Designed to assist the teacher who wishes to use marine organisms for biological laboratory investigations, this manual includes general information on maintaining marine aquaria and collecting marine organisms as well as five tested laboratory exercises. The exercises deal with the measurement of oxygen consumption (giving techniques for measuring relative rates of respiration in fish and a manometric technique for direct measurement of oxygen consumption), population density, fertilization in the sea urchin, salinity tolerance, and food webs of shore organisms. Following each exercise are suggestions for further research, and a list of related references for the teacher. A general bibliography is included. This work was prepared under an ESEA Title III contract. (EB)
LABORATORY EXPERIENCES IN MARINE BIOLOGY
FOR UPPER ELEMENTARY AND SECONDARY SCHOOL GRADES

Teachers Edition

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ACKNOWLEDGEMENTS

I would like to thank Dr. Douglas Roberts, Temple University, Philadelphia, Pennsylvania for his valuable assistance and guidance in the formulation of this manuscript. I am also indebted to Gordon Chan, College of Marin, Kentfield, California; Peter Pollack, Montclair High School, Montclair, California, and Maxwell Cohen, Far Rockaway High School, New York for carefully reviewing and testing the exercises in this manuscript and for their most helpful editorial comments. I wish to express my thanks to my diligent wife who typed the original manuscript.
PREFACE

In the past several years there has been an increased emphasis upon science in the elementary and secondary schools. A particular emphasis upon marine science has also become evident (8,18,22). If one examines more closely this recent development, it can be found that there has been an increased emphasis upon the quantitative open-ended laboratory experiences in which the student himself becomes the investigator rather than the recipient of a demonstration or a given set of facts (9). This has not only been true of the Biological Sciences Curriculum Study (B.S.C.S.), but it is also evident in other publications of school science texts of recent date (10,13,15,20).

It is the intention of this manual to bring to the teacher a tool, in the form of a series of laboratory experiences in marine biology, which fulfills these long awaited changes in the teaching of science. It is hoped that these experiments will give the student the ability to discover for himself not only the methods and tools of science but the facts that can be derived from experiencing an unending and meaningful investigation.

The following experiences are not necessarily related although the teacher can adapt these to one specific organism to provide for an ecological problem-solving approach. Many of the experiences do not possess specific answers or at least always invite another question.

The experiences described in this manual have been thoroughly tested and are currently in use by schools in California, Florida, Chile and Saipan.
General Information Regarding Marine Organisms

Marine organisms can be used to demonstrate many of the very same principles of reproduction, physiology, evolution, genetics, etc., in biology that have heretofore been demonstrated with other organisms. Selecting marine organisms in certain cases is justifiable, however, because they demonstrate these principles with greater ease or with greater clarity. The following information might be helpful to the teacher in dealing with marine forms.

Most marine organisms may be kept in aquaria for several months provided certain conditions are met to insure their survival. If the teacher has access to the ocean, he may use natural sea water which is best for maintaining these organisms. Artificial sea salts can be obtained from Ward's Natural Science Establishment, Incorporated, P.O. Box 1712, Rochester, New York, 14603 or Ward's of California, P.O. Box 1749, Monterey, California, 93942. The teacher might desire to make his own artificial sea water; a chart (page 4) describes various methods.

The following are some general hints in maintaining marine aquaria (6, 7):

1. Aquaria must not be metal.

2. Glass, plastic or marine plywood are suitable materials for the construction of marine aquaria, and they may be sealed with an epoxy resin.

3. Glass gallon jars make fine small aquaria.

4. Metal instruments must never be introduced into the aquaria as they will upset the delicate balance of the metallic ions (23). This will shorten the lives of the organisms that are introduced into the tank.

5. Only use wooden instruments for cleaning the tank, removing dead organisms and feeding of live forms.

6. Remove leftover bits of food from the tank after feedings.

7. Feed the organisms no more than twice a week. Do not overfeed.
8. Keep the water temperature cool - between 15°C and 25°C. Always check the temperature of the water at the site of the collection of organisms and try to duplicate this in the laboratory or classroom as closely as possible. This may be done by placing ice, sealed in a plastic bag, into the tank, or by constructing a simple refrigeration unit (5, 25). Ward's Natural Science Establishment supplies self-contained refrigerated systems that work extremely well.

9. Never use red or brown algae in the tank as they decay rapidly. Use the green algae Ulva (sea lettuce) as it is the best oxygenator.

10. Watch the water level in the tank and as water evaporates, add distilled water to maintain the tank’s original level.

11. Replace approximately 25% of the total volume of sea water or artificial sea salts each two to three weeks (5). This will prevent excessive accumulation of organic wastes from the organisms that might become toxic to them, and will re-establish ionic balances.

12. Keep the tanks well aerated by using a good pump.

13. Do not place tanks in direct sunlight.

14. Do not overcrowd the tanks with organisms (23), density ratio should be three average size organisms (about the size of a ping pong ball), per gallon of sea water.
# OUNCES OF CHEMICALS TO MAKE 100 GALLONS OF SEA WATER BY VARIOUS FORMULAE

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<tr>
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How And Where To Obtain Marine Organisms

1. Small Marine Fish:

Small marine fish like the Killi-fish, *Fundulus heteroelitus*, can be purchased live during the warmer months of the year, from May through October, from most live bait dealers. However, it is better to have the students construct a seine from gauze or cheesecloth, some corks, lead fishing weights and twine and collect these organisms themselves during a field trip to the shore. These fish can be found in abundance in shallow waters along the beaches and in bays. They can be transported back to the laboratory in plastic buckets or bags and should be kept out of direct sunlight and kept as cold as possible. If it is more convenient, seining nets may be purchased from most biological supply houses.

![Seine Diagram](image)

2. Fiddler Crabs:

The fiddler crab, *Uca*, may also be purchased from live bait dealers from about May through October, but again it is best to have the students collect these organisms themselves. Fiddler crabs can be found along mud flats near the high tide mark in burrows in the mud near the Eel grass. Dig fast with a trowel or small shovel about six to ten inches down and shake the animals out of the mud. A gardener's bulb digger, plunged about the fiddler crab tunnel, insures your getting fiddler crab tunnel and all. Males can be recognized by the fact that they possess one larger cheliped (pincher) which they use in attracting females during reproduction, while females possess chelipeds of equal size. Fiddler crabs may be maintained in the laboratory by transporting some of the mud clumps and placing them with these clumps in larger glass containers. If the specimens were purchased they may be maintained on ordinary beach sand. They should be dampened frequently with sea water and kept cool and moist. Feed the crabs every day with small bits of raw fish or shrimp.
3. Sea Urchins:

Sea urchins can be obtained from Pacific Bio-Marine Supply Company, P.O. Box 282, Venice, California, 90293 or from Woods Hole Marine Biological Laboratory, Woods Hole, Massachusetts. Sea urchins can be found at low tide on sandy bottoms, living near rocks and pilings in clear and uncontaminated water. They can often be dredged up from deeper waters as well. They can be kept easily in a tank and fed bits of shrimp or fish. In transporting them from the beach to the laboratory, keep them uncrowded and generally dark as well as submerged in sea water.

4. Artemia salina:

Artemia can be purchased as eggs from any pet supply dealer. Artemia are easily cultivated from the dried eggs in about twenty-four hours. A 3.5 grams-per-liter solution of sodium chloride is used as a hatching medium, and about ½ gram of the eggs should be spread on the surface of the solution in a shallow pan. As the animals hatch, if a lamp is placed at one end they will congregate in that area and can be separated with a fine mesh net. A 70° room temperature seems optimum. Keep the culture near daylight as this facilitates hatching(21).
Suggested Daily Activities

1. General Introduction and Discussion

2. Construction of Nets and other Collecting Equipment.

3. Field Trip to the Shore to Obtain Specimens.

4. Observation and General Biology of Collected Forms either at the Beach or upon returning to the Laboratory.

5. Discussion of the Experiment and Construction of the Necessary Apparatus.

6. Performing the Experiment.

7. Calculations and Discussion of the Data Collected.

8. Suggestions for Additional Experiments to be Conducted by Small Groups Independent of Class Research.

The preceding eight items are recommended as the sequence of activities for any of the following laboratory experiences. Any one item may take more than one "science period." It is suggested that, if real excitement is generated by any activity, it should not be terminated in order to fit into a particular time period.
The Measurement of Oxygen Consumption at Various Temperatures

Purpose

The purpose of this investigation is to measure the oxygen consumption of any organism at varying temperature conditions. This investigation enables the student to perform a relatively simple experiment with only one variable: temperature. He can then observe the effects of temperature on respiratory rate. It gives the student an initial inquiry into an organism's environment-related problems but may hopefully lead to a wide range of subsequent questions based upon the experimental results obtained.

How To Get Started

One way to initiate an activity such as this is to ask the class where they spend their summer vacations. Ask how many have ever been to the seashore. What organisms do they know that they have found while at the shore? A display of marine life might be appropriate at this time, or an appropriate film.

Seashore life can generally be obtained for display purposes, on a loan basis, from local museums or universities. The teacher might ask about the importance of the oceans and how man may one day utilize them. A discussion of the problems that organisms encounter in the sea and on land might prove fruitful and arouse interest. If man is to utilize the sea effectively he might have to breathe under water. This proposition could lead to discussions of how one might measure the respiration rate of organisms, and here we have the ideal time slot for the first laboratory experience.

The teacher would be wise to have the students collect their own organisms for this experiment, as well as to construct their own equipment. Take the class to the shore for this activity. A first hand experience always adds much to their desire to learn and makes it a rather enjoyable way to learn. In so doing, they will attain other skills as well.
The Measurement of Oxygen Consumption

Methods and Materials

The methods to be utilized in this experiment vary with the organisms selected for investigation. Most fish enable direct readings to be taken on the relative rate of respiration. By observing the movement of the operculum of an isolated fish in a tank one can estimate whether the fish is breathing fast or slow.

Fish derive their oxygen through extraction from the water. The gills behave as a semipermeable membrane for gaseous exchange much as human lungs do. The thin tissues allow only certain molecules to pass through. Oxygen goes in and carbon dioxide goes out. The class should repeat this investigation on a number of fish at the same temperature as well as a range of temperatures. The class might be divided into teams of three or four and each group could select a temperature for investigation.

To calculate the oxygen consumption, a number of variables must be considered. The volume of oxygen consumed by an organism over a period of time will vary with the weight of the organism (26) as well as the temperature of the water. Since a greater amount of gas is soluble in colder waters, one might expect that the number of beats that the operculum will make will generally increase as the temperature of the water increases. The fish should also be weighed and oxygen consumption per gram body weight should be calculated. This is most easily accomplished. Place the fish in a beaker of water and weight the beaker and water alone. Subtract, and we have the weight of the fish. Divide the number of beats by the gram weight and you have a number which represents the number of beats per gram body weight per minute.

This will not give an absolute value for the amount of oxygen consumed but only a relative number indicative of the oxygen consumption rate (3).
The Measurement of Oxygen Consumption

Collection of the Data and Calculations

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<tr>
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<tr>
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<td>10</td>
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</table>

A = Total # Beats  
B = Total Weight  
C = Total Beats / Total Wt.

Total Beats = # Beats/gm. body Wt./Min.  
Total Wt.
The Measurement of Oxygen Consumption

The following exercise may be performed also and could be used as a follow-up to the preceding or in place of it. The teacher will note that the preceding exercise is less quantitative than that which follows.

Another organism that lends itself very well to this sort of an investigation is the fiddler crab, Uca. A direct observation method can not be used with Uca but the exact amount of oxygen consumed must be calculated. If the crabs are weighed collectively and placed in a respirometer constructed as follows, a reading is possible.
The Measurement of Oxygen Consumption

The potassium hydroxide (KOH) absorbs all of the carbon dioxide given off by the crabs and, as oxygen is consumed, a vacuum tends to be created and the tinted liquid moves up the tube. Several minutes should be allowed after the organisms have been introduced into the chamber to allow the system to reach stability. Record the level of the liquid at the beginning of the experiment and again after one minute. Record the following data:

1. Total weight of the crabs in grams.
2. Total oxygen consumed/minute in milliliters.
4. Temperature of respirometer.

Since the volume of a gas varies with the temperature and pressure changes of the atmosphere, one must adjust for these variations. This can be done by calculating what the relative volume would be under standard conditions of temperature and pressure.

\[ V_2 = V_1 \cdot \frac{P_2}{P_1} \cdot \frac{T_1}{T_2} \]

- \( V_2 \) = Actual volume of oxygen consumed in one minute per gram body weight.
- \( V_1 \) = Observed volume of oxygen consumed.
- \( P_2 \) = Atmospheric pressure at time of experiment.
- \( P_1 \) = 760 mm, which is standard pressure.
- \( T_1 \) = Temperature in °C at time of experiment + 273.
- \( T_2 \) = 273, correction factor for absolute temperature.

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Actual volume of oxygen consumed per gram body weight per minute at standard conditions of temperature and pressure.
The Measurement of Oxygen Consumption

Further Research

By performing this exercise the student has learned some basic techniques, not only in the construction of apparatus and collection of actual specimens, but also the collection and organization of data and the measurement of a physiological mechanism. The student may now ask why temperature affects respiratory rate. This may lead to a variety of investigations such as the relationship between dissolved oxygen content and temperature or between size and metabolic rate. How may metabolism be controlled by various chemicals or hormones? Menidea, (spearing) are often netted along with killi-fish. Spearing rarely live more than a few hours. Is this a related respiratory problem? He may wish to compare marine and fresh water metabolic rates. It is hoped, too, that prior to the utilization of any organism some of the general biology and anatomy concerning it will be studied.

Additional References Helpful to The Teacher


What Is A Population?

Purpose

The purpose of this investigation is to have the student gain valuable experience in measurement techniques and sampling procedures, as well as some understanding about populations and their measurement. This investigation could certainly give rise to a number of exciting classroom discussions and subsequent laboratory experiences.

How to Get Started

The teacher might begin discussions of communities and plant–animal interrelationships. One might discuss how single organisms affect the entire community, just as one person affects other persons. The teacher should then bring up the human population explosion and how man might solve this problem, if a student has not already done so. This could lead to discussions of the fact that, if man is going to control his own population, he must deal effectively with animal and plant population. This really means he must be able to measure and control plant and animal populations.

Methods and Materials

A number of organisms could be used to illustrate the principles gained by the execution of this exercise. Seaweeds, beach grasses, fish, shrimp, etc. could be used. Fiddler crabs have been chosen as they are excellent laboratory animals and can be used in a variety of ways. Organize class into teams and have the students collect the organisms themselves, as this again lends much to the experience. Measurements could be taken in the field or upon returning to the laboratory.

The method of collecting these animals is important. Have the students find a large colony of fiddler crabs. Have them mark off squares two meters by two meters in different areas, working in teams of about five students. They should dig out all of the fiddler crabs to a depth of twenty-five centimeters. The fiddler crabs should be placed in a plastic bucket. Upon returning to the laboratory or directly in the field, have each team determine the sex of the crab, the length of the chela, the weight of each crab and the total number of specimens found in the two meter area.
What is A Population?

These specimens should be returned to the collection site after all the measurements are taken, so as not to deplete the area and to avoid upsetting the natural balance that exists. Have them record the data and make sure that each team works independently.

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</tbody>
</table>

UCA (male)

CHELIPED
What is A Population?

Total # of crabs
Total # of male crabs
Total # of female crabs
Mean weight of crabs
Mean chela length

Volume = length x width x depth
Length = 2.0 meters
Width = 2.0 meters
Depth = 0.25 meters
V = 2.0 x 2.0 x 0.25
V = 1.0 meter³

Density of the population = \( \frac{\text{mass}}{\text{volume}} \)

\[ D = \frac{\text{#of crabs}}{1 \text{ meter}^3} \]

Further Research

After this experiment is performed, there are many fruitful areas to investigate for further research. The population of fiddler crabs could be measured over a period of years and the data compared. The relative density in different areas of the mud flat could be measured during various seasons of the year. The teacher will find that the students will probably recommend many fine experiments.

Additional References Helpful To The Teacher


Fertilization In The Sea Urchin

Purpose

The purpose of this laboratory experience is to demonstrate to the student the basic process of reproduction - fertilization and early development. This exercise gives the student a first-hand account of the process of fertilization. He is able to see the sperm cell fertilize the egg and then observe the fertilized egg undergo a series of cell divisions until it reaches the pluteus larvae stage. This demonstration certainly should make the entire process of reproduction more understandable. It could also serve as a springboard for exciting classroom discussions.

How to Get Started

This experience is specifically designed to be used during a discussion of development. After the study of populations is completed, (if it ever is), the teacher should then focus upon the stability of populations. A population remains stable when the number of births equals the number of deaths. Then the class should discuss how organisms are produced. What is reproduction? How does it take place? Why does it take place?

Methods and Materials

The sea urchin, Arbatia, lends itself well for this type of investigation. Here, too it is best to have the class collect the forms. Sea urchins can be found at low tide in shallow waters, in and around rocks and sandy bottoms. The best time of the year to collect sexually mature forms of Arbatia is between May and September (11). If these months are not convenient, the sea urchins should be brought to the laboratory and placed in sea water that is gradually brought up to 16°C and given eight to ten hours of light for a period of about ten days (1,2). Collect large numbers of organisms but do not crowd them into the same tank. Two average-size sea urchins can be kept in a gallon jar (4). This procedure increases the probability of sexual maturity, but is not always reliable. The teacher should consult the local marine research station for breeding times of local varieties.
Fertilization in the Sea Urchin

To perform this experiment, the animal should be cut around the test or shell and the top half lifted off. The gonads will adhere to the underside of the top of the shell. Use a wooden or plastic instrument to scoop out the gonads. The ovaries are reddish in color and the testes are more yellow. The gonads should be placed in cool filtered sea water in a bowl and gently teased apart. Remember not to use metal instruments. A wide mouth pipette could be used to suck the gonads in and out several times. This will cause a release of the sex cells. If the students work in pairs, as one looks at a suspension of eggs under high power of the microscope, the other can place a drop of the sperm suspension at the edge of the cover slip and observe the fertilization reaction (19).

The zygotes or fertilized eggs can be kept easily until the eggs attain the pluteus larvae stage.

If this technique fails to produce good results, a second can be employed. Use a weak electric current of about 10 volts and not more than 15 volts. Dry cells should be employed. Place the sea urchins in a shallow dish of sea water, oral surface down, and place the electrodes on opposite sides of the animal.
Fertilization in the Sea Urchin

In ten to twenty seconds the gametes should ooze out of the aboral side. The eggs are orange and the sperms are white. Remove the wires after eggs and sperms are released.

A third technique (12) can also be employed to induce the production of sperms and eggs. The injection of two milliliters of a 0.5 molar potassium chloride salt solution, (74.5 grams of KCl/liter distilled water), into the body cavity through the soft membrane around the mouth should induce the desired results.

### Table of Development Time in Sea Urchin Reproduction

<table>
<thead>
<tr>
<th>Stage</th>
<th>Time</th>
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</thead>
<tbody>
<tr>
<td>Fertilization</td>
<td>2 to 5 minutes</td>
</tr>
<tr>
<td>1st Cleavage</td>
<td>50 to 75 minutes</td>
</tr>
<tr>
<td>2nd Cleavage</td>
<td>75 to 100 minutes</td>
</tr>
<tr>
<td>3rd Cleavage</td>
<td>100 to 150 minutes</td>
</tr>
<tr>
<td>Blastula</td>
<td>6 hours</td>
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<tr>
<td>Gastrula</td>
<td>12 to 20 hours</td>
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<tr>
<td>Pluteus Larvae</td>
<td>24 to 48 hours</td>
</tr>
</tbody>
</table>

### Further Research

If this experiment has stimulated real interest, a number of problems will present themselves for further research. The student could use similar techniques with other animals or with some plants. There are numerous problems connected with embryology that could be investigated. The teacher should consult some of the following references.

### Additional References Helpful to the Teacher


Fertilization in the Sea Urchin


Salinity Tolerance

Purpose

The purpose of this investigation is to allow students to investigate the effect of salinity variations on the mortality rate of a population of organisms. This investigation has implications of the evolution of organisms because of environmental pressures that could be placed upon them, as well as the ecological range of that species. Students have been exposed previously to a population and its measurement. This experiment could be a logical extension.

How to Get Started

Since the students have just completed an investigation into fertilization, the teacher might very well raise the question of how many animals, once fertilized, shall survive to maturity and reproduce again. What are some of the factors that could account for their death? Certainly, viral and bacterial infection, predator-prey relationships and chance should be considered as changes in environmental conditions. The class could measure the effect of a single factor - salinity - on mortality rate.

Methods and Materials

The class could use brine shrimp, Artemia salina, as is suggested here, but any marine organism that is convenient to handle or easily obtained could effectively be utilized. The teacher should not hesitate to make a substitution.

Working in teams, students should be given eggs to hatch about two to three days before. Allow each team to observe these eggs during various stages of development. This period will allow some familiarity with the organism and its developmental patterns. Artemia (14) have a wide salinity tolerance range, so the teacher should allow much latitude in experimentation by the students. The salinity hatching range tolerance of Artemia (24) is between 0.1% and 6.0%, so the selected range should be from distilled water to above 6.0%. 

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Salinity Tolerance

In order to test for salinity tolerance, students should prepare a salt solution of normal seawater that has a salt content of 35\%/00 or 3.5\%. To accomplish this, sodium chloride could be used. Use 3.5 grams of sodium chloride and 96.5 milliliters of distilled water. Students should then vary the salinity to make higher or lower salt concentrations as desired. The organism along with the solution could be placed into large finger bowls and the mortality rate recorded hourly or daily. The animals need not be fed during this experiment. At least twenty-five animals per salinity concentration should be used and they should all be the same age. The water in the dishes could be at room temperature.

Further Research

This experiment raises many questions as to the actual cause of the mortality. Since the animals were not fed during the experiment, would feeding them have changed the mortality rate? Would a change in temperature rate effect mortality? Is there a relationship between dissolved oxygen and salinity (16)? If there is, could this affect mortality? All of these questions and many more that students are likely to raise could be tested if further research is desired.

Additional References Helpful to the Teacher

Salinity Tolerance

Additional References – cont’d


Special Ecological Problem

The problems of daily life in the marine environment in perspective are varied and awesome; particularly the problems of obtaining food and avoiding being eaten as food by another 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 - fish, sea urchins, shrimp, clams, etc. Upon your return to the laboratory, examine the stomach contents by removing the animals' stomach. Cut each stomach open and wash the contents into a dish of water. Examine the contents both macroscopically and microscopically to establish what was in the animal's stomach. Make a list of what each animal eats. Have the class assemble all the data they can and tabulate it. When this is complete, you might try to establish a food web like that which is illustrated.

In addition, identify and tabulate the materials found within the stomachs of the organisms. It might be fruitful to have the student identify any special adaptations of the organism which enabled it to capture that food. A good source for some of the material might be the local fisherman who could easily supply the class with black fish, mackerel, snappers, whiting and others.
Literature Cited


Literature Cited


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