: moving from left to right, the first field is only 2 years old, the second is about 10 years old, and the third has been abandoned for more than 100 years. You will notice that succession can be characterized by discrete stages, although there is, of course, considerable overlap in species composition between adjacent stages as a community moves from one stage to another.

Notice the openness of the youngest "old field." The first colonists are grasses and herbs. Think about seed production—do grasses make many seeds or few? Think about seed movement—do grasses disperse well or poorly? Think about resources such as light or nutrients—are they likely to be limited, and will residents have to compete for them?

Notice that the 10-year-old field has shrubs. Think about plant size, density, and reproductive rates. Think about resource competition in comparison to that in the younger field. Is a new grass plant likely to establish itself (recruit) in this field?

Notice that the 100-year-old field has trees with little undergrowth. The plants that are present in the undergrowth are ferns—what is the typical light regime for a fern? Notice that there is some open space beneath the trees. Why is this space open? How has the habitat for potential colonists changed? Is it more likely that potential colonists cannot reach the habitat or that they could not survive if they did reach it?

The rhetorical questions just posed were designed to help you think analytically about succession. Using the information and ideas outlined above, in conjunction with what you have learned in lecture, answer the numbered questions throughout the sections below:

Pre-lab Questions—Record Your Answers on the Online Version

Species at each stage of succession often share many traits in common. Recall from earlier in the quarter our discussion of different life-history strategies.

1. Would you expect early successional species to be r-selected or K-selected?
2. Why?
3. What about species that dominate later in succession? Would you expect them to be r-selected or K-selected?

   Why do early successional species give way to later species? This is usually because they are poor competitors, and they either alter the environment in a way that reduces their own success or eventually succumb to competition from other species. Why don't late successional species dominate from the start? There are several possibilities. One is that it may take a long time for colonization by these species to occur because of poor dispersal abilities and slow growth rates. This is called neutral or tolerance succession. In contrast, early successional species can either facilitate or inhibit succession. The example of succession of plants on Mount St. Helens following a volcanic eruption is an example of facilitative succession because early colonizers modify their environment by adding nitrogen, which facilitates or enhances the ability of other species to colonize. Without lupines, later successional species would become established at a much slower rate.

   In inhibition succession, early successional species suppress later colonists and succession proceeds only when some other agent of mortality (such as changing physical conditions, or predators) causes early colonists to die. Under inhibition succession, late successional species would become abundant more quickly in the absence of the early colonizing species. This occurs most often when early colonists preempt resources by occupying all available space, light, or other limiting resource, thereby

inhibiting the establishment or growth of later successional species. We will see examples of this in lecture.

In the first lab you were interested in characterizing patterns of species diversity within a community. Because of succession, community composition changes over time. How do you think that species diversity changes over time as succession proceeds? From the list of potential causal hypotheses above, you can see that making a prediction may not be straightforward. Early successional communities might be diverse because few species have been excluded and resources are abundant, or they might be species-poor because conditions are sufficiently stressful and few species can tolerate these conditions (as in the Mount St. Helens example). Similarly, more time might allow more species to colonize, but on the other hand, this also might allow more time for competitive exclusion to happen. A resolution of these two ideas suggests that diversity might be greatest in communities of intermediate age because these have sufficient time for many species to become established but not enough for competitive exclusion to have resulted in declines in diversity. Look back at the photographs of old fields of different ages—can you see any evidence in support of this hypothesis?

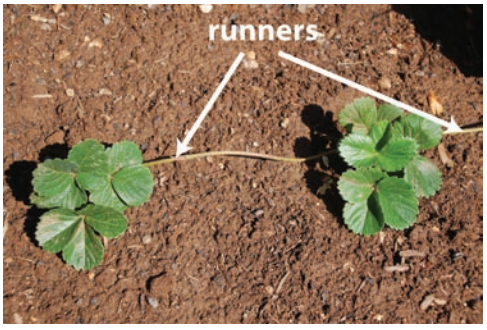


Figure 6-2

In the lab exercise for this week, you will look for elements of succession in a community of marine organisms that encrust hard surfaces in ocean waters. You will use the comparative method by examining the species present on hard surfaces placed in the ocean for different lengths of time. You will see communities that differ in age: approximately 3 months, 6 months, and 9 months old.

At first glance, many of the animals you will study may remind you of plants. Many adult marine animals are sessile—they attach to hard surfaces and most cannot move once they have settled into a particular place. Some sessile animals are **unitary**—they grow by adding new cells to an existing individual, but most are **modular** and grow by *copying feeding units* of the body many times. Perhaps you have seen a spider plant, Bermuda grass, or a strawberry grow in this way.

Figure 6-3

When the first strawberry plant is established, it sends **runners** out across the soil (Figure 6-2).

New plants develop when the runners put out **roots** (Figure 6-3).

The new plants are attached to the first plant, but once they have roots, they can survive if the runners break.

This modular growth strategy allows a single genetic individual to exploit a large area of the habitat.

From lab #2, you know something about a plant's physiological needs. Plants have to collect resources and compete with other plants for access to those resources. Imagine a **modular plant**, like a strawberry, grown in the same planting box with a **unitary plant**, like a daisy. Both start from seedlings. Both plants need light, but their growth and competitive strategies differ.

4. Two resources are given below. Add a third of your choice, and *briefly discuss the relative costs and benefits* of having a modular growth strategy for each resource:

   (a) Competition for light:

   (b) Competition for soil nutrients:

   (c) Competition for _____.

5. Is a modular growth strategy better for capturing concentrated resources or widespread (dilute) resources? Briefly explain your thinking.

Modular animals have some similarities to modular plants, but they also have some important differences such as the ability to feed once they are overgrown. Below are thumbnail sketches of a few common types of encrusting marine unitary and modular organisms that you are likely to see in lab. You do not have to memorize any taxonomic terms, but a passing familiarity with common names may make it easier to discuss what you see with your classmates.

A unitary (solitary) **tunicate**, also called a **sea squirt** is shown in Figure 6-4. The important thing to notice is the bloblike body with two siphons at the top. The animal feeds by collecting food particles from the water around it; water enters the body through one siphon, passes through an internal mesh basket where particles are filtered out, and exits via the other siphon. You may see several species.

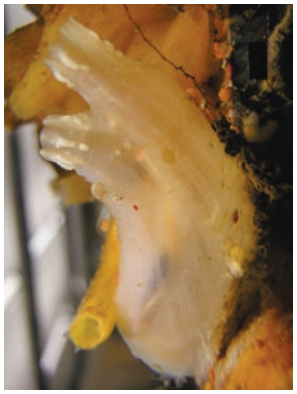
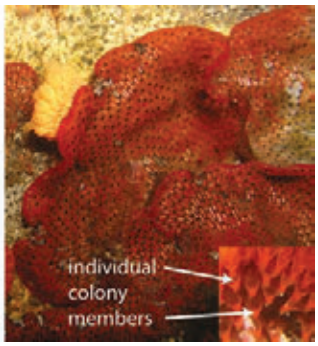


Figure 6-4

Modular sea squirts develop as one animal buds to produce another. The buds remain attached to each other. Such animals are called **colonial tunicates**, and each budded module is usually very small. The colony has a firm, fleshy appearance and is covered with small pores where the siphons of each module open to the colony surface (Figure 6-5). The siphon openings are visible and are sometimes arranged in pathways or clusters. Many tunicate colonies have an extremely low surface pH; this might affect colonization or overgrowth by other species?

Figure 6-5

Bryozoans are stiff, somewhat crunchy, encrusting animals. They have a modular growth pattern: the first organism makes a series of buds, and each bud makes others, until a colony is formed. The shape of the colony can be treelike, a flat crust, or something in between (Figure 6-6). Bryozoans feed by collecting tiny particles from the surrounding water on a ring of tentacles.



You cannot see the individual modules in these photos.

Figure 6-6

Some of the other unitary organisms you may encounter:

Tube-dwelling worms collect particles on a retractable crown of tentacles. The mouth is in the center of the tentacle crown. The animal in Figure 6-7 has a flexible tube (see arrow), whereas the animal in Figure 6-8 has a hard tube (see arrow) made of calcium.

Figure 6-7

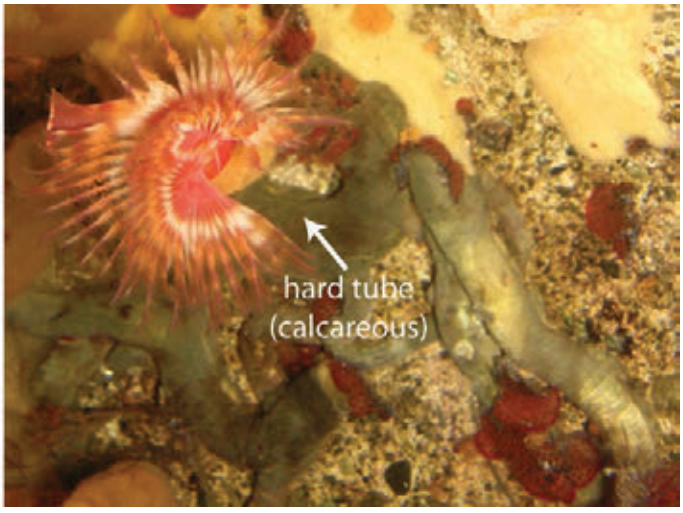
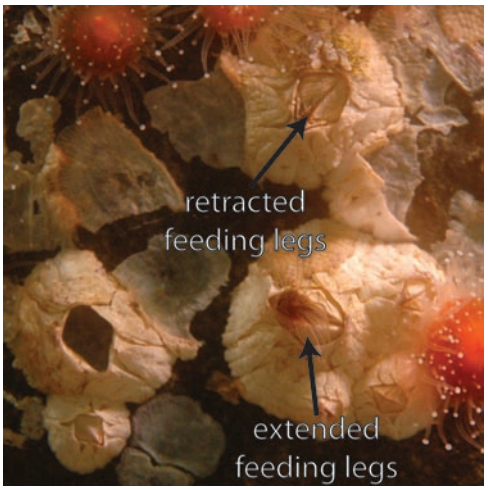


Figure 6-8

Barnacles collect food by combing a feathery structure through the water above their bodies. The feeding structure, a set of legs, can be pulled into the hardened shell (as in the upper animal in Figure 6-9) or extended through the slit at the top of the body (lower animal).

Mussels attach to hard surfaces by a set of strong threads (byssal threads). They have two shells with a small **gape**, or opening, between them. Water, containing food and oxygen, enters via the gape (see arrows in Figure 6-10) between shells and food particles are collected on an internal gill covered with mucus.



Barnacles with feeding legs retracted and extended

Figure 6-9

Figure 6-10

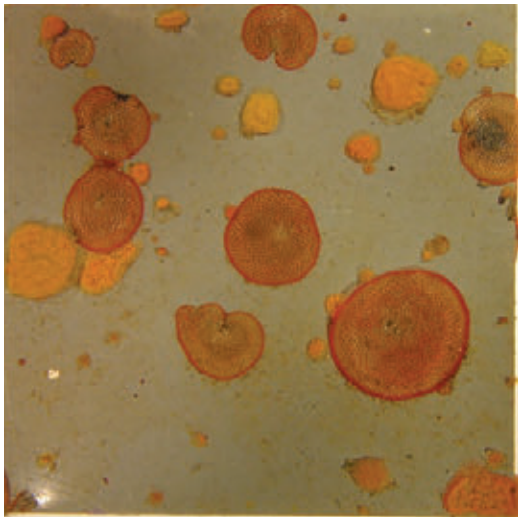


Figure 6-11

Figure 6-12

6. What do most sessile animals eat?

7. Briefly describe one way animals could compete for this kind of food source.

Figure 6-11 depicts a panel similar to those you will see in lab this week. The panel is hard, so animals do not penetrate it as roots do with soil. This panel was placed in the ocean only a short time ago, so much of it is still bare (the gray area).

8. What kind of animal is the largest orange crust?

9. For what resource might sessile animals compete?

The panel in Figure 6-12 has been left in the ocean for many months.

10. Name two kinds of animals that are visible:

(a)

(b)

Space is clearly at a premium on the older panel shown in Figure 6-12.

11. Describe one growth strategy you see here that was not visible on the younger panel:

12. What can you see that indicates competition among organisms?

13. Why might it be advantageous to have a modular growth pattern in this situation?

Have you started to wonder how animals get onto the panels in the first place? Animals colonize the panels as free-swimming larvae. Some larvae feed in the swimming stage and others do not. Those that do not feed have only a short time in which to find a settlement site—if they delay settlement, they will not have sufficient energy reserves left to metamorphose into adults after they settle.

All newly exposed panels are empty, so the development of the community is affected by which larvae are in the water at the time the panels are exposed. The presence of larvae in the water depends on which species in the surrounding community are reproductively active at the time space is available. Reproductive activity in animals varies seasonally in the same way that some wildflowers bloom in late winter and others bloom in mid-summer. However, unlike seeds that can remain dormant for some time, the larvae of encrusting marine organisms begin growing as soon as they settle. Once the blank panel is filled, there is an opportunity for new settlers to land on top of existing animals and for competition among organisms to affect community composition.

14. List one factor that you imagine would limit settlement on top of living organisms.

The panels hang vertically from the side of a dock. Animals such as crabs and snails live on the dock and can crawl onto the panels. The ocean can be stormy at times, and heavy rains mix freshwater into the ocean surface waters.

15. Use your imagination to describe two ways in which disturbance of the sort just described might affect the animals that live on the panels and amount of free space available.

(a)

(b)

16. In what way might *growth form* (shape) of the panel inhabitants affect the likelihood of surviving on the panels?

17. Are modular organisms more or less likely to be part of the *long-term* community than are unitary organisms? Explain your thinking.

In lab this week you will need to observe patterns of species distribution and to develop testable mechanistic explanations for these patterns. You may want to look back at the organisms just described to refresh your memory on their natural history and lifestyles.

The laboratory exercise begins on the next page.

Lab 6: Succession in a Marine Fouling Community

In lab, you and a partner will catalog the number and relative abundances of species in communities of three different ages living on 10 cm × 10 cm panels. Find at least three more pairs of students to pool your data with.

Select a panel and place it in a large plastic tub filled with seawater. Be sure to randomize your selection using the dice provided. Use the string grid, similar to the one in the picture below to estimate percent cover for the different species. Do not count any growth that extends beyond the panel. *Your grid will divide the panel surface into 25 squares each equal to 4% of the total surface area***.** Use the fraction of a square filled by a particular organism to estimate percent cover. For example, the colonial tunicate outlined in red in the upper right of the panel in Figure 6-13 covers about 100% of the total area in 4 squares, or about 16% of the total surface area (4 squares @ 4% each).

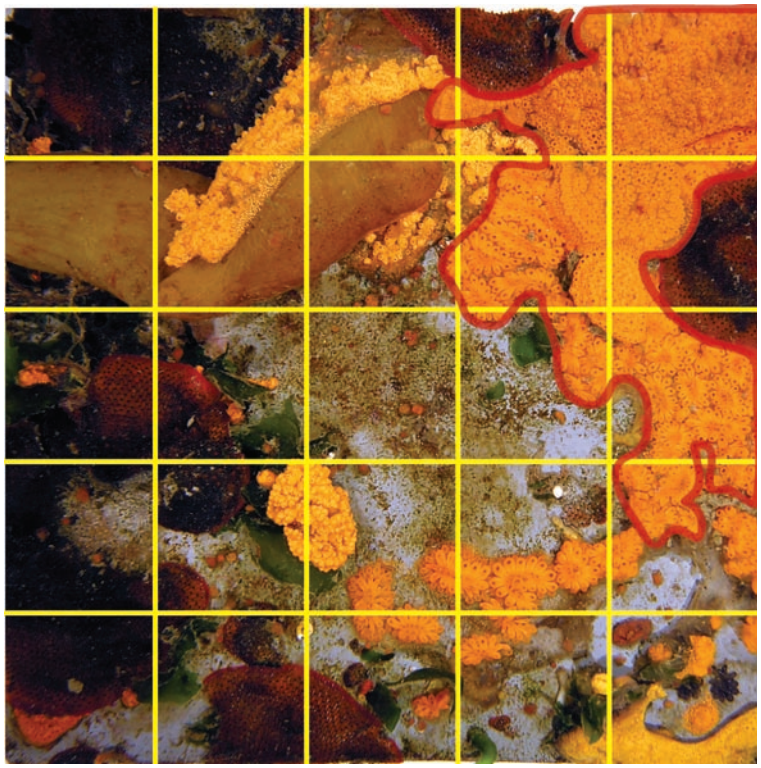


Figure 6-13

Using what you just learned about the basic kinds of encrusting animals and the photographic key provided in lab, count the number of *sessile* species *on one panel of each age*. You will distinguish between the number of species on the primary substrate (the panel) and those on secondary substrate (growing on other organisms = overgrowth) on each panel.

Examine the panels at the lab bench; you can put them under the dissecting microscope if you wish. This may be necessary for panels that look bare—they are not! Be gentle with the animals as they are delicate and will be handled by many students this week. It is *not* important that you correctly identify each species with its proper scientific name; the keys provided are meant only as a guide. What is important is that you are consistent for all the panels you count and clearly delineate the characteristics

of your species. For example, the outlined colony in the figure above is *Botrylloides violaceous*. You will have a picture key in lab that will clearly identify this. However, whether you call it "*Botrylloides violaceous*" or "orange encrusting blob" on your data sheet does not matter much, so long as you always identify this organism in the same way. Meet with the other three partners in your group to standardize your identifications. Your TA will give you some other hints on how to identify and operationally classify "species" for this lab. Use your experience in delineating "operational taxonomic units" for your survey in lab #1 as a guide.

Record Your Data on the Grids on Pages 159 to 161

(1) Estimate the relative abundance (as percent cover) of each species on each of the panels; be consistent about what you call a species. First, record the total cover of each species on the panel. Place these data in column A of the data grid. Then record the percent cover of each species that is growing *directly* on primary substrate (the panel), rather than on top of other organisms. Record these data in column B of the data grid. The total area occupied by all species may be greater than 100% because of overgrowth, but the total amount of primary space occupied should not exceed 100%.

(2) Count the number of species on each panel.

(3) Use number of species and percent of total cover to calculate the diversity of each panel with the Shannon-Wiener index you learned in lab #1.

(4) Calculate the *percent overgrowth* for each species on each panel by *subtracting* the percent on primary substrate (column B) from the percent of total area (column A) for each species.

Instructions for further calculations and the lab report follow the data sheet pages. **Instruction (5) is on the top of page 162.**

When you have finished with a panel, put it back in the flume exactly where you found it.

Data Collection in Lab

Panel Age

Species	Common name or identifier	Total % cover (A)	P_i	% Cover on panel surface (B)	% Overgrowth (= A – B)

Total number of species =

Total % cover =

Shannon-Wiener Index value =

Total % on primary surface =

Total % overgrowth =

Data Collection in Lab

Panel Age

Species	Common name or identifier	Total % cover (A)	P_i	% Cover on panel surface (B)	% Overgrowth (= A – B)

Total number of species =

Total % cover =

Shannon-Wiener Index value =

Total % on primary surface =

Total % overgrowth =

Data Collection in Lab

Panel Age

Species	Common name or identifier	Total % cover (A)	P_i	% Cover on panel surface (B)	% Overgrowth (= A – B)

Total number of species =

Total % cover =

Shannon-Wiener Index value =

Total % on primary surface =

Total % overgrowth =

(5) Pool your data with the other three pairs of students in your group for a reasonable sample size. Calculate **average** values for number of species, total percent cover, the Shannon-Wiener index, percent cover on panel, and percent overgrowth **for panels of each age**.

Panel age	Number of species	% cover	H′ value	% cover on panel SFC	% cover as overgrowth
3 months					
3 months					
3 months					
3 months					
Average:					
SD					

Panel age	Number of species	% cover	H′ value	% cover on panel SFC	% cover as overgrowth
6 months					
6 months					
6 months					
6 months					
Average:					
SD					

Panel age	Number of species	% cover	H′ value	% cover on panel SFC	% cover as overgrowth
9 months					
9 months					
9 months					
9 months					
Average:					
SD					

Name: _____ TA: _____ Date: _____

Laboratory Report

(6) Plot each of the five values (number of species, total percent cover, Shannon-Wiener index, percent cover on panel, and percent overgrowth) on the y-axis against panel age on the x-axis (5 plots total). Your TA will provide graph paper. You can make one set of plots per pair of students and photocopy them; otherwise make one set per individual. (**½ point per plot**)

(7) Describe the results shown in each plot in a few sentences. Write the sentences on the side of *each graph*. (**½ point per description**)

(8) Select one result that interests you and your partner. Develop a **potential explanation** for this result that is consistent with the observations.

For example, you might observe a decline in the number of species over time but notice that all the species on the oldest panels were colonial tunicates. Perhaps you would like to consider what factor(s) are responsible for the decline in species richness and why colonial tunicates dominate late successional communities.

You might hypothesize that the preponderance of tunicates was due to superior competitive ability, or better food capture rates, or a great abundance of tunicate larvae, or . . . There are several potential explanations on pages 149–150; ***weave these ideas together with information gained from the pre-lab text on succession.***

Results: (**½ point**)

Proposed hypothesis to explain this result: (**½ point**)

(9) Develop a testable prediction of your hypothesis.

Our hypothesis predicts: (**½ point**)

Our mathematical prediction: (**½ point**)

Briefly **outline a feasible experiment** that would test your hypothesis. Outline the essential features of the design by describing what you would do and which data you would collect.

We could test our hypothesis by: (**½ point**)

(10) Describe one experimental *outcome* that would *support your hypothesis* and *one outcome that would refute your hypothesis*. Remember to refer to your prediction.

Our hypothesis would be supported if the experimental outcome was: (**½ point**)

Our hypothesis would be refuted if the experimental outcome was: (**½ point**)

(11) Find another pair of students and ask them to explain the result they elected to study. Listen to their proposed explanation for the result. Develop an *alternative explanation* for the result this pair of students elected to study. *They will do the same thing for your hypothesis.*

My classmates, _____, observed:

They hypothesized:

Their prediction was:

An alternate explanation and prediction for their result is: (**½ point**)

(12) Examine the alternate explanation that the other pair of students has developed for *your* result.

My classmates thought *my result* could be explained by:

Their prediction was:

Would your planned experiment indicate that only one hypothesis was correct? (explain—could you rule out the other hypothesis?) (**½ point**)

(13) Assume that the experiment that *you and your partner* outlined went as planned. What conclusion (one or two sentences) would you be able to draw? The conclusion must be based on the data you intended to collect. (**½ point**)

If the experiment went as planned, I would conclude that:

Lab: Succession in a Marine Fouling Community

Course: BIS2B--Introduction to Evolution and Ecology

University of California, Davis

Pat Randolph
Dept of Evolution and Ecology

Big Questions (Lab Goals):

What is succession?

How can we determine if succession is occurring?

How might we quantify a change in a community through time?

What mechanisms can explain WHY there is a change in community structure? (develop a hypothesis)

How would we test our hypothesis?

Basic Premise:

Community structure changes through time

Colonizers (pioneer species: r-selected species)

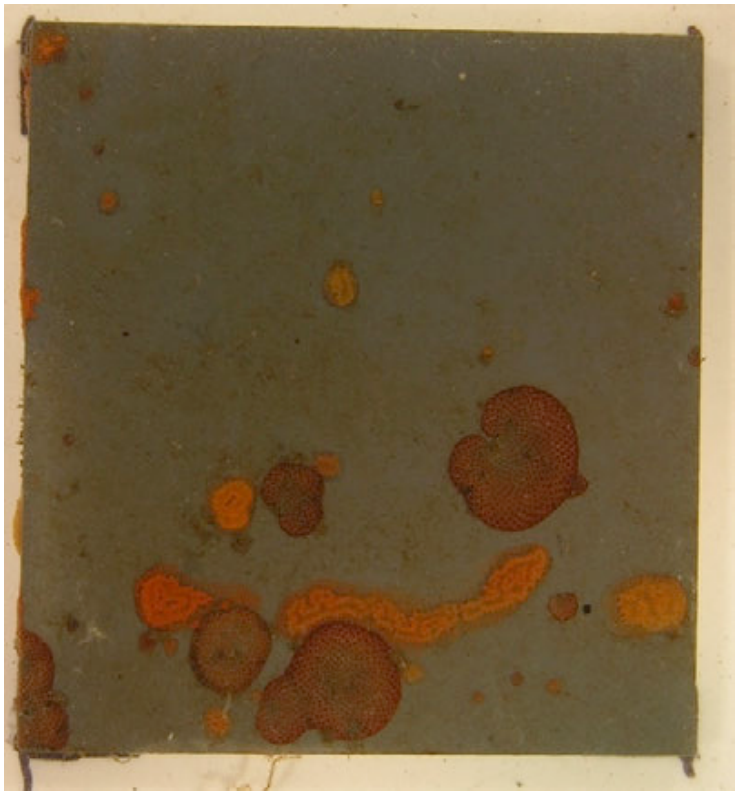
Mid-successional species (mix of r- and K-selected species)

Late successional species (climax community: mostly K-selected species)

What system would let us test the idea of change in the community in a 3-hour lab?

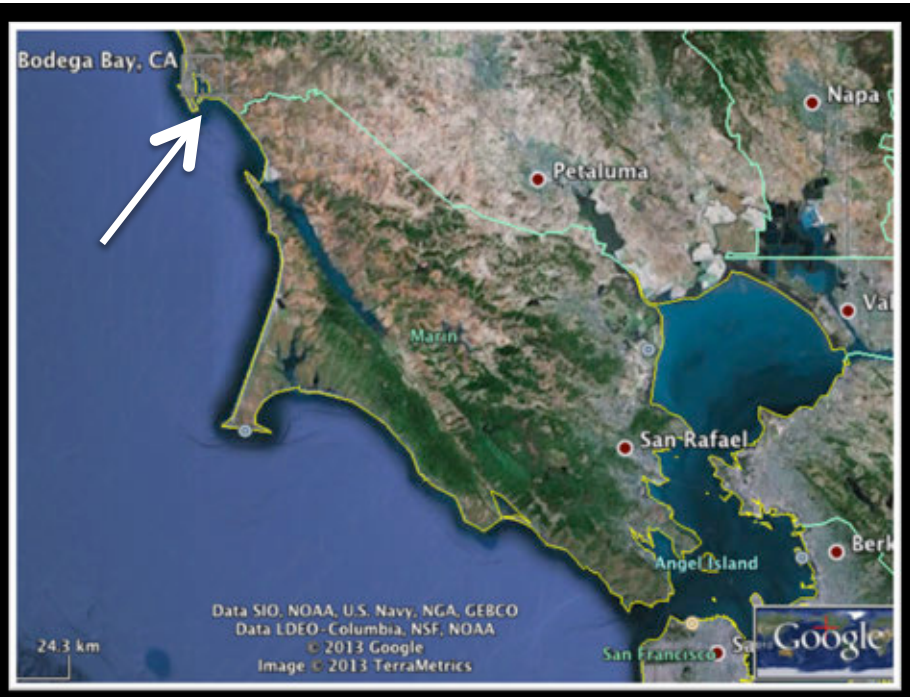
Our System:

Marine organisms growing on a floating dock in Bodega Bay

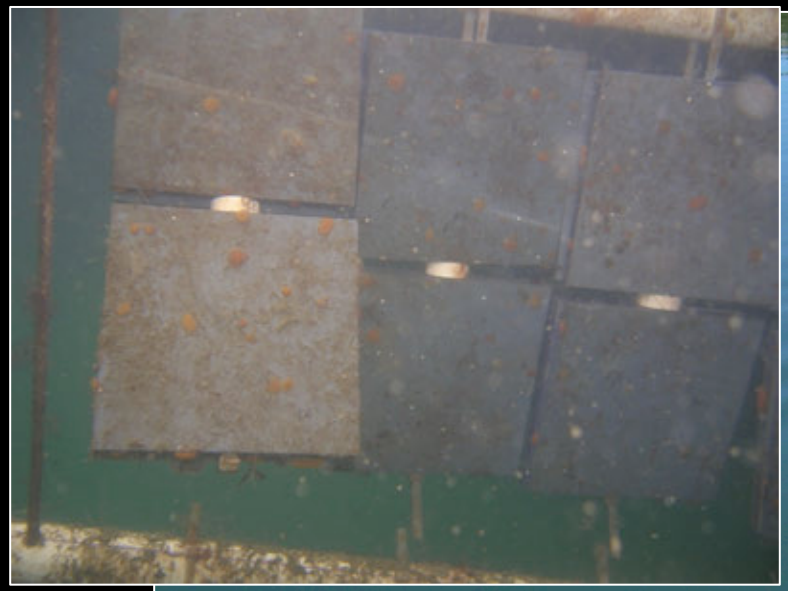


3 month old plate

9 month old plate

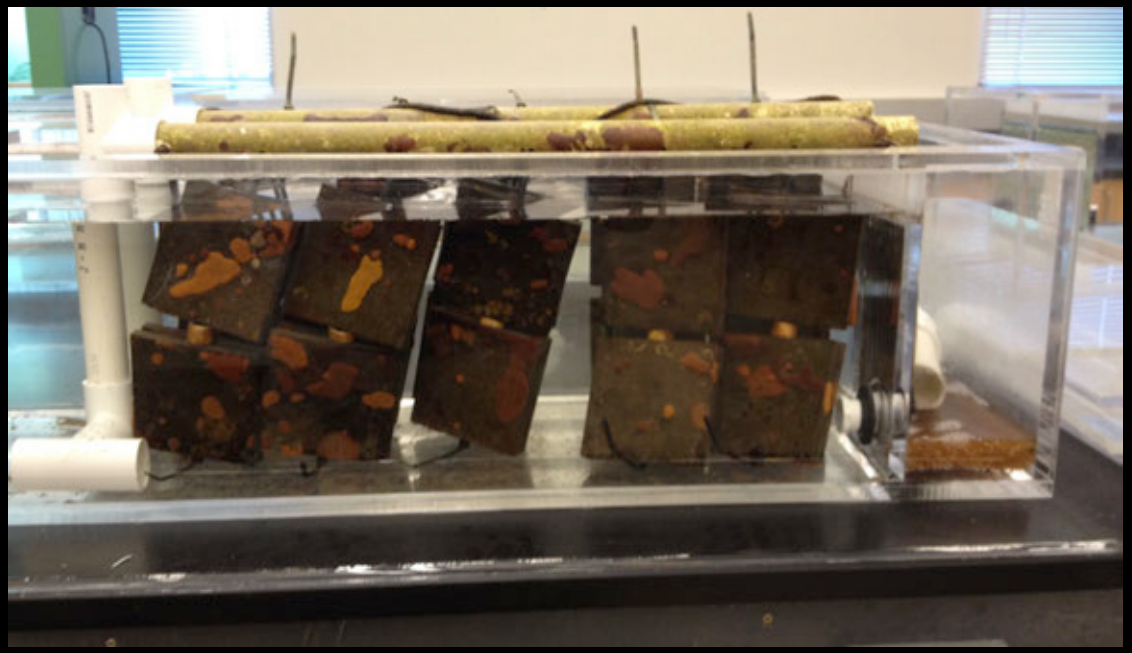

Bodega Bay, CA
Napa
Petaluma
Marin
San Rafael
Angel Island
San Francisco
Data SIO, NOAA, U.S. Navy, NGA, GEBCO
Data LDEO-Columbia, NSF, NOAA
© 2013 Google
Image © 2013 TerraMetrics
24.3 km
Google


Panels hang here




igloo

http://coursecontent.ucdavis.edu/BIS/2B/Lab06/player.html




UCDAVIS

The Lab—Part I Data Collection

-Groups of 2
-Each group samples 3 plates
    (3 month, 6 month, 9 month).
-Data collected:
    number of species
    percent cover for each species (includes overgrowth)
    percent of each species on the panel surface only
-Calculations:
    Shannon Diversity Index
    Percent overgrowth

The Lab—Part I: Thinking Part

Hypothesis Development:

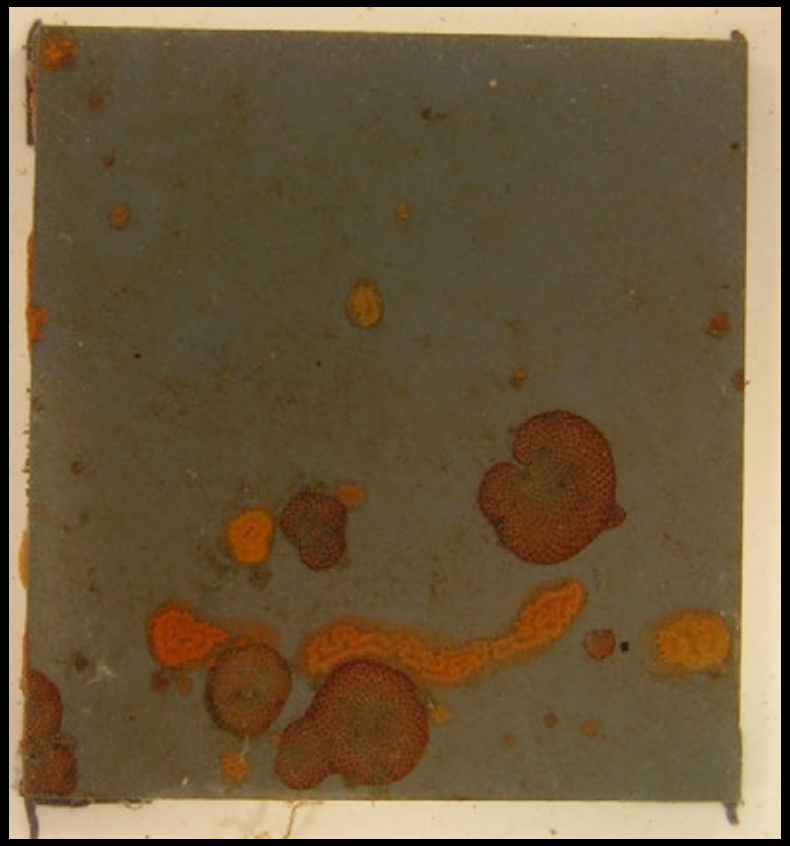
-pick a result (observation) that was interesting

-develop a mechanistic hypothesis to explain this result

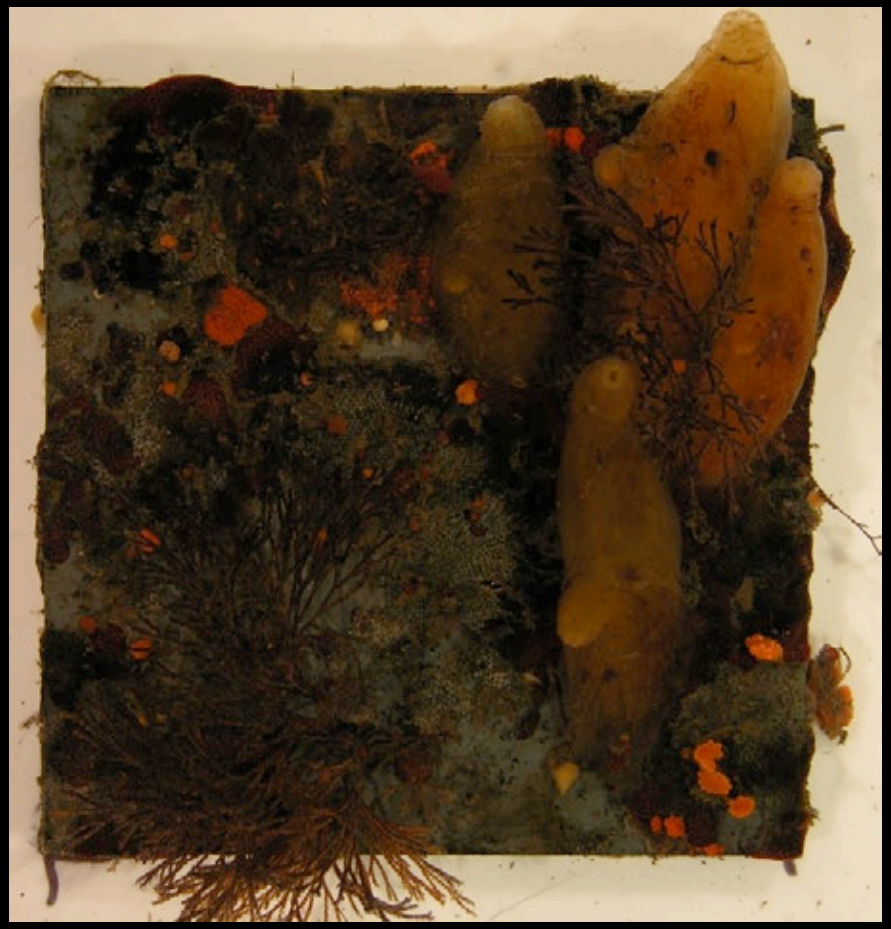
-include a prediction, mathematical prediction

-Outline an experiment to test hypothesis

-Exchange ideas with another group—are there other hypotheses that could explain the data?

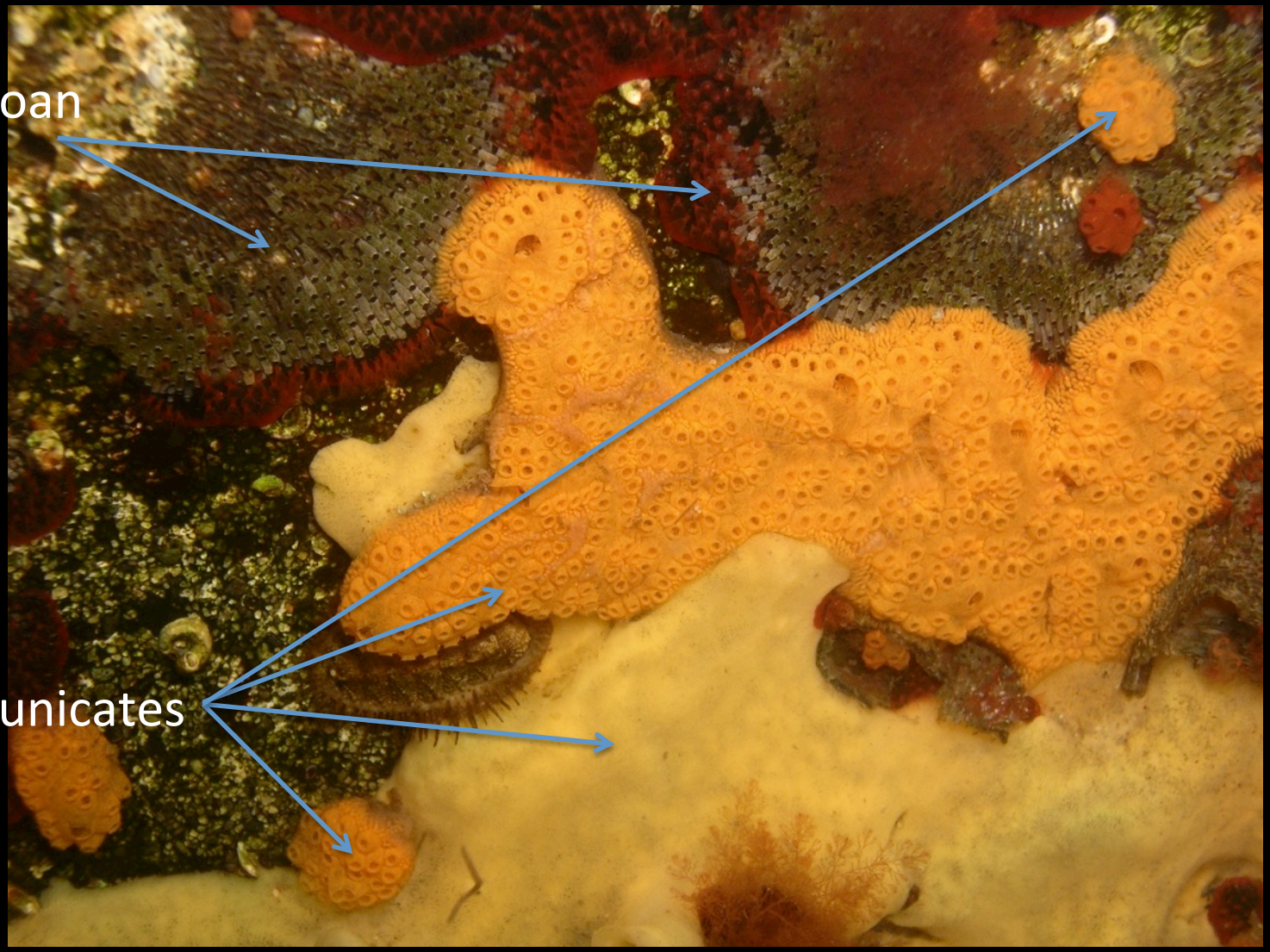


Typical 3 month old panels

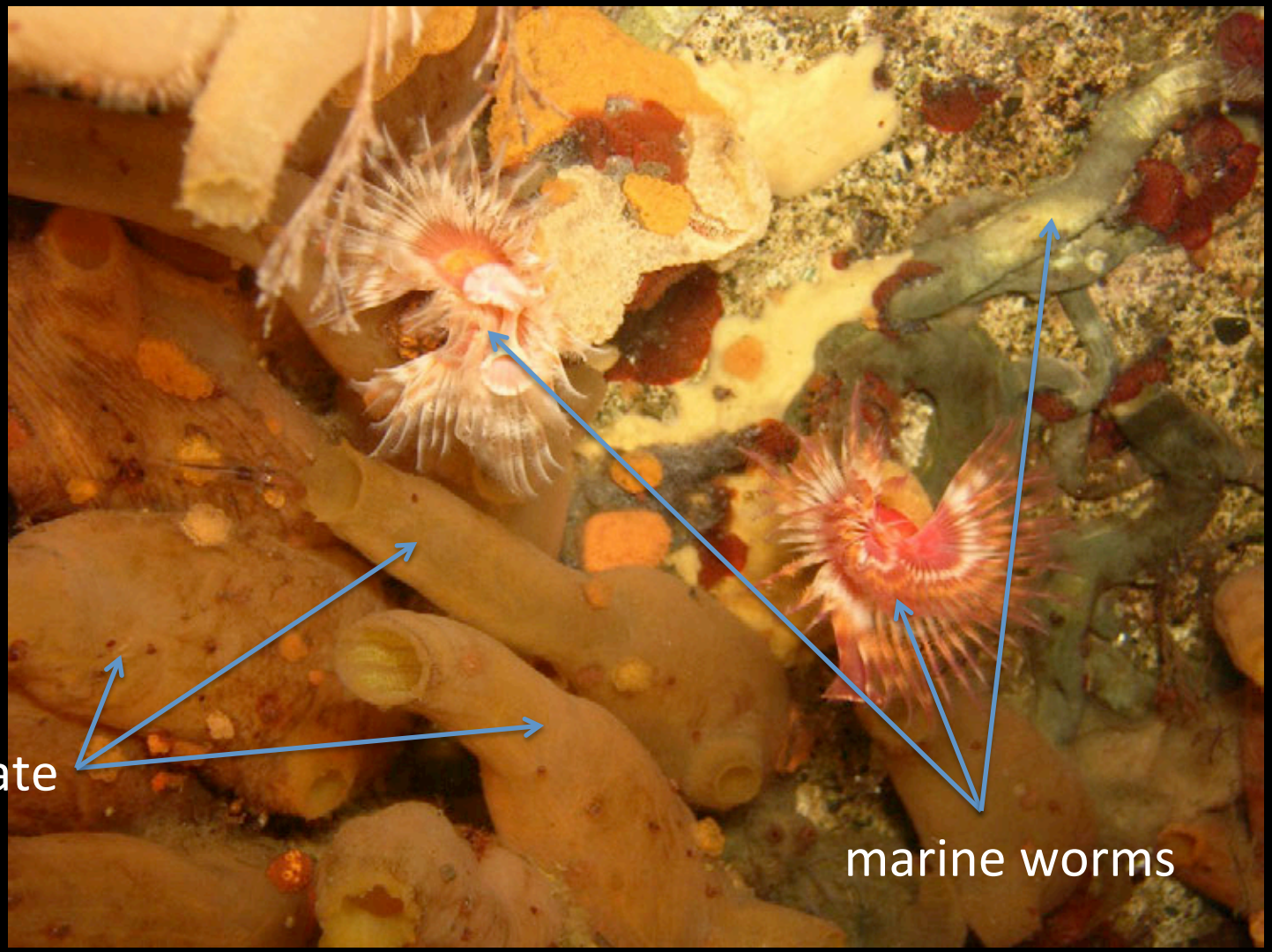


9 month old panels

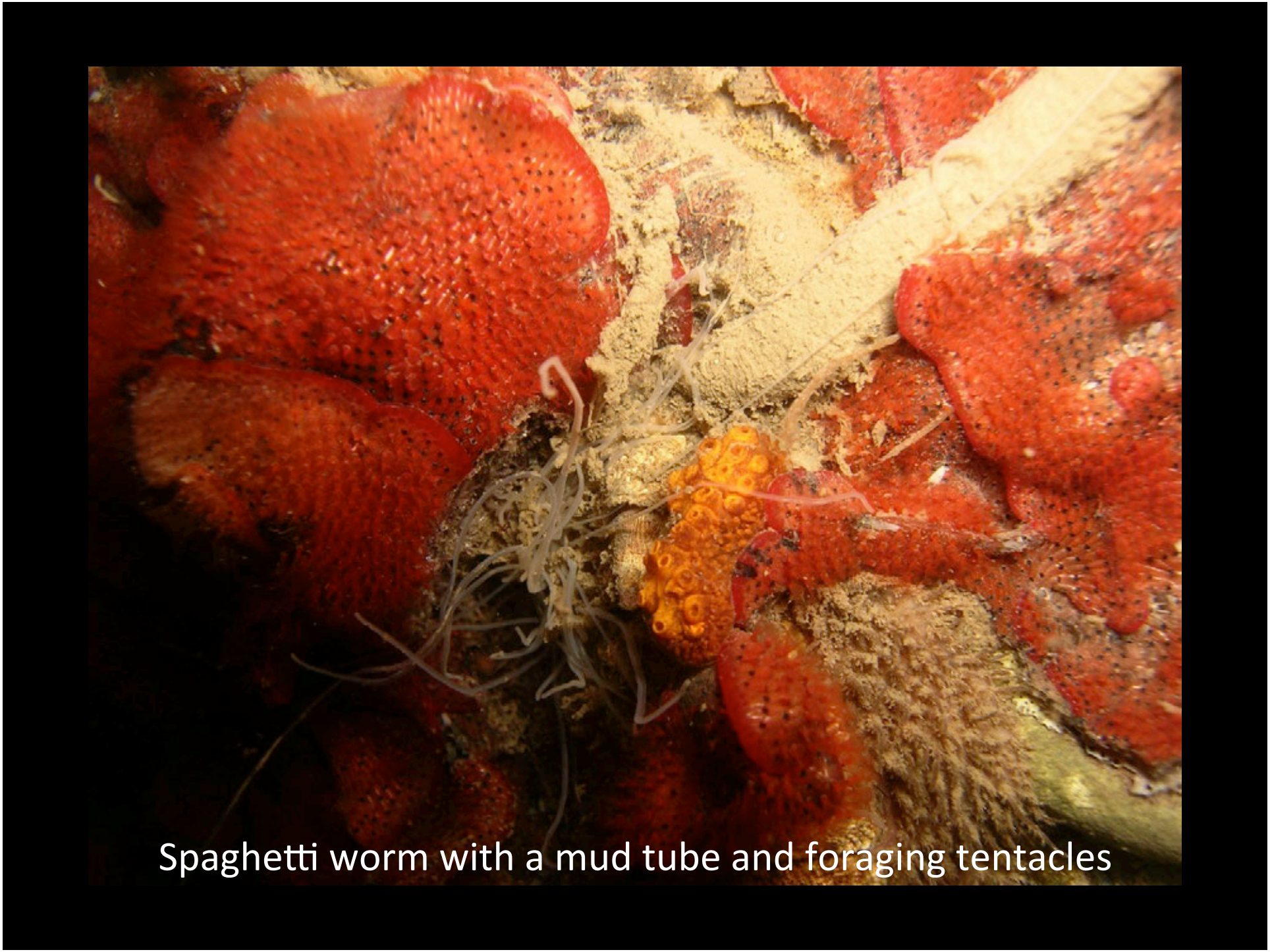
Quick run through of some of the organisms



Mostly Modular Members



Unitary animals



Spaghetti worm with a mud tube and foraging tentacles

Solitary tunicate

Barnacles- unitary

Sponge-gray area between modular and unitary

Plumose anemone--unitary

Workshop 12

RABLE 2015

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UCI RABLE
February 21, 2015

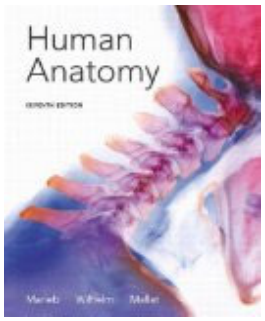
Insights and activities from a human anatomy lab without cadavers

Background

- Bio Sci D170 (Applied Human Anatomy) is a six-unit upper-division elective targeted for students that want to apply to health professional programs.
- The pre-requisite for this course is a human physiology lecture with a C or better.
- The majority of students that take the class are junior and senior biological sciences, pharmaceutical sciences, or public health sciences majors. Sophomore level nursing science students also enroll in the course.
- The course meets for three 50-minute lectures a week (in class size of 90 to 120 students) and one three-hour lab section a week (in sections of 24 students).
- The course is taught with a systems anatomy approach (as compared to a regional approach used by medical schools) and covers all major organ systems of the body, including skeletal, muscular, nervous, cardiovascular, respiratory, digestive, urinary, reproductive, and integumentary.

Course materials

- Bio Sci D170 is taught using the following materials:
  - Textbook: Human Anatomy, 7e by Marieb et al.
  - MasteringA&P: an online learning management system that provides students with opportunities for interactive assignments and quizzes, videos, tutorials, the eTextbook, and a study area
  - Practice Anatomy Lab (PAL): PAL is a virtual cadaver that students can use to see real human cadavers that can be "virtually dissected" to uncover deeper structures of the body.
  - A variety of models used in lab (described below).



MasteringA&P®

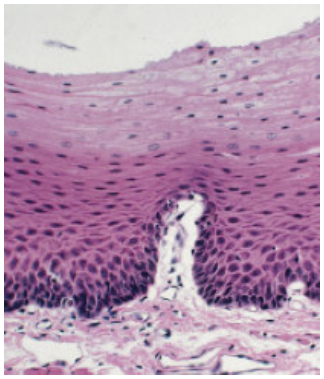
Course structure for the lecture component

- Bio Sci D170 is taught as a highly structured course, meaning that students are actively required to participate in the learning process before, during, and after each day of class. The breakdown for course activities and assignments is below:
  - For each day of class, students are provided with an optional reading guide that contains a series of questions that can be answered by reading the textbook. The reading guides are intended to help students focus on specific portions of the textbook and to help them become active note-takers.
  - Prior to each day of class, students are assigned a pre-class assignment on MasteringA&P that includes multiple choice, matching, and labeling activities. These assignments are intended to give students a chance to be familiar with the material prior to coming to class.
  - During class, students work alone or in groups to answer a series of questions, including multiple-choice, matching, labeling, drawing, or short-answer. Lecture is minimal and is provided during transition points or to clarify student misconceptions or difficult material. The course is taught via active learning to get students to participate in the learning process and to give them chances to practice using their knowledge that they acquired from pre-class assignments.
  - After class, students are required to complete weekly timed review quizzes on MasteringA&P. These quizzes are intended to model the exams in the course and to keep students up-to-date with studying the course material.
  - Major assessments include three midterm exams and a final exam, all of which contain cumulative portions.

Course structure for the lab component

- This lab is taught without cadavers, so instead students gain hands-on experience with plastic human anatomy models (whole skeletons, muscle limbs, torsos, hearts, etc), dissecting animal organs (sheep brain, heart, lung, eye, etc), and the virtual cadaver through PAL.
- During lab sessions, students work in groups of three to five to complete lab activities. Activities range from identifying and labeling models, making models, drawing anatomical structures, or feeling their own bodies to identify structures.
- Students turn in a worksheet or take a quiz at the end of each lab session.
- Students also complete an online PAL assignment that is related to the organ systems explored during that week of lab.
- Major summative assessments include a midterm lab practical exam and a final lab practical exam. During these exams, students rotate through ~25 stations to answer questions related to anatomical models.

Activities for this workshop



1. Epithelial and connective tissue identification
    - Students are exposed to tissues early in the course to give them a foundation on the structure of organs in the body.
    - Students gain experience classifying types of tissues in the body through the use of microscopic images from their textbook and also from PAL.
    - In this activity, students work in groups to classify “unknown” images of epithelial and connective tissues. They also develop a flowchart that will help them organize their knowledge on tissue types.

2. Plotting cranial and spinal nerves through the body
    - Students often have difficulty transferring their knowledge of organ systems in the body from 2-D textbook pages to 3-D models, and many studies have suggested that the nervous system is especially challenging.
    - In this activity, students use string or other craft materials to plot the pathways that cranial and spinal nerves take through the human body. Students apply their “nerves” to human skeleton models to visualize how the nerves coarse through the body in three dimensions.
    - NOTE: This activity has been published in CourseSource and can be accessed through the link below.

Plotting Cranial and Spinal Nerve Pathways in a Human Anatomy Lab

Justin F. Shaffer[1*]

CourseSource

http://www.coursesource.org/courses/plotting-cranial-and-spinal-nerve-pathways-in-a-human-anatomy-lab

Activity 1 - Tissue identification

- You should have a stack of five images for your group. Each image is a microscopic image of a tissue from the human body.
- As a group, determine which type of tissue each image is showing by filling in the following table. You might not need / be able to fill in each column for each image. Use your textbook for help if you need it.

Image	Number of cell layers	Types of cells	Shapes of cells	Types of fibers	Other distinguishing features	What tissue is this and how do you know?
1						
2						
3						
4						
5						

- Answer these questions below:
  - In these images, what is colored blue (by hematoxylin)? What is colored pink (by eosin)? (Hint: see chapter 1 for help)
  - How can you tell the difference between micrographs of elastic and dense regular connective tissues?
  - The epithelium of the intestine needs to secrete lots of digestive enzymes and needs lots of surface area for molecular absorption to occur. What type of epithelium lines the intestine and what structures should the cells contain? Justify your answer.

Using the information from the above table, come up with a logical flowchart or guide to help you distinguish between the types of epithelial tissue and connective tissue proper. **Draw this flowchart on a piece of paper and show your instructor before moving on.**

Activity 2 – Nerve pathways through the body

- In your lab envelope you have many pieces of yellow mason line – these will be used to model the pathways that nerves travel through the body. PLEASE be careful with them, as the ends fray easily and other groups will need to use them!
- There are six total nerves; two cranial and four major nerves that are parts of the nerve plexuses of the body. Some of the nerves have more than one ending – these will represent attachment points to spinal nerves. Those that have multiple endings thus are spinal nerves, and those with a single ending are cranial nerves. It is up to you to figure out which nerve to use for the following exercises. Examine their lengths and number of endings to figure out which is which.
- **NOTE:** this model will not be completely accurate, as we are not modeling the nerve plexuses; rather the nerves will attach directly to the spinal nerves. Also remember that each nerve is paired – there is a left and right of each nerve. You are only going to model the left or the right, not both.

- Spread your six nerves out on your lab bench and compare their lengths and number of spinal nerve attachment points. Can you tell which ones are cranial nerves and which ones are spinal nerves?

- Optic nerve
    - Take the brain from the torso and identify the origination point of the optic nerve.
    - Identify the optic nerve from the envelope of nerves and tape one end to the attachment point on the brain.
        - Where does the optic nerve originate from the brain? _____
    - Remove the skullcap from the human skeleton skull model, and identify the foramen that the optic nerve passes through.
        - What foramen does the optic nerve pass through? _____
    - Carefully place the brain in the cranial fossa and thread the cranial nerve through its foramen.
    - Remove the left eye from the torso and carefully place it in the left orbital socket of the skull. The optic nerve is now “attached” to the eye!

- Vagus nerve
    - Remove the brain from the skull and identify the origination point of the vagus nerve. (leave the optic nerve attached!)
        - Where does the vagus nerve originate from the brain? _____
    - Identify the vagus nerve from the envelope of nerves and tape one end to the attachment point on the brain.
    - In the skull model, identify the foramen that the vagus nerve passes through.
        - What foramen does the vagus nerve pass through? _____
    - Carefully place the brain in the cranial fossa and thread the cranial nerve and vagus nerve through their foramina.
    - Thread the vagus nerve to the approximate location where it would innervate the heart.

Show your instructor your optic and vagus nerves before moving on. Do not remove the nerves until the end of the lesson.

- Phrenic nerve
    - Identify the phrenic nerve from the envelope of nerves.
        - Which spinal nerves make up the phrenic nerve? _____
        - What plexus does the phrenic nerve originate from? _____
    - On the human skeleton model, identify the spinal nerves that make up the phrenic nerve.
    - Tape the ends of the phrenic nerve to its appropriate spinal nerves.
    - Thread the phrenic nerve from its attachment points at the spinal nerves to its most distal point – use tape to help keep it in place.

- Ulnar nerve
    - Identify the ulnar nerve from the envelope of nerves.
        - Which spinal nerves make up the ulnar nerve? _____
        - What plexus does the ulnar nerve originate from? _____
    - On the human skeleton model, identify the spinal nerves that make up the ulnar nerve.
    - Tape the ends of the ulnar nerve to its appropriate spinal nerves.
    - Thread the ulnar nerve from its attachment points at the spinal nerves to its most distal point – use tape to help keep it in place.

Show your instructor your phrenic and ulnar nerves before moving on. Do not remove the nerves until the end of the lesson.

- Obturator nerve
    - Identify the obturator nerve from the envelope of nerves.
        - Which spinal nerves make up the obturator nerve? _____
        - What plexus does the obturator nerve originate from? _____
    - On the human skeleton model, identify the spinal nerves that make up the obturator nerve.
    - Tape the ends of the obturator nerve to its appropriate spinal nerves.
    - Thread the obturator nerve from its attachment points at the spinal nerves to its most distal point – use tape to help keep it in place.

- Tibial nerve
    - Identify the tibial nerve from the envelope of nerves.
        - Which spinal nerves make up the tibial nerve? _____
        - What plexus does the tibial nerve originate from? _____
    - On the human skeleton model, identify the spinal nerves that make up the tibial nerve.
    - Tape the ends of the tibial nerve to its appropriate spinal nerves.
    - Thread the tibial nerve from its attachment points at the spinal nerves to its most distal point – use tape to help keep it in place.

Show your instructor your obturator and tibial nerves before moving on. Take a photo of your completed skeleton model before removing your nerves.

- Answer the following questions about the nerves of the body:
    - What rami make up the plexuses? Dorsal or ventral or both or neither?
    - What muscle(s) does the phrenic nerve innervate?
    - What muscle groups does the ulnar nerve innervate? What cutaneous regions does it innervate?
    - What muscle groups does the tibial nerve innervate? What cutaneous regions does it innervate?
    - If you were to see the radial nerve on a skeleton in the final lab practical, how would you know that it is the radial nerve?