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ABSTRACT

This manual contains instructions for laboratory exercises using marine organisms. For each exercise a problem is defined, materials are listed, possible ways to solve the problem are suggested, questions are asked to guide the student in interpreting data, and further reading is suggested. The exercises deal with the measurement of oxygen consumption in fish, population density, fertilization in the sea urchin, salinity tolerance, and food webs of shore organisms. This work was prepared under an ESEA Title III contract. (EB)

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LABORATORY EXPERIENCES IN MARINE BIOLOGY

Student Edition

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by Roger J. Raimist

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INTRODUCTION

At the present time man is learning more about the sea. The study of marine biology can be a fascinating and absorbing subject.

This laboratory manual contains a number of interesting activities and exercises that you might like to perform. You should read through the entire laboratory exercise before beginning. However, please note that you are not given step by step directions. These exercises have been written so that you will be required to do a great deal of thinking and rely on your own judgment. You may have to do some additional reference reading in the library. You should be able to think of a number of other experiments which you could perform and and you should try them. Your teacher will be able to give you some help but you must do all the thinking and research yourself. It is hoped that you enjoy these experiences and learn something about marine biology.

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The Measurement of Oxygen Consumption at Various Temperatures

The Problem

Animals breathe oxygen from the air if they are land dwellers like rabbits, elephants, people. If animals live in the water they obtain oxygen dissolved in the water by organs called gills.

Did you ever stop to think how much oxygen you use?

How much oxygen do you think a fish or a crab uses?

Do larger animals use more oxygen than smaller ones?

Do animals use more oxygen if they are warm or cold?

The initial problem confronting you in this exercise is how to measure oxygen consumption. For the moment let us measure oxygen consumption under varying conditions of temperature.

Materials Needed

Several small marine fish
Two centigrade thermometers
5-10 gallon tank filled with tap water
Net to handle fish
Smaller container or beaker with sea water to fit inside larger tank
Ice
Beam Balance Scales calibrated in grams

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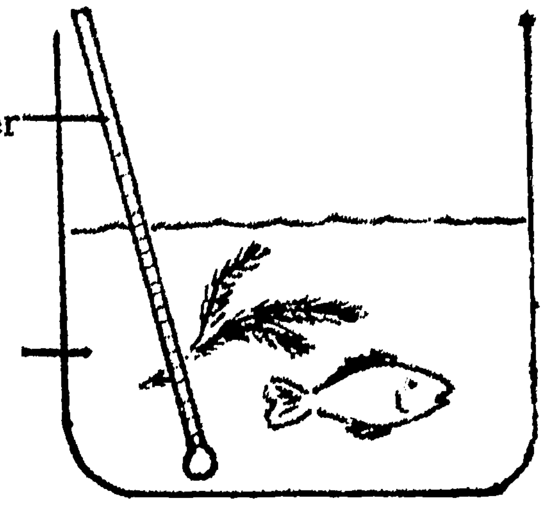
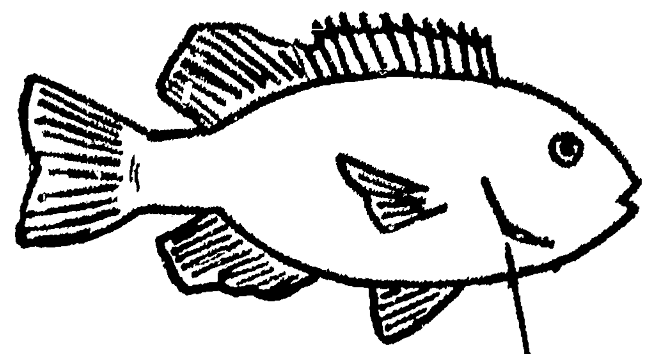
The Measurement of Oxygen Consumption





Possible Ways to Solve This Problem

Let us first observe some fish in a tank of water. Pick out one in the tank. Is he breathing? How do you know? How does be obtain his oxygen? Look at the fish being used by others in the class. Are all the fish alike? Are they exactly alike? Make a list of all the differences that you can observe.

Can you think of a way to determine if your fish uses more oxygen at some times than at others? Place some water into a tank or large container.

Would 50°C be a good temperature at which to take measurements? Why?
Would 90°C be a good temperature? Why?

What guidelines are you going to use in selecting a temperature? Select a specific temperature that you wish maintained. Why is it necessary to maintain this temperature at a steady point? Is the temperature of the room the same as that which you have chosen? What might happen to the temperature of the water over a long period of time? How would this affect the experiment?

Add warm water or cold water to maintain the steady temperature. You might use ice in a plastic bag to cool the water. Place a jar of sea water containing the fish into a larger tank and when both the tank and the jar reach the same temperature, record it. Now count the number of beats of the operculum or gill cover per minute and record it.

The Measurement of Oxygen Consumption

Again, look at the fish used by others in the class. Did they obtain the same results? Can you think of an explanation? If all the students used the same temperature do you think they would get the same results? Try using the same temperature and see. If you still fail to obtain the same results can you think of anything else about each fish that is different? Might it be a good idea to obtain the weight of the fish? Place some seawater in a jar and weigh it. Then add the fish and weigh it again. What is the weight of the fish? What effect does the weight have on respiratory rate? How could you adjust for this difference? Why would a ratio between weight and the number of operculum beats adjust for this difference?

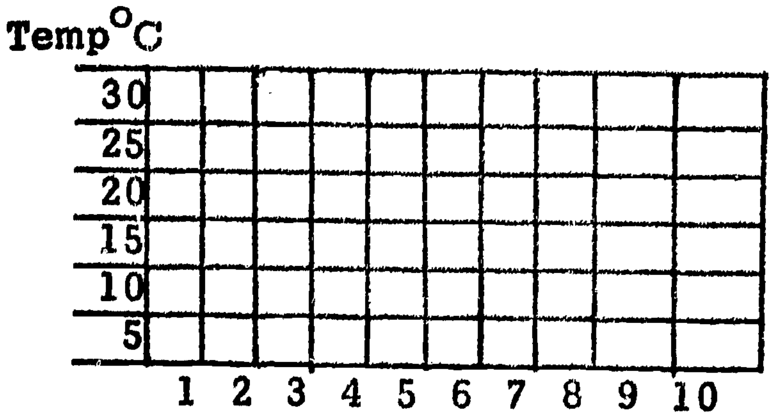
Repeat this at the same temperature ten times using a different fish each time. Why should this be repeated so often? Calculate the average number of beats and record it. Repeat this entire procedure at different temperatures or have others in the class perform this experiment at different temperatures. Graph the results of the entire class's experiments.

Temp °C	1	2	3	4	5	6	7	8	9	10	T_b	T_w	T_b/T_w
5													
10													
15													
20													
25													
30													

b = beats
w = weight
T_b = total beats
T_w = total weight

The Measurement of Oxygen Consumption

Relation Between Temperature and Operculum Beats/Minute



$\frac{\text{total beats}}{\text{total wt.}}$ = # beats/gm body wt./min.

Legend - number of operculum beats/min.

Conclusions and Further Research

What does your graph indicate? Why do you think it behaves this way? Did you actually measure the amount of oxygen consumed? What did you measure? What is the relationship between the amount of oxygen consumed and the number of beats per minute? Can you think of another way to perform this experiment using fiddler crabs which do not breathe under water? What other kinds of experiments might give you more information to better understand this exercise?

Additional References Helpful to The Student

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2. Huber, O., A Simple Procedure for Estimation of Metabolism in Small Animals, Turtox News: 40:36-37: January, 1962.
3. Knudsen, J.W., Biological Techniques, Harper and Row, New York, 1966.
4. Mahoney, R., Laboratory Techniques in Zoology, Butterworths, Wash., 1966.
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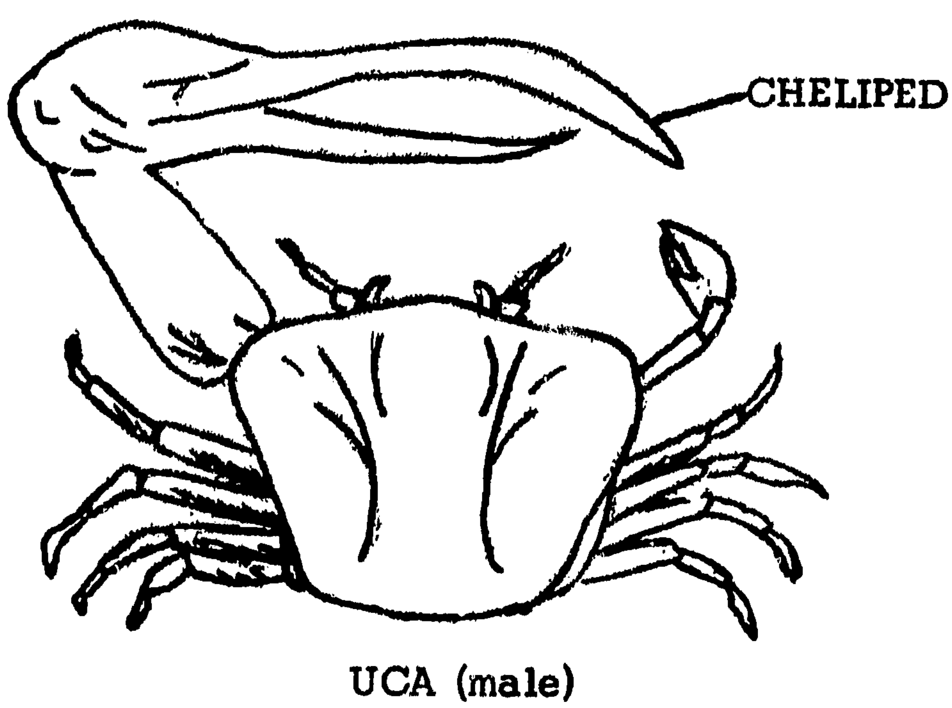
What Is A Population?

The Problem

Often we think of one animal or one plant while performing an exercise in the laboratory. In nature, plants and animals do not live alone just as people do not live alone. Organisms can be studied as populations. What is a population? How does it behave? How can it be measured? These are some questions to think about while performing the following exercise in population measurement.

Materials Needed

Three shovels per team
Ten meters of twine per team
One meter stick per team
Four wooden stakes per team
One hammer per team
One plastic bucket per team
Five small plastic centimeter rulers per team
One gram scale per team - should be able to measure tenth of a gram
One 500 milliliter glass beaker per team



UCA (male)

What is a Population?

Possible Ways to Solve This Problem

One way we can study a population is to obtain one. For this purpose, let us use fiddler crabs. You may wish to use something else. Feel free to do so. You may work as a member of a team, or independent from the group, but never go collecting alone. At the beach, at a crab nesting area, mark off a two meter square area and collect all the crabs in that area to a depth of 25 centimeters. Record their sex, weight in grams and the length of the chela in centimeters. How do you tell the sex of a fiddler crab? What is the chela?

Selected Characteristics of Fiddler Crabs

	Sex	Wt. in Grams	Chela Length in Cm.
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

Total # of crabs ____________
Total # of male crabs________
Total # of female crabs______

Mean weight of crabs__________
Mean chela of crabs___________
Volume of mud examined________

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Conclusions and Further Research

Consider the implications of some of the following questions. Does your data agree with that obtained by the other teams? Why does it agree or not? What is a sample? What is meant by a random sample? How can you tell? What is a ratio? What is the ratio of male to female crabs? Is there a relationship between the weight of the crab and the chela length? What is meant by density? Which is more dense, jello or water? Why is it more dense? If you are not sure, devise a means to measure the comparative densities of jello and water. How can you measure the density of your population? Can you estimate the entire population of the beach? What else would you like to know about the population? How could you find out?

Additional References Helpful to The Student

1. Hardin, G., Population, Evolution and Birth Control, W H. Freeman, California, 1964.

2. Reid, G.K., Ecology of Inland Water and Estuaries, Rinehold, New York, 1965.

3. Smith, R.L., Ecology and Field Biology, Harper and Row, New York, 1966.

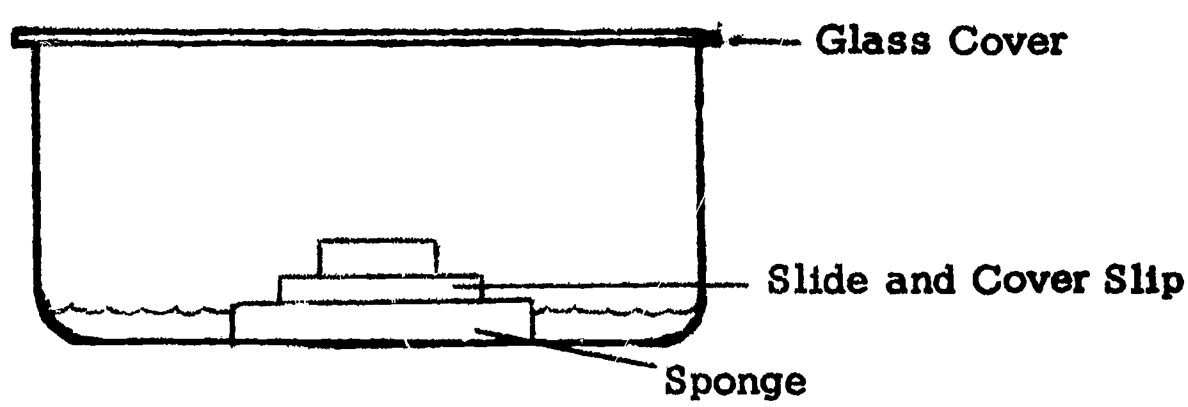
Fertilization in the Sea Urchin

The Problem

It is interesting to think of populations of organisms but ultimately each member of that population is an individual. Where did that individual come from? Where do all organisms come from? There are two necessary cell types for the development of any individual whether plant or animal. What are the cell types? What is fertilization? How does an animal develop? Let us investigate these cells and see if the process of fertilization and development can be observed first hand.

Materials Needed

One sharp-pointed scissor
Several wooden tongue depressors
Several sea urchins
Several glass bowls containing filtered sea water
Several pipettes or eye droppers
Slides and coverslips
Microscope



Fertilization in the Sea Urchin

Possible Ways to Solve this Problem

Obtain some sea urchins and examine them with a partner. Can you tell on which side the mouth is located? This side is called the oral surface and the opposite side is called the aboral surface.

Cut the hard shell of the sea urchin along the equator halfway between the oral and aboral surface. Remove the aboral end. What seems to be sticking to the underside of the aboral surface? These gonads, or reproductive glands, are producers of sperm cells in males and egg cells in females. Look at the sea urchins that others in the class have opened. Open several yourself and scoop out the gonads and place them in separate dishes of filtered sea water. Can you determine the sex of your animal? Using a separate pipette for each sea urchin, suck the gonad material in and out several times to release the sex cells or gametes. Place a drop of the cloudy liquid on a slide and coverslip the slide. Look at it under the high power lens of the microscope. What do you observe? Are they typical cells? Ask your partner to observe a single egg cell under the high power lens. As he does so, you should place a drop of the sperm suspension on the edge of the cover slip. What did he see? Now you try it. Save these slides by placing them in a dish with a sponge moistened with sea water. Cover it tightly. Observe this slide regularly and note what takes place. You might wish to mix large quantities of sperm and egg cells and observe fresh specimens each day.

Conclusions and Further Research

Did all the students make the same observations? What would happen if the salinity (the salt content) of the water were changed? Have your teacher supply you with 2% solutions of lithium and thiocyanate salts. Observe what happens to the embryos in these solutions. Are there other animals that you could use to observe fertilization and development? Try separating each cell at the 2 and 4 cell stage. Will they each grow into complete organisms? What else would you like to know about fertilization and development? Can you design an experiment that will answer your questions?

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2. Harvey, E.B., Electrical Method of Determining the Sex of Sea Urchins, Nature, 173:86, 1954.

3. Rugh, R., Vertebrate Embryology, Harcourt, Brace and World, New York, 1964.

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Salinity Tolerance

Problem

Organisms are affected by their environment. When temperature conditions change in your environment, how does it affect you? Can you think of other ways you are affected by changes in your environment?

Imagine that you are a small marine shrimp-like animal, and you live in the water near some beach. What kinds of changes in your environment might affect you? Could you be affected by a change in light, temperature, salt content, pollution?

If you lived near the mouth of a river and conditions became poor in the ocean could you move upstream? If you did, what would be the biggest change in the conditions you could expect? In this experiment you will be specifically concerned with measuring an organism's ability to survive at various conditions of salinity or salt content.

Materials Needed

Sodium Chloride
Large finger bowls with glass covers
Spatula
Eyedroppers
Distilled water
Gram scale
Graduated cylinder
Artemia salina

Possible Ways to Solve This Problem

An excellent organism to use for this investigation is Artemia salina, the common brine shrimp. If you wish to use another organism feel free to do so.

Obtain some brine shrimp eggs and add them to a pan of sea water. Use about half a teaspoon of shrimp eggs for each quart of water. Keep the pan near sunlight and at room temperature. The animals will hatch in 48 hours. Observe some eggs microscopically each day as they hatch to familiarize yourself with Artemia.

What is the salinity of sea water? Use sodium chloride as the salt solution in this investigation as this is a single important salt in the sea. What is the salinity of distilled water? Make up a range of salinities that you feel you would like to work with. What guidelines are you going to use to determine the concentration of the solutions you intend to work with?

Place solutions in equal quantities in finger bowls and mark each bowl clearly, indicating the salinity. Why are you using equal quantities of salt water? If the bowls are kept at room temperature and are left exposed to the air, what might happen to the water? What would happen to the salinity? How could you prevent this? How many organisms are you going to place in each bowl? Why is this a good number? After the animals are introduced into the bowls, count them each day in the morning and again in the afternoon and record the number of live animals. Graph the results in each bowl on the same graph using different colors for each salinity.

		SALINITY		
Day	A	B	C	D
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

Conclusions and Further Research

What information does the graph supply you with? In which salinity did the animals die fastest? Is there any relationship between salinity and survival in *Artemia*? Which salinity seems to be the best for keeping *Artemia* alive? Could other factors have caused their death? Could crowding have an effect on mortality? Would they have survived longer if you used fewer animals in each bowl? Would they have lived longer at a different temperature? Is there a relationship between salinity and oxygen dissolved in the water? How could you find out? Would this have an effect on survival?

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Special Ecological Problem

The problems of daily life are awesome and varied, particularly the problem of obtaining food and avoiding being eaten as food by another larger organism. Have you ever wondered what fish eat? What do starfish eat? What do shrimp eat? It might be interesting to establish interrelationships of animals in terms of their food requirements -- a food web.

While your class is at the shore, collect all sorts of animals from one location - fish, sea urchins, shrimp, clams, etc. Upon your return to the laboratory, examine the stomach contents by removing each animal's stomach. Cut it open and wash the contents into a dish of water and examine the contents both microscopically and macroscopically to establish what they are. Make a list of what each animal eats. Have your classmates assemble all the data they can and tabulate it. When this is complete you might try to establish a food web chain like that which is shown.