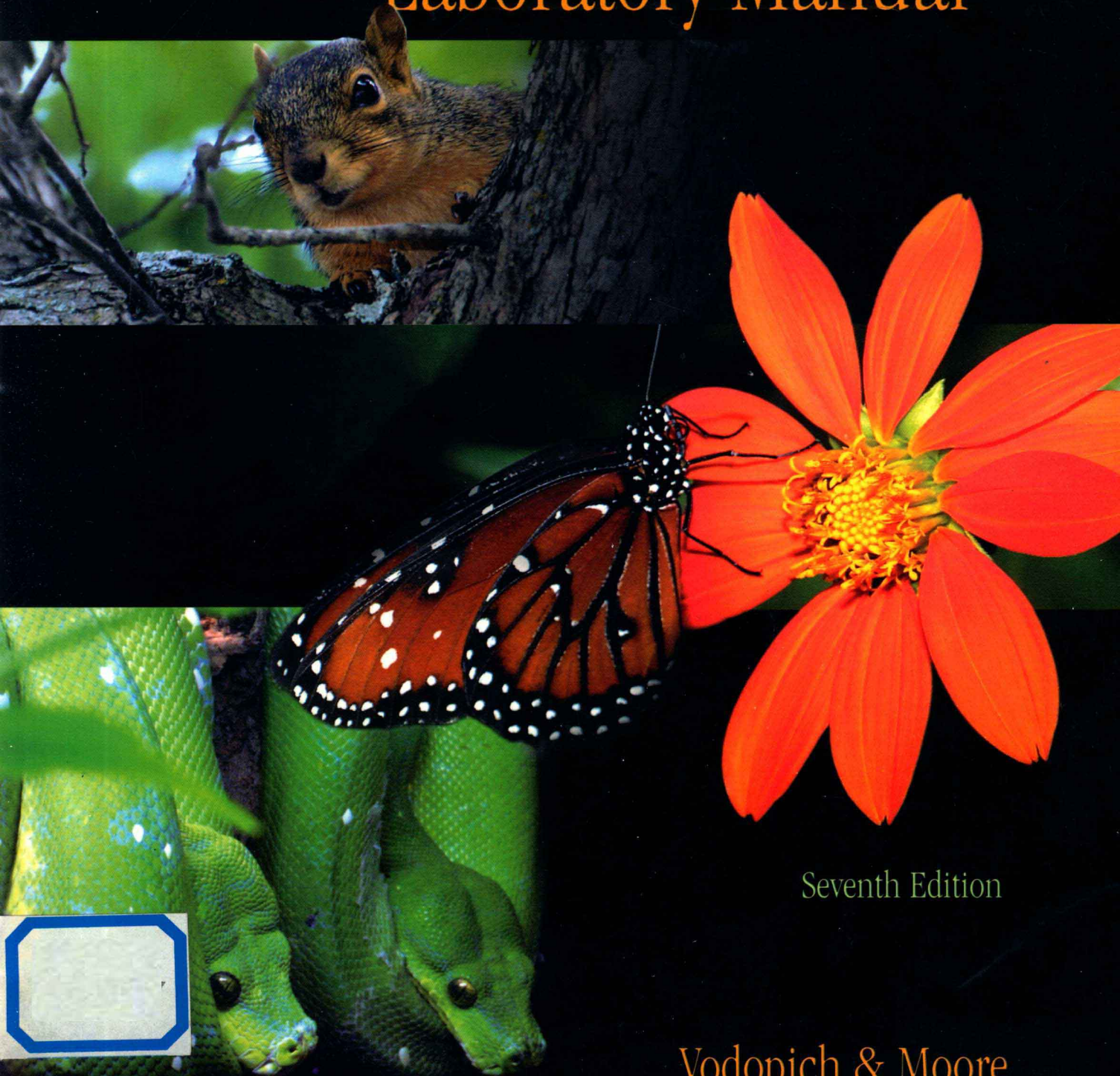


BIOLOGY

Laboratory Manual



Seventh Edition

Vodopich & Moore

seventh edition

Biology

Laboratory Manual

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Higher Education

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BIOLOGY LABORATORY MANUAL, SEVENTH EDITION

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Some of the laboratory experiments included in this text may be hazardous if materials are handled improperly or if procedures are conducted incorrectly. Safety precautions are necessary when you are working with chemicals, glass test tubes, hot water baths, sharp instruments, and the like, or for any procedures that generally require caution. Your school may have set regulations regarding safety procedures that your instructor will explain to you. Should you have any problems with materials or procedures, please ask your instructor for help.

Table 25.2 Characteristics of the Six Kingdoms

	Archaeobacteria and Bacteria	Protista	Plantae	Fungi	Animalia
					
Cell Type	Prokaryotic	Eukaryotic	Eukaryotic	Eukaryotic	Eukaryotic
Nuclear Envelope	Absent	Present	Present	Present	Present
Transcription and Translation	Occur in same compartment	Occur in different compartments	Occur in different compartments	Occur in different compartments	Occur in different compartments
Histone Proteins Associated with DNA	Absent	Present	Present	Present	Present
Cytoskeleton	Absent	Present	Present	Present	Present
Mitochondria	Absent	Present (or absent)	Present	Present	Present
Chloroplasts	None (photosynthetic membranes in some types)	Present (some forms)	Present	Absent	Absent
Cell Wall	Noncellulose (polysaccharide plus amino acids)	Present in some forms, various types	Cellulose and other polysaccharides	Chitin and other noncellulose polysaccharides	Absent
Means of Genetic Recombination, If Present	Conjugation, transduction, transformation	Fertilization and meiosis	Fertilization and meiosis	Fertilization and meiosis	Fertilization and meiosis
Mode of Nutrition	Autotrophic (chemosynthetic, photosynthetic) or heterotrophic	Photosynthetic or heterotrophic, or combination of both	Photosynthetic, chlorophylls <i>a</i> and <i>b</i>	Absorption	Ingestion
Motility	Bacterial flagella, gliding or nonmotile	9 + 2 cilia and flagella; amoeboid, contractile fibrils	None in most forms; 9 + 2 cilia and flagella in gametes of some forms	Both motile and nonmotile	9 + 2 cilia and flagella, contractile fibrils
Multicellularity	Absent	Absent in most forms	Present in all forms	Present in most forms	Present in all forms
Nervous System	None	Primitive mechanisms for conducting stimuli in some forms	A few have primitive mechanisms for conducting stimuli	None	Present (except sponges), often complex

Biology

Laboratory Manual

Preface

We designed this laboratory manual for an introductory biology course with a broad survey of basic laboratory techniques. The experiments and procedures are simple, safe, easy to perform, and especially appropriate for large classes. Few experiments require more than one class meeting to complete the procedure. Each exercise includes many photographs, traditional topics, and experiments that help students learn about life. Procedures within each exercise are numerous and discrete so that an exercise can be tailored to the needs of the students, the style of the instructor, and the facilities available.

TO THE STUDENT

We hope this manual is an interesting guide to many areas of biology. As you read about these areas, you'll probably spend equal amounts of time observing and experimenting. Don't hesitate to go beyond the observations that we've outlined—your future success as a scientist depends on your ability to seek and notice things that others may overlook. Now is the time to develop this ability with a mixture of hard work and relaxed observation. Have fun, and learning will come easily. Also, remember that this manual is designed with your instructors in mind as well. Go to them often with questions—their experience is a valuable tool that you should use as you work.

TO THE INSTRUCTOR

This manual's straightforward approach emphasizes experiments and activities that optimize students' investment of time and your investment of supplies, equipment, and preparation. Simple, safe, and straightforward experiments are most effective if you interpret the work in depth. Most experiments can be done easily by a student in two to three hours. Terminology, structures, photographs, and concepts are limited to those the student can readily observe and understand. In each exercise we have included a few activities requiring a greater investment of effort if resources are available, but omitting them will not detract from the objectives.

This manual functions best with an instructor's guidance, and is not an autotutorial system. We've tried to guide students from observations to conclusions, to help students make their own discoveries, and to make the transition from observation to biological principles. But discussions and interactions between student and instructor are major components

of a successful laboratory experience. Be sure to examine the "Questions for Further Thought and Study" in each exercise. We hope they will help you expand students' perceptions that each exercise has broad applications to their world.

THE SEVENTH EDITION

All exercises in this edition were critiqued by a review panel of current users, and their suggested revisions were carefully considered and incorporated. Classifications schemes presented in the diversity exercises have been updated to reflect the onslaught of molecular data being used in modern systematics. Introductory material has been reviewed and revised for completeness. The number of tables and figures has been extended with more than 70 figures being either revised or replaced.

Darrell S. Vodopich
Randy Moore

Reviewers

We thank the following reviewers for their helpful comments and suggestions during the preparation of this new edition.

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Welcome to the Biology Laboratory

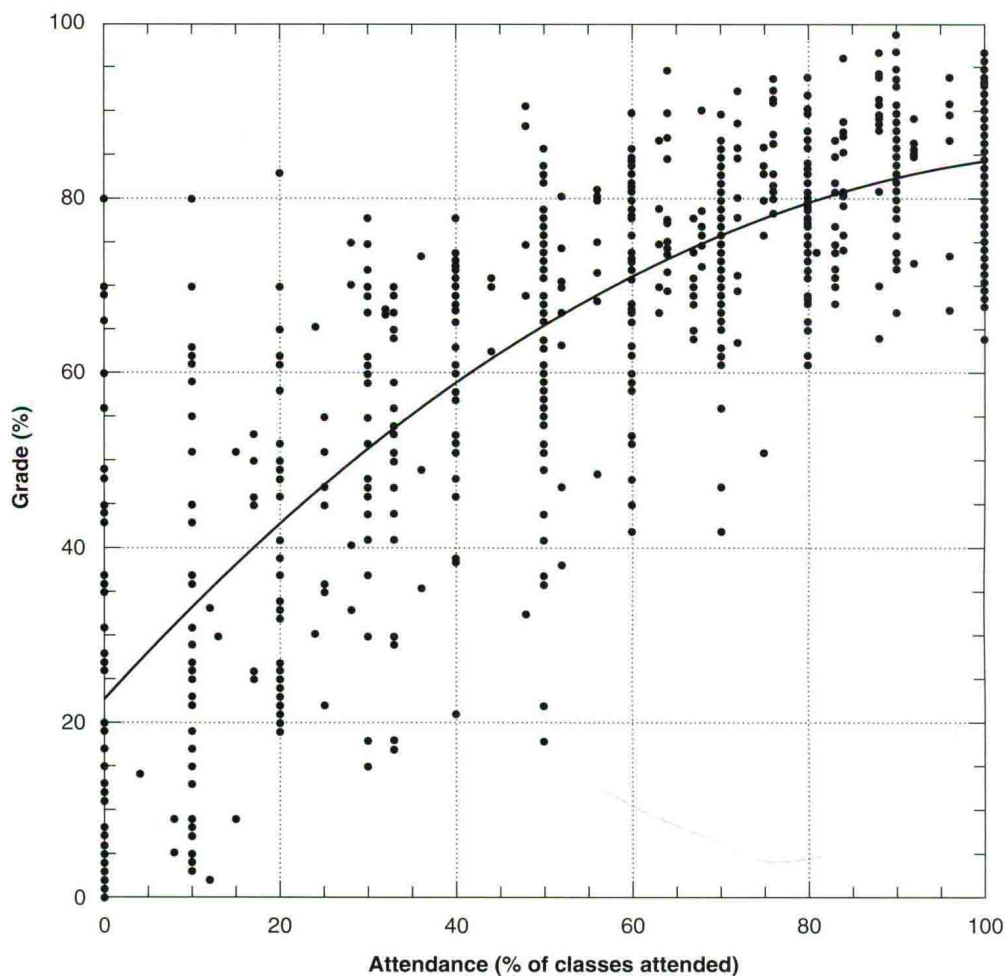
Welcome to the biology laboratory. Although reading your textbook and attending lectures are important ways of learning about biology, nothing can replace the importance of the laboratory. Indeed, in lab you'll get hands-on experience with what you've heard and read about biology—for example, you'll observe organisms, do experiments, test ideas, collect data, and make conclusions about what you've learned. That is, you'll *do* biology.

You'll enjoy the exercises in this manual—they're interesting, informative, and can be completed within the time limits of your laboratory period. We've provided questions to test your understanding of what you've done; in some of the exercises, we've also asked you to devise your own experiments to answer questions that you've posed. To

make these exercises most useful and enjoyable, follow the guidelines discussed below:

THE IMPORTANCE OF COMING TO CLASS

Biology labs are designed to help you experience biology firsthand. To do that, you must attend class. Indeed, if you want to do well in your biology course, attend class and pay attention. To appreciate the importance of class attendance for making a good grade in your biology course, examine the following graph of how students' grades in an introductory biology course relate to their rates of class attendance. Data are from a General Biology class, University of Minnesota, 2003.



In the space below, write your interpretation of these data.

BEFORE COMING TO LAB

Read the exercise before coming to lab. This will give you a general idea about what you're going to do, as well as why you're going to do it. Knowing this will not only save time, it will also help you finish the experiments.

WHEN IN LAB

1. Don't start the exercise until you've discussed the exercise with your laboratory instructor. She/he will give you specific instructions about the lab and tell you how the exercise may have been modified.
2. Stay focused as you work. You'll be able to finish each exercise within the allotted time if you stay busy. You'll not be able to finish the exercise if you spend your time talking about this weekend's party or last week's big game.
3. Discuss your observations, results, and conclusions with your instructor and lab partners. Perhaps their comments and ideas will help you better understand what you've observed.

4. Always follow instructions and follow safety guidelines presented by your instructor.
5. If you have questions, ask your instructor.

SAFETY IN THE LABORATORY

The exercises in this manual were designed with safety as a top priority. Always follow these safety precautions:

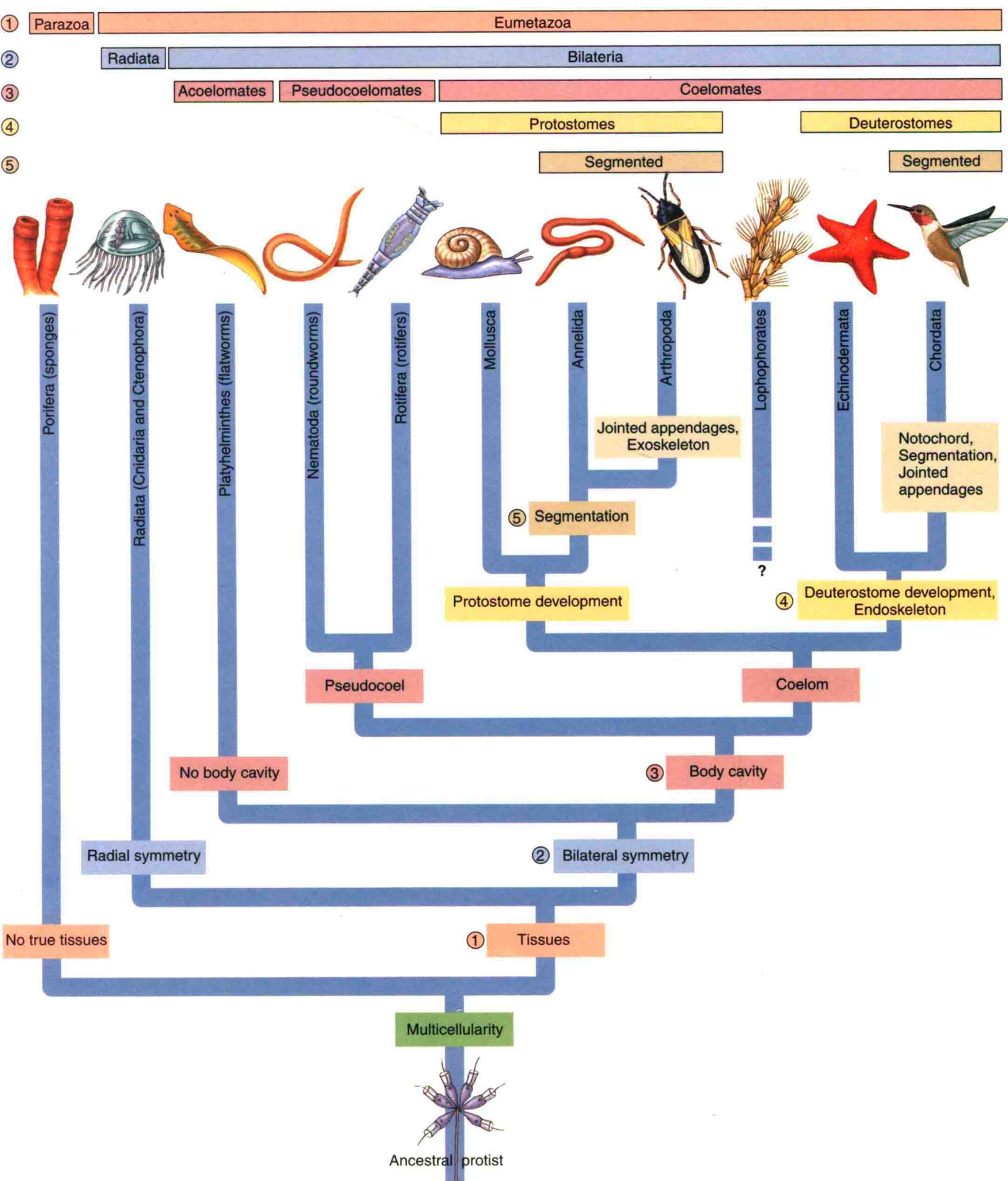
1. Do not eat, drink, smoke, or apply cosmetics when in the lab.
2. Use the equipment properly. If you have any questions or problems, contact your instructor.
3. Clean up spills immediately.
4. Report all injuries—no matter how minor—immediately to your instructor.
5. If you have long hair, tie it back. If you use open flames, roll up loose sleeves.
6. Never taste any substance or solution. Do not put anything in the lab into your mouth.
7. Treat all live animals gently and with respect.
8. The locations of lab safety equipment will be pointed out to you during the first lab. Familiarize yourself with the location and operation of this equipment.
9. At the end of each lab, clean your work area, wash your hands thoroughly with soap, and return all equipment and supplies to their original locations.

AFTER EACH LABORATORY

Soon after each lab, review what you did. What questions did you answer? What data did you gather? What conclusions did you make?

Also note any questions that remain. Try to answer these questions by using your textbook or visiting the library. If you can't answer the questions, discuss them with your instructor.

Welcome to the laboratory!



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Measurements in Biology

The Metric System and Data Analysis

Objectives

By the end of this exercise you should be able to:

1. Identify the metric units used to measure length, volume, mass, and temperature.
2. Measure length, volume, mass, and temperature in metric units.
3. Convert one metric unit to another (e.g., grams to kilograms).
4. Use measures of volume and mass to calculate density.
5. Practice the use of simple statistical calculations such as mean, median, range, and standard deviation.
6. Analyze sample data using statistical tools.

Every day we're bombarded with numbers and measurements. They come at us from all directions, including while we're at the supermarket, gas station, golf course, and pharmacy, as well as while we're in our classrooms and kitchens. Virtually every package that we touch is described by a measurement.

Scientists use a standard method to collect data as well as use mathematics to analyze those data. Measuring things is a must before we can objectively describe what we are observing, before we can experiment with biological processes, and before we can predict how organisms respond, adjust to, and modify their world. Once we have made our measurements, we can analyze our data and look for variation and the sources of that variation. Then we can infer the causes and effects of the biological processes that interest us.

THE METRIC SYSTEM

Scientists throughout the world use the **metric system** to make measurements. The metric system is also used in everyday life virtually everywhere except the United States. With few exceptions (e.g., liter bottles of soda, 35-mm film), most measurements in the United States use the antiquated English system of pounds, inches, feet, and so on. Check with your instructor about bringing to class common grocery store items with volumes and weights in metric units, or examining those items on display.

Scientists make all of their measurements in the metric system; they do not routinely convert from one system to another. Thus, this exercise will not involve conversions from the English to metric systems (if you want to know those conversions, see Appendix III). Rather, this exercise will introduce you to making metric measurements of length, mass, volume, and temperature. During this lab, you should spend your time making measurements, not reading background information. Therefore, *before lab, read this exercise carefully to familiarize yourself with the basic units of the metric system.*

Metric units commonly used in biology include:

meter (m)—the basic unit of length

liter (L)—the basic unit of volume

kilogram (kg)—the basic unit of mass

degree Celsius (°C)—the basic unit of temperature

Unlike the English system with which you are already familiar, the metric system is based on units of ten. This simplifies conversions from one metric unit to another (e.g., from kilometers to meters). This base-ten system is similar to our monetary system, in which 10 cents equals a dime, 10 dimes equals a dollar, and so forth. Units of ten in the metric system are indicated by Latin and Greek prefixes placed before the base units:

Prefix (Latin)		Division of Metric Unit	
deci	(d)	0.1	10^{-1}
centi	(c)	0.01	10^{-2}
milli	(m)	0.001	10^{-3}
micro	(μ)	0.000001	10^{-6}
nano	(n)	0.000000001	10^{-9}
pico	(p)	0.000000000001	10^{-12}

Prefix (Greek)		Multiple of Metric Unit	
deka	(da)	10	10^1
hecto	(h)	100	10^2
kilo	(k)	1000	10^3
mega	(M)	1000000	10^6
giga	(G)	1000000000	10^9

Thus, multiply by:

- 0.01 to convert centimeters to meters
- 0.001 to convert millimeters to meters
- 1000 to convert kilometers to meters
- 0.1 to convert millimeters to centimeters

For example, 620 meters = 0.620 kilometers = 620,000 millimeters = 62,000 centimeters.

Question 1

Make the following metric conversions:

- 1 meter = ___ centimeters = ___ millimeters
- 92.4 millimeters = ___ meters = ___ centimeters
- 10 kilometers = ___ meters = ___ decimeters

Length and Area

The **meter** (m) is the basic unit of length. Units of area are squared units (i.e., two-dimensional) of length.

$$1 \text{ m} = 100 \text{ cm} = 1000 \text{ mm} = 0.001 \text{ km} = 1 \times 10^{-3} \text{ km}$$

$$1 \text{ km} = 1000 \text{ m} = 10^3 \text{ m}$$

$$1 \text{ cm} = 0.01 \text{ m} = 10^{-2} \text{ m} = 10 \text{ mm}$$

$$470 \text{ m} = 0.470 \text{ km}$$

$$1 \text{ cm}^2 = 100 \text{ mm}^2 \text{ (i.e., } 10 \text{ mm} \times 10 \text{ mm} = 100 \text{ mm}^2)$$

To help you appreciate the magnitudes of these units, here are the lengths and areas of some familiar objects:

Length

Housefly	0.5 cm
Mt. Everest	8848 m
Diameter of penny	1.9 cm
Toyota Camry	4.7 m

Area

Total skin area of adult human male	1.8 m ²
Football field (goal line to goal line)	4459 m ²
Surface area of human lungs	80 m ²
Central Park (New York City)	3.4 km ²
Ping-Pong table	4.18 m ²
Credit card	46 cm ²

Procedure 1.1

Make metric measurements of length and area

Most biologists measure lengths with metric rulers or metersticks.

- Examine intervals marked on the metric rulers and metersticks available in the lab.
- Make the following measurements. Be sure to include units for each measurement.

Length of this page	_____
Width of this page	_____
Area of this page (Area = Length \times Width)	_____
Your height	_____
Thickness of this manual	_____
Height of a 200-ml beaker	_____
Height of ceiling	_____

Question 2

What are some potential sources of error in your measurements?

Volume

Volume is the space occupied by an object. Units of volume are cubed (i.e., three-dimensional) units of length. The liter (L) is the basic unit of volume.

$$1 \text{ L} = 1000 \text{ cm}^3 = 1000 \text{ mL}$$

$$1 \text{ L} = 0.1 \text{ m} \times 0.1 \text{ m} \times 0.1 \text{ m}$$

$$1 \text{ cm}^3 = 0.000001 \text{ m}^3$$

To help you appreciate the magnitudes of these units, here are the volumes of some familiar objects:

Chicken egg	60 mL
One breath of air	500 cm ³
Coke can	355 mL

Scientists often measure volumes with pipets and graduated cylinders. Pipets are used to measure small volumes, typically 25 mL or less. Liquid is drawn into a pipet using a bulb or pipet pump (fig. 1.1). Never pipet by mouth.

Graduated cylinders are used to measure larger volumes. To appreciate how to make a measurement accurately, pour 40–50 mL of water into a 100-mL graduated cylinder



Figure 1.1

A pipet is used to extract and dispense volumes of liquid. A suction bulb (shown in green on the left) draws fluid into the pipet, and graduated markings on the pipet allow precise measurement of a fluid's volume. Never use your mouth to suck fluid into a pipet.

and observe the interface between the water and air. This interface, which is called the **meniscus**, is curved because of surface tension and the adhesion of water to the sides of the cylinder. When measuring the liquid in a cylinder such as a graduated cylinder, always position your eyes level with the meniscus and read the volume at the lowest level (fig. 1.2).

Procedure 1.2

Make metric measurements of volume

1. Biologists often use graduated cylinders to measure volumes. Locate the graduated cylinders available in the lab to make the following measurements. Determine what measurements the markings on the graduated cylinder represent. Be sure to include units for each measurement.
2. Measure the milliliters needed to fill a cup (provided in the lab). _____
3. Measure the liters in a gallon. _____

Procedure 1.3

Measure the volume of a solid object by water displacement

1. Obtain a 100-mL graduated cylinder, a thumb-sized rock, and a glass marble.
2. Fill the graduated cylinder with 70 mL of water.

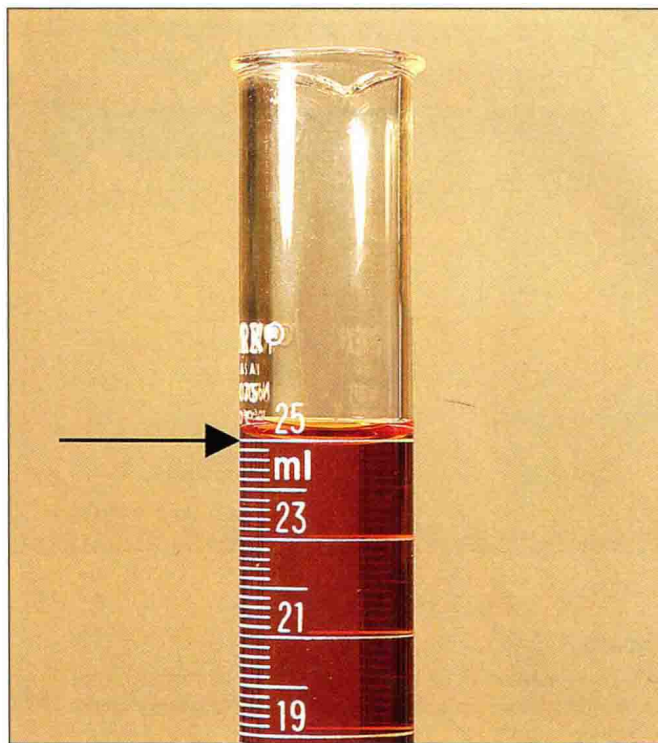


Figure 1.2

When measuring the volume of liquid in a graduated cylinder, always measure at the bottom of the meniscus. The bottom of the meniscus in this photograph is indicated by the arrow. The correct volume is 25 mL.

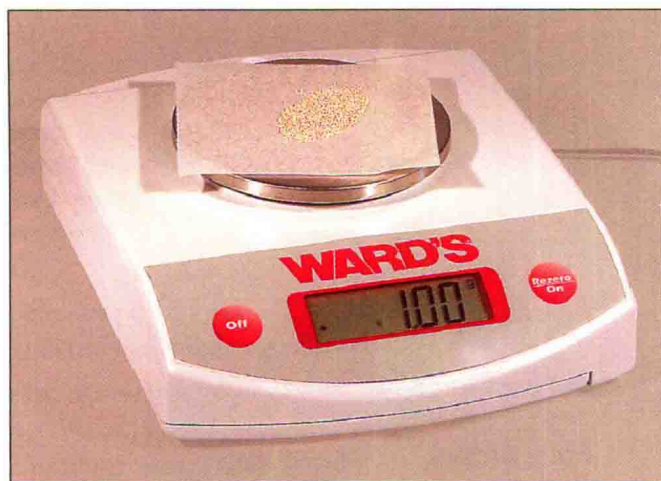
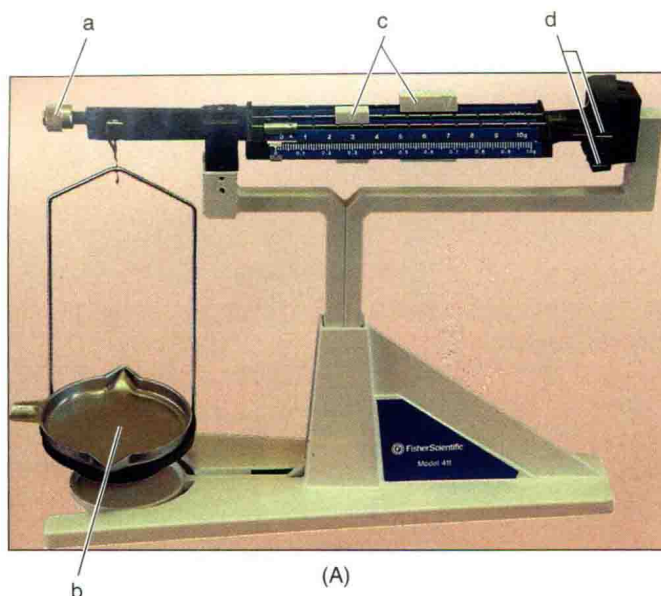
3. Submerge the rock in the graduated cylinder and notice that the volume of the contents rises.
4. Carefully observe the meniscus of the fluid and record its volume.
5. Calculate and record the volume of the rock by subtracting the original volume (70 mL) from the new volume.
Rock volume _____
6. Repeat steps 2–5 to measure and record the volume of the marble.
Marble volume _____

Biologists use pipets to measure and transfer small volumes of liquid from one container to another. The following procedure will help you appreciate the usefulness of pipets.

Procedure 1.4

Learn to use a pipet

1. Add approximately 100 mL of water to a 100-mL beaker.
2. Use a 5-mL pipet with a bulb or another filling device provided by your instructor to remove some water from the beaker.
3. Fill the pipet to the zero mark.



(B)

Figure 1.3

Biologists use balances to measure mass. (A) The parts of a triple-beam balance include (a) the zero-adjustment knob, (b) measuring pan, (c) movable masses on horizontal beams, and (d) balance marks. (B) The top loading balance has a measuring pan, a power switch, and a zero calibration button.

4. To read the liquid level correctly, your eye must be directly in line with the bottom of the meniscus.
5. Release the liquid into another container.

Question 3

What volume of liquid did you measure?

Mass

The **kilogram** (kg) is the basic unit of mass.¹ A kilogram equals the mass of one thousand cubic centimeters (cm³) of water at 4°C. Similarly,

$$1 \text{ kg} = 1000 \text{ g} = 10^3 \text{ g}$$

$$1 \text{ mg} = 0.001 \text{ g} = 10^{-3} \text{ g}$$

Here are the masses of some familiar objects:

9V battery	40 g
Ping-Pong ball	2.45 g
Basketball	0.62 kg
Quarter	6.25 g

Biologists often measure mass with a triple-beam balance (fig 1.3), which gets its name from its three horizontal beams. Suspended from each of the three beams are movable masses. Each of the three beams of the balance are marked with graduations: the closest beam has 0.1-g graduations, the middle beam has 100-g graduations, and the farthest beam has 10-g graduations.

¹ Remember that mass is not necessarily synonymous with weight. Mass measures an object's potential to interact with gravity, whereas weight is the force exerted by gravity on an object. Thus, a weightless object in outer space has the same mass as it has on earth.

Before making any measurements, clean the weighing pan and move all of the suspended weights to the far left. The balance marks should line up to indicate zero grams; if they do not, turn the adjustment knob until they do. Measure the mass of an object by placing it in the center of the weighing pan and moving the suspended masses until the beams balance. The mass of the object is the sum of the masses indicated by the weights on the three beams.

Procedure 1.5

Make metric measurements of mass

1. Biologists often use a triple-beam balance or a top loading scale to measure mass. Locate the triple-beam balances or scales in the lab.
2. Measure the masses of the following items. Be sure to include units for each measurement.

Nickel	_____
Paper clip	_____
Pencil	_____
Rock (used in procedure 1.3)	_____
100-mL beaker (empty)	_____
100-mL beaker containing 50 mL of water	_____

Question 4

- a. **Density** is mass per unit volume. Use data that you've gathered to determine the density of water at room temperature.

Density of water = (mass/volume) = _____

- b. What is the density of the wooden pencil? Does it float? Why?
- c. What is the density of the rock? Does it sink? Why?

Temperature

Temperature is the measure of the kinetic energy of molecules—that is, the amount of heat in a system. Biologists measure temperature with a thermometer calibrated in degrees Celsius ($^{\circ}\text{C}$). The Celsius scale is based on water freezing at 0°C and boiling at 100°C . You can interconvert $^{\circ}\text{C}$ and degrees Fahrenheit ($^{\circ}\text{F}$) using the formula $5(^{\circ}\text{F}) = 9(^{\circ}\text{C}) + 160$. Here are some typical temperatures:

40°C	a very hot summer day
30.6°C	butter melts
75°C	hot coffee
-20°C	temperature in a freezer
37°C	human body temperature

Procedure 1.6

Make metric measurements of temperature

- Obtain a thermometer in the lab. Handle a thermometer with care. If it breaks, notify your instructor immediately.
- Determine the range of the temperatures that can be measured with your thermometer by examining the scale imprinted along the barrel of the thermometer.
- Measure the following temperatures:
 Room temperature _____ $^{\circ}\text{C}$
 Cold tap water _____ $^{\circ}\text{C}$
 Hot tap water _____ $^{\circ}\text{C}$
 Inside refrigerator _____ $^{\circ}\text{C}$

UNDERSTANDING NUMERICAL DATA

Statistics offer a way to organize, summarize, and describe data—the data are usually samples of information from a much larger population of values. Statistics and statistical tests allow us to analyze the sample and draw inferences about the entire population. Consequently, the use of statistics enables us to make decisions even though we have incomplete data about a population. Although this may seem unscientific, we do it all the time; for example, we diagnose diseases with a drop of blood. Decisions are based on statistics when it is impossible or unrealistic to analyze an entire population.

Let's say that you want to know the mass of a typical apple in your orchard. To obtain this information, you could analyze one apple, but how would you know that you'd

Hints for Using the Metric System

- Express measurements in units requiring only a few decimal places. For example, 0.3 m is more easily manipulated and understood than 300000000 nm.
- When measuring pure water, the metric system offers an easy and common conversion from volume measured in liters to volume measured in cubic meters to mass measured in grams: $1\text{ mL} = 1\text{ cm}^3 = 1\text{ g}$.
- The metric system uses symbols rather than abbreviations. Therefore, do not place a period after metric symbols (e.g., 1 g, not 1 g.). Use a period after a symbol only at the end of a sentence.
- Do not mix units or symbols (e.g., 9.2 m, not 9 m 200 mm).
- Metric symbols are always singular (e.g., 10 km, not 10 kms).
- Except for degree Celsius, always leave a space between a number and a metric symbol (e.g., 20 mm, not 20mm; 10°C , not 10°C).
- Use a zero before a decimal point when the number is less than one (e.g., 0.42 m, not .42 m).

picked a “typical” sample? After all, the batch from which you chose the apple may contain many others, each a little bit different. You'd get a better estimate of “typical” if you increased your sample size to a few hundred apples, or even to 10,000. Or, better yet, to 1,000,000.

The only way to be certain of your conclusions would be to measure all the apples in your orchard. Since this is clearly impossible, you must choose apples that *represent* all of the other apples—that is, you must be working with a *representative sample*. A statistical analysis of those sample apples reduces the sample-values to a few characteristic measurements (e.g., mean mass). As you increase the size of the sample, these characteristic measurements provide an ever-improving estimation of what is “typical.”

There are a variety of software programs that perform statistical analyses of data; all you have to do is enter your data into a spreadsheet, select the data that you want to analyze, and perform the analysis. Although these software packages save time and can increase accuracy, you still need to understand a few of the basic variables that you'll use to understand your numerical data. We'll start with the mean and median:

The **mean** is the arithmetic average of a group of measurements. Chance errors in measurements tend to cancel themselves when means are calculated for samples that are relatively large; a value that is too high because of random error is often balanced by a value that is too low for the same reason.

The **median** is the middle value of a group of measurements.

The median is less sensitive to extreme values than is the mean. To appreciate this, consider a sample consisting of 14 leaves having the following lengths (all in mm):

80 69 62 74 69 51 45 40 9 64 65 64 61 67

The mean length is 58.6 mm. However, none of the leaves are that length, and most of the leaves are longer than 60 mm.

Question 5

- Does the mean always describe the “typical” measurement? Why or why not?
- What information about a sample does a mean *not* provide?

Determine the median by arranging the measurements in numerical order:

9 40 45 51 61 63 64 64 65 67 69 69 73 80

The median is between the seventh and eighth measurement: 64 mm. Note that in this sample, the mean differs from the median.

Question 6

- What is responsible for this difference between the mean and median?
- How would the median change if the 9-mm-long leaf was not in the sample?
- How would the mean change if the 9-mm-long leaf was not in the sample?
- Consider these samples:
Sample 1: 25 35 32 28
Sample 2: 15 75 10 20
What is the mean for Sample 1? _____
What is the mean for Sample 2? _____

In most of the exercises in this manual, you’ll have time to make only one or two measurements of a biological structure or phenomenon. In these instances, a mean may be the only

descriptor of the sample. However, if your class combines its data so that there are many measurements, you’ll need to know how to do a couple of other calculations so that you understand the variation within your sample.

Variability

As you can see, the samples in Question 6d are different, but their means are the same. Thus, the mean does not reveal all there is to know about these samples. To understand how these samples are different, you need other statistics: the range and standard deviation.

The **range** is the difference between the extreme measurements (i.e., smallest and largest) of the sample. In Sample 1, the range is $35 - 25 = 10$; in Sample 2 the range is $75 - 10 = 65$. The range provides a sense of the variation of the sample, but the range can be artificially inflated by one or two extreme values. Notice the extreme values in the sample of leaf measurements previously discussed. Moreover, ranges do not tell us anything about the measurements between the extremes.

Question 7

- Could two samples have the same mean but different ranges? Explain.
- Could two samples have the same range but different means? Explain.

The **standard deviation** indicates how measurements vary about the mean. The standard deviation is easy to calculate. Begin by calculating the mean, measuring the deviation of each sample from the mean, squaring each deviation, and then summing the deviations. This summation results in the **sum of squared deviations**. For example, consider a group of shrimp that are 22, 19, 18, and 21 cm long. The mean length of these shrimp is 20 cm.

Sample Value	Mean	Deviation	(Deviation) ²
22	20	2	4
19	20	-1	1
21	20	1	1
18	20	-2	4

Sum of Squared Deviations = 10

The summary equation for the sum of squared deviations is:

$$\text{Sum of squared deviations} = \sum_{i=1}^N (x_i - \bar{x})^2$$