

TESTED STUDIES FOR LABORATORY TEACHING

Volume 20

Proceedings of the 20th
Workshop/Conference of the Association
for Biology Laboratory Education (ABLE)

Edited by

Susan J. Karcher

Department of Biological Sciences
Purdue University

Chapter 13

The Roles Of Living Marine Organisms And Field Work In Teaching Invertebrate Biology

Anne Rudloe

Gulf Specimen Marine Laboratory

Panacea, Florida 32346

Phone: (850) 984-5297

e-mail: gspecimen@sprintmail.com

Anne Rudloe received her Ph.D. from Florida State University. Her research interests include behavioral ecology of marine species, aquaculture of marine species for research use, and the biology of sea turtles. She operates Gulf Specimen Marine Laboratory, a non-profit biological supply service, environmental education center, and aquarium. She teaches at Florida State University's Center for Professional Development.

© 1999 Anne Rudloe

Contents

Introduction	238
Eco-Sortments: Collections of Species from Various Coastal Habitats	241
Experiment 1: Brittle Star Chemical Reactions to Food	244
Experiment 2: Sand Dollar Feeding	245
Experiments with Salt Marsh Snails	248
Appendix A: Material from Hopkins and Smith	251

Introduction

It is not possible for a biology student to comprehend the true diversity of life on earth without a good knowledge of the totally or predominately marine groups. All the forms that are usually so casually lumped together as "the invertebrates" vastly outnumber both in species number and in morphological diversity the vertebrates that so many students unthinkingly and chauvinistically consider to be biology's main story.

So, invertebrate survey courses are taught, but as we all know, for the student, they can easily become a mind-numbing litany of obscure little things with hard to pronounce names and endless memorization of anatomical diagrams that blur into an indigestible lump. Or, these groups may be introduced in a marine biology class where there is insufficient time to present more than the rudiments of the major phyla. In both cases, it can be difficult for a student to ever grasp either the scientific sweep or the esthetic beauty of the biological diversity that is represented.

Live animals in the classroom can provide a major improvement in dealing with the problem. With adequate classroom aquarium holding facilities, the animals can be maintained for lengthy periods. A collection of exercises using abundant, hardy, and easily obtainable species is provided below. With such simple exercises, the student may observe living forms and conduct laboratory exercises that illustrate key aspects of the organisms' behavior and physiology.

These exercises have been developed by the author and/or contributed by colleagues over many years. The exercises on sponges, sea urchins, and fiddler crabs are by P. M. Hopkins of the University of Oklahoma. They are reproduced with the author's permission from Hopkins, P.M. and D. Smith, *Introduction to Zoology: A Laboratory Manual* 3rd ed. [ISBN 0-89582-358-6] (Morton Publishing Company, Englewood, CO. phone 1-800-348-3777) and were developed based on material from Gulf Specimen Marine Laboratory. L. Hagan of the University of Colorado, also using material from Gulf Specimen, contributed the exercise on periwinkle snails. An independent non-profit organization, Gulf Specimen has been supplying live marine animals to university biologists since 1963.

Yet, even with the use of live specimens, the student may still have difficulty relating the assorted laboratory exercises to a larger context. What is the relationship of the sand dollar used

in one week's lab to the grass shrimp that was studied the week before? If the student never connects individual animals to the sweeping story of the evolution and diversification of life throughout geological time, then he or she may still remain lost in the same old ocean of isolated and seemingly unconnected details, and still never grasp the sweep and grandeur of life on earth that these marine forms collectively display. In that case, the aquarium may not be much of an improvement over the old preserved material labs, despite all the extra effort taken by the teacher.

The critical task for the teacher is to guide the student's vision beyond the plethora of details in the individual lab exercises to a clear picture of the sweep of biological diversification. The task of the teacher is to share, in a compelling and evocative way, his or her own vision of the wonder of life on earth and its endless adaptive strategies. An excellent first step is to load everybody into vans, head in small groups to a coastal marine laboratory and get wet, sunburned, and collecting as quickly as possible. But if you are simply too far from a good location to do that, what is the next best thing?

In my opinion, the next best thing is to set up the aquarium second and order the marine animals third. Before doing any of that, get **YOURSELF** wet and sunburned, immerse yourself in the vast reality of the sea and its myriad of rainbow and crystalline creatures and then take that back in **YOUR** pictures and **YOUR** words to your students. Your first hand narratives will bring the subject alive like no video or prepackaged set of transparencies ever could. The lab exercises will come alive when you add to them your own insights and connections forged from first hand experience. It has the potential to make the subject of life on earth as exciting for your students as it is for you.

Based on this premise, a group of participants at the ABLE Conference held in June, 1998 at Florida State University spent the first part of a workshop day at the Gulf Specimen Marine Laboratory's aquarium observing a wide array of fish and invertebrates from the northern Gulf of Mexico. We then did a field trip into the varied estuarine habitats from which these animals come, allowing participants to collect with seines and take slides to develop personal background material for later classroom use. Habitats utilized included salt marshes, estuaries, barrier beach, and oyster reefs. At the end of the day, the group convened to address the following questions:

1. What basic concepts are we trying to convey in undergraduate invertebrate zoology or marine biology classes, and how are they best conveyed?
2. How is living material best used in achieving the above?
3. What is the value of field experience and how can it be replaced if unavailable?
4. How much emphasis should be placed on classification in an introductory class?
5. How differently do we teach majors and non-majors?
6. Is incorporating ecology and behavior into a survey of phyla effective or does it make it even more difficult to cover the material adequately?

7. Since taxonomy attempts to reflect common descent and evolutionary theory is central to making biology coherent, how do you handle the creationism issue?

Points on which the group developed consensus are listed below:

1. For non science majors, the primary goal should be to engage them, to light the spark of personal interest in biological diversity, and ,hopefully, to produce scientifically literate voters.
2. As to whether field experience should precede or follow extensive classroom work, the group was divided. All agreed that the best format for field experience is a self-guided experience in which students must engage in problem solving, using the instructor as a resource.
3. Most workshop participants did integrate ecology into their presentations, particularly the concepts of succession and the role of disturbance in ecosystems.
4. There can never be too much living material or hands-on experience in the classroom. Even a series of campus nature walks can be used to generate a teaching collection.
5. In teaching classification, limit the discussion of characteristics that separate groups to higher taxonomic levels. Otherwise the focus may be lost for the student.
6. Creationism generated the most discussion. Many participants preferred to acknowledge the issue at the beginning of the class and then come back to it after some background had been developed. Most found their students appreciative of the teacher's willingness to bring up the subject because the students had never previously had a venue in which to discuss it dispassionately, to hear any of the biological arguments involved, or to consider the differing roles of science and religion in society.

Teaching the scope, diversity, and beauty of life on earth, doing it in a sufficiently rigorous way, and doing it within the constraints of a short course can be a daunting prospect. Further, any discussion of religion and science requires preparation in ethics, history, and philosophy as well as science. But conveying excitement and enthusiasm for what may be argued to be the most remarkable natural phenomenon known to all of science is a lot more fun than simply droning on through the phylum characteristics until time runs out and exams begin.

Eco-Sortments: Collections of Species from Various Coastal Habitats

There's more to the ocean than salt water. Just as there is a wide range of terrestrial environments, including mountains, deserts, forests and plains, the sea has wave pounded rocky shores, sandy beaches, mangrove swamps, mud flats and meadows of sea grass. Each habitat shelters a variety of species that have evolved special adaptations to survive and reproduce in that habitat. Some dig into the sand, others use protective coloration. By obtaining live samples of species from each habitat, the instructor may demonstrate some of these habitat specific adaptations and interactions.

Salt Marshes

The waving green marshes that lie between land and sea, interwoven with meandering creeks, provide food and shelter for small fish, shrimp and crabs. Shallow marshes are the ocean's nursery, where most estuarine species must spend the early part of their life cycles, developing from juveniles to adults in levels of food and shelter found in few other marine habitats. Decomposing grasses nourish the estuaries with nutrient rich detritus as well. Southern marshes swarm with juvenile blue crabs; shrimp, mullet, croakers, trout and ladyfish that in turn attract snowy egrets, herons and a diverse array of other wading birds. Every student of ecology or marine biology should see directly the diversity of life there. Species available to the classroom include several species of marsh grass, oysters, periwinkles, white shrimp, grass shrimp, fiddler crabs, killifish, juvenile fish spp. and detritus.

Fouling Community

Any fixed object - wharf piling, boat bottom, or dock - placed in the ocean rapidly becomes the site of an unusual assortment of animals known as the fouling community. First barnacles settle out of the plankton, then hydroids, sponges, bryozoans, oysters and other sessile forms. These in turn provide a vast habitat for a multitude of small organisms including bizarre skeleton shrimp, tube building amphipods, isopods, and small crabs. Algae and tunicates appear. In this world flatworms, nudibranchs and errant polychaetes crawl over the hydroids. Sometimes small nemerteans and nematodes appear, or tiny pink anemones. These fouling animals are a rich source of food and attract large numbers of fish such as sheepshead, drum, redfish and spadefish.

Under the dissecting microscope this collection provides a large diversity of invertebrate form and demonstrates the concept of the artificial reef.

The Sponge Community

Lissodendoryx isodictyalis is a large shaggy yellowish sponge that grows on muddy bottoms along the Atlantic and Gulf Coasts. Throughout its entire range it is usually overgrown

and infested with other marine organisms. A large variety of commensal polychaete worms, nematodes, hairy brittlestars, xanthid and anomuran crabs, nudibranchs and flatworms dwell within the vascular canals, partaking of the food bearing currents that are pumped throughout the colony. Some forms such as pistol shrimp and amphipods, highly modified in both form and color, spend their lives deep within the protective canal system entirely dependent upon their host sponge for food, oxygen and waste removal. The outside of the sponge may be overgrown with tunicates, hydroids, gorgonians and bryozoan colonies, adding to the diversity.

Tearing the sponge apart, locating, removing and examining the commensal, parasitic and associated organisms of this living self-contained habitat makes a fascinating laboratory session. Since the organisms are generally concealed within the sponge, neither the quantity nor the presence of specific organisms is known in advance.

The ability of the sponge pieces to thrive and grow despite being broken up allows the instructor to make clear the fundamental anatomical difference between sponges and phyla with interdependent tissues and organs.

The Sea Grass Habitat

Waving green meadows of turtle grass, *Thalassia testudinum* flourish in the tropics wherever sunlight can penetrate down through the waters. They are among the most productive habitats on earth. Not only does the dense foliage of this marine angiosperm provide food and protection for a huge community of invertebrates and fish, but the decomposing leaves are a major source of detritus which nourishes planktonic organisms far out at sea. Few creatures in the community feed directly upon the turtle grass itself, except for the green sea turtle and sea urchins. However, many fish, crabs, shrimp and other creatures feed upon the hundreds of species of epiphytic algae and encrusting zoophytes such as serpulid worms, bryozoans, ascidians and sponges. The silt-catching leaves provide a haven for filter feeders such as scallops, millions of amphipods, parchment worms and sea cucumbers. Predatory speckled trout, spiny boxfish, pigfish and others lurk in the vegetation, feeding rapaciously on crustaceans and mollusks.

Turtle grass itself is truly amazing. As a marine angiosperm, it accomplishes pollination, germination and fruit formation underwater and spreads by creeping rhizomes, which helps anchor the substrate.

Species available from this habitat include sea cucumbers, grass shrimp, caridean shrimp, orange bryozoans, dwarf hermit crabs, sea urchins, tunicates, scallops, pipefish, boxfish, and sea robins.

The Sandy Shore

Few environments on earth are as harsh and unstable as sandy shores. Despite the shifting sands, eroding and accreting beaches, the exposure of the tidal flats to the broiling sun, frost and torrential rains, a large and specialized community of organisms exist along most sandy shorelines. The communities vary greatly from wave swept outer beaches to sheltered inlets and bays. Bivalves, annelids, sea cucumbers, whelks, conchs and even worm eels can be found down in the burrows. They can be detected by trails in the sand and burrows. Some are predators emerging at night to seek their prey on the flats, others remain down in their burrows extending tentacles or siphons to the sea above, drawing in both food and oxygen. Mole crabs and amphioxus have tremendous burrowing powers and can disappear before your eyes. Coquinas pull themselves down in the sand with spasmodic jerks and annelids slide through the sand. Flatfishes use camouflage, changing color with the sands.

Species available include coquinas, mole crabs, bristleworms, hogchokes, flounders, brittlestars, dogwhelks, beach hoppers, amphipods, amphioxus, sand dollars, horseshoe crabs and calico crabs.

Laboratory Exercises Using Live Marine Species

Serpent Star, *Ophioderma brevispinum* and Sand Dollar, *Melita quinquesperforata*

Ophioderma brevispinum is extremely abundant on sandy subtidal bottoms, often occurring in association with the sand dollar *Melita quinquesperforata*. It ranges from Cape Cod to Brazil, occurring from the low tide mark to depths of 200 meters. *Ophioderma* is negatively phototactic and may often be found under rocks or massed in empty shells.

The serpent star is a relatively large hardy species that does not automotize its arms readily. Thus it may be handled far more easily than most ophiuroids. A vigorous scavenger, it is excellent for observing chemotactic responses. If a bit of flesh is placed in an aquarium containing serpent stars, previously motionless animals begin to lash vigorously about, searching for food. The bait is soon covered with a writhing mass of serpent stars. *Ophioderma* is suitable for experiments dealing with chemotaxis and is also good for examining locomotion in ophiuroids.

Experiment 1: Brittle Star Chemical Reactions to Food

Materials: 4 brittle stars

Large Petri dish with sand and seawater

Small particle of flesh (hamburger, fish, etc.)

Procedure 1:

1. Place brittle stars in dish
2. Add food at a distance
3. Observe time for any behavioral response and time for stars to contact food
4. Measure water temperature
5. Repeat at various temperatures and plot temperature vs. response time

Questions:

1. Did the stars have to touch the food before responding to it?
2. How rapid was the response?
3. Do stars share, compete, or ignore others when food is present?

Procedure 2:

1. Sketch a star and number its arms.
2. As stars move observe whether the arms move in coordinated pairs or separately.
3. Is there an anterior end?
4. Do any arms drag?
5. Observe how the animals move around various obstacles.
6. When upside-down, what sequence of arms is used to right the star?

The sand dollar, *Melita quinquesperforata* is an irregular echinoid. It is basically a highly flattened burrowing sea urchin. Ranging from Nantucket to Brazil, it is most abundant south of Cape Hatteras and in the Gulf of Mexico. No species of this genus is found outside the Americas. In the northern Gulf of Mexico, it occurs on sandy bottoms from the intertidal zone, where it congregates in slight depressions, to a depth of approximately 15 meters. Thousands of individuals per square meter may occur in late summer. Sand dollars normally lie buried just beneath the surface of the sand and crawl forward, making characteristic U shaped trails in the sand. *Mellita* has a distinct anterior-posterior orientation as it moves through the sand.

A ciliary mucus feeder, the sand dollar picks up particles below 20 micrometers as it moves forward. A constant shower of sand grains and other particles fall on the aboral surface. Larger particles are carried across the surface of the test until they reach one of the 5 lunules or slits in the test where they are carried through the lunule and deposited on the substrate. Small particles fall between the spines and are carried by ciliary action to and through the lunules to the oral surface where they are carried by food tracts and oral ambulacral grooves in mucus filaments to the mouth. The cilia are located on the epithelial covering of the spines. The lunules are formed first as notches on the margin of the test of young animals. As the test grows, it

encloses the lunules. The spines fringing the lunule are protective, preventing large particles from entering and blocking the opening.

Pipetting a suspension of carmine particles or yeast cells stained with Congo Red over the sand dollar allows a demonstration of feeding. Observations of the oral surface may be made by placing the animals on a thin film of sand on a glass bottom and mounting a mirror beneath it.

The long thick ambulatory spines on the oral surface accomplish locomotion. When at the surface of the sand, *Melita* buries by advancing into the sand at a slightly downward angle and needs sufficient room to move forward as it buries. If placed in too small a container, it may be unable to bury. It changes direction by rotating the test away from the obstacle until it can again move forward. It may move either clockwise or counterclockwise. This may be demonstrated by placing small sticks in front of a sand dollar. If upside-down, the animal may display a righting response in which the spines on the posterior end pile sand on the edge of the test until the animal is tilted upward. When at approximately a 45-degree angle, wave action will then topple it into the proper position.

Substrate preference may be demonstrated by setting up an aquarium with both silty and sandy areas of substrate. Sand dollars will aggregate in the coarser substrate. This is correlated with their mode of feeding since smaller particles would clog the feeding apparatus.

Experiment 2: Sand Dollar Feeding

Materials: shallow glass container with sand and seawater
sand dollars
suspension of carmine red particles or stained yeast cells
pipette
mirror and stopwatch

Procedure:

Place a sand dollar in the dish, barely covered with seawater and pipette a suspension of cells or particles over the oral or aboral surface.
Observe the movement of the particles under a dissecting microscope.

Questions:

1. By what means can you detect if the animal is alive?
2. How fast do the particles move?
3. What is the purpose of the 5 grooves radiating from the mouth on the oral surface?
4. How does the sand dollar move, right itself, or get around obstacles?
5. What substrate type does it prefer? How does substrate affect feeding efficiency?

Sea Urchin Embryology

Fertile sea urchins are widely used to demonstrate fertilization and early cell cleavage stages. Fertility is typically seasonal and is affected by water temperature and nutritional state of the animals among other factors. Urchins typically feed on diatoms, algae, sea grass and sea grass detritus. *Arbacia punctulata* and *Lytechinus variegatus* are both good subjects for such exercises and may be induced to spawn by injection with 0.5M KCl. Eggs are orange and sperm are white. Collect 0.2 cc (about 2 drops) of sperm in about 10 cc of seawater. It remains active for about 20 minutes. Add 2 drops of this sperm mixture to a finger bowl of seawater with eggs. Sperm penetration is rapid and occurs anywhere on the egg. Temperature determines the rate of cleavage. At about 20 degrees C, first cleavage is in about 50 minutes, second is 100 minutes and 3rd in about 145 minutes.

Grass Shrimp, *Palaemonetes pugio*

The grass shrimp species of the genus *Palaemonetes* are abundant small shrimps of the marshes and estuaries. They are separated ecologically largely by salinity preferences, from fully marine to freshwater areas. They are widely used in comparative endocrinology and also in water quality and toxicity evaluation. Much work has been done on hormonal control of the distal retinal pigment of the compound eye as well as on hormonal control of the chromatophores.

Grass shrimp are of great ecological importance, constituting a major part of the diet of fishes such as speckled trout, *Cynoscion nebulosus*. As detrital grazers in marshes, they are significant in transferring energy from detrital sources into other food webs.

P. pugio has a life cycle of approximately one year, reaching maturity in several months. In the northern Gulf of Mexico, it is ovigerous from March to October with water temperatures from about 17-38 degrees C.

The small size and hardiness of this species makes it useful for laboratory demonstrations of crustacean swimming, escape and feeding behaviors. The transparency of the body allows easy observation of a crustacean open circulatory system. If the animal is immobilized with methyl cellulose, the rate of the heart beat may be observed and measured relative to varying temperatures, salinities, or concentrations of dissolved oxygen and carbon dioxide. Chromatophore functioning may be observed by placing shrimp against dark and light colored backgrounds and noting the overall color and response of chromatophores under a dissecting microscope. To observe the functioning of the distal retinal pigment, the eyes of shrimp held in darkness for 12-24 hours may be compared to those of animals kept in lighted aquariums. This must be done quickly before the eyes readjust.

Common Fiddler Crab, *Uca pugilator*

The sand fiddler crab is a brightly colored small semi-terrestrial crab, which occurs in large colonies along sheltered marshy shores of the Atlantic and Gulf coasts. The males are easily distinguishable by a vastly enlarged claw, which they wave vigorously in front of their burrows to attract a female. The waving displays of the various species of the genus are species specific and serve as isolating mechanisms, preventing interbreeding of congeneric populations. At night, when visual displays are ineffective, male crabs produce sounds by tapping their large claw against the substrate.

U. pugilator has been widely used in the study of endogenous biological rhythms. Its conspicuous circadian rhythm of melanophore activity was first reported in 1937. The cells expand by day and contract at night, causing the crab to appear much lighter at night. This rhythm persists under constant light or darkness. There is also a tidal rhythm of melanophore activity superimposed over the daily pattern. Metabolic patterns also display endogenous circadian rhythms. Color changes also occur in response to temperature changes. Crabs become lighter as the temperature rises, providing cooler body temperatures. The species has 4 basic chromatophore types: black, red, white, and yellow. They are not innervated but are regulated by hormones secreted in the eyestalks and brain. They may easily be observed in intact fiddler crabs under low magnification.

Color change is also influenced by substrate. Place 5 fiddlers in a white container and 5 in a black container. After an hour, examine and measure chromatophores of various colors in a walking leg or on the carapace. Then exchange the pans and observe the chromatophores at 15 minute intervals. Plot degree of expansion of chromatophores versus time.

Marsh Periwinkle, *Littorina irrorata*

The intertidal marsh periwinkle, *Littorina irrorata*, adorns salt marshes from New York to Texas. At low tide when the marsh sediments are uncovered, periwinkles crawl about, grazing on plant detritus, diatoms and filamentous algae. When the tide rises, the snails ascend blades of marsh grass to a point above the water level. A number of species of the genus orient to polarized light, with photopositive snails crawling at right angles to the plane of vibration of the light. Periwinkles distinguish and follow mucoid trails of other individuals of the same species. This can be demonstrated by running snails through a Y shaped tube and recording the number of choices of each branch of the Y. This is then repeated with one branch designated "clean" and scrubbed with sea water after each traverse by a snail. The majority of snails then choose the other branch, following the intact trail laid by their predecessors. It can also be demonstrated in air by tracing with a marker the trails made on a beaker as snails crawl up the sides.

The snail can also be used to demonstrate positive phototaxis. Place several snails in the middle of a large glass tube and allow them to adhere to it. Then seal it with plastic wrap and place it inside a cardboard tube. Position a light source at one end. After about 1 hour, note the

distribution of snails in the tube and compare to a control in which both ends were darkened. In both air and water, the response is positive. Use various light intensities as measured with an exposure meter. The same method can be used to demonstrate geotaxis by removing the light and orienting the tube vertically. The snails move upward, more rapidly in water than in air.

The functioning of the molluscan foot is readily examined in this species as well. Place the snail on a piece of glass, and when it begins to crawl, invert it over a container of salt water. Observe the movement of the foot under low magnification and note the roles of cilia and mucus in locomotion.

Experiments with Salt Marsh Snails, *Littorina irrorata*

(This exercise is from L. Hagan of the University of Colorado and is used with permission.)

Background:

The salt marsh snail is a common inhabitant of salt marshes. Population densities of the snail can reach from 100 to 200 individuals per meter. At high tide the snails are visible along the water line. When the tide recedes and exposes the marsh surface, snails climb down grass stalks and feed on lower decaying portions of plant stalks and algae mats. Upon being covered by water on the next incoming tide, snails climb back up again to resume their position above water line. See Figure 13.1.



Figure 13.1. Salt marsh snails on grass stalks. (After Oceanography for Landlocked Classrooms. 1990. NABT.)

Purpose:

Students will observe climbing behavior in snails in order to understand how one sea organism adapts to the changing water level during high and low tides.

Materials

Two plastic jars

Hydrometer

Salt marsh snails--(Live periwinkles may be purchased from Gulf

Specimen Marine Laboratory,

Panacea, FL.)

Tape

Data charts

Dried marine algae

Salt

Tap water in tall bottle

Dechlorinating liquid

Measuring spoons

Timer

Ruler

Sharpie marker

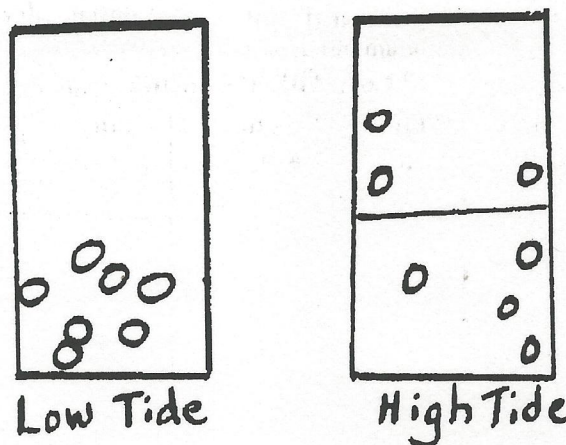


Figure 13.2. Jars representing high and low tide.

Procedures:

1. Fill tall water bottle with tap water. Add one drop of dechlorination liquid.
2. Add 2 level tablespoons of sea salt. Close the bottle tightly and shake the bottle.
3. Test the water with the hydrometer. Add small amounts of salt until the salinity level reaches 1.020-1.025. This is the salinity of ocean water--the optimum salinity for these snails. The green band on the hydrometer should float at the meniscus line.
4. Use the Sharpie and ruler to label the jars every 2 centimeters. Label one jar low tide and one jar high tide.
5. Add four snails to the bottom of each jar. One of the jars will represent high tide and the other low tide. Cover the jars with lids. Leave the jars undisturbed. Do not move the jars during the experiment.

Living Marine Organisms

6. Add sea water to the 2 cm line on the high tide container. Begin recording the snails movement every 10 minutes for 40 minutes. Fill in the charts on the Salt Marsh Snails worksheet to the nearest 2 cm every 10 minutes.

Salt Marsh Snails Worksheet

High Tide (Sea water added to 2 cm)

Minutes	0 cm # snails	2 cm # snails	4 cm # snails	6 cm # snails	8 cm # snails	10 cm # snails	12 cm # snails
0	4						
10							
20							
30							
40							

Low Tide (No water added)

Minutes	0 cm # snails	2 cm # snails	4 cm # snails	6 cm # snails	8 cm # snails	10 cm # snails	12 cm # snails
0	4						
10							
20							
30							
40							

Analysis:

1. Write down your observations.
2. Interpret your observations.
3. What behaviors allow this animal to survive?

Appendix A

The following pages are reprinted by permission of Morton Publishing, 925 West Koneon Ave., Unit 12, Englewood, CO 80110 from Introduction to Zoology: A Laboratory Manual, Hopkins, P.M. and Smith, David G. Copyright 1997, pp 40, 42, 43, 119, 120, 138, 139, 141.

119

Endocrine System. Certain specialized nerve cells can produce hormones. These special nerve cells look exactly like impulse-conducting nerve cells: they have dendrites, a cell body, and axons. The hormone-producing neurons differ from the nerve impulse-conducting neurons by the termination sites of their axons. Impulse-conducting neurons make contact with other nerve cells or with effector organs. The hormone-producing neurons, however, have axons that terminate on specialized regions of the circulatory system. These neurons release chemicals (called neurohormones) into the blood, and the circulatory system delivers the hormones to the entire body.

All animals that have a nervous system have neurohormones. Even the cnidarians, with their simple nerve nets, have neurohormones. It is believed that the endocrine system and the nervous system evolved simultaneously. Some neurons evolved to handle rapid and short-term information transfer. Other neurons, such as the neurosecretory neurons, evolved to handle long-term information transfer.

Neurohormones are produced in the crayfish by a number of neurons located in the eyestalk ganglia (Fig. 10-10B), the brain, the subesophageal ganglion, and possibly, in other ganglia in the abdomen.

The nerve cell bodies that produce the neurohormones are located in the X-organ in the eyestalk ganglia (Fig. 10-10B). Long axons from these cell bodies leave the X-organ and meet at a blood space called the sinus gland. The hormones are produced in the cell bodies, transported down the long axons and released into the circulation at the ends of the axons.

Several different neurohormones are produced in the X-organ. One neurohormone controls the levels of sugar in the blood of the crayfish, another controls osmotic concentration of the blood and still another controls color changes. We will look at one of these hormones in a later section of this chapter.

The arthropod endocrine system is unique among the invertebrates because it uses two types of endocrine control. The arthropods have a non-nervous gland that produces a steroid hormone called ecdysone. Indeed, it is believed that the arthropods are the only invertebrate group to use steroid hormones. Ecdysone controls arthropod growth and development. Ecdysone is produced in the crustaceans by a gland called the Y-organ (Fig. 10-9A). Locate the Y-organ in your dissected crayfish. It is attached to the epidermis on the body wall above the mandibles. The Y-organ is an oval disc of tissue (but may be somewhat difficult to find).

When you have finished the exercises in this section, fill in the sections of the Review Charts in Appendix E that correspond to the crayfish.

Hormonal Control Of Color Change In Crabs

Many crustaceans can change color in response to background colors or light conditions. Color changes are effected by means of specialized cells in the epidermis. These highly branched cells (called chromatophores, see Fig. 2-1B) contain pigment granules. These pigment granules can be concentrated near the center of the cell or the pigment can be dispersed into the branched processes of the cell. Depending on the color of the pigment, dispersal causes a lightening or a darkening of the animal. The movement of the pigment granules is under the control of neurohormones produced in the eyestalk X-organ and released via the sinus gland.

Obtain six fiddler crabs (*Uca pugilator*). Chill the crabs by immersion in ice for about 5 minutes (or until the crabs become totally lethargic). Remove the crabs' eyestalks by cutting the eyestalk as close to the body as possible (the exoskeleton is very thin on this portion of the eyestalk). Save the eyestalk for later use. Each crab should be kept in a separate beaker or paper cup and identification numbers assigned to each one.

To extract the hormone from the eyestalk tissues (extracts can be made ahead of time, frozen, and stored):

1. Grind the eyestalks in a cooled mortar and pestle in approximately 1.0ml of 0.85% NaCl.
2. Transfer this solution to a centrifuge tube and place in a boiling water bath for 2 minutes.
3. Centrifuge the solution for 2 minutes.
4. Draw the red supernatant into a 1.0ml tuberculin syringe. (Note and record the total volume of the supernatant for later calculations.)

Observe the hind leg of the eyestalkless crabs using a dissecting microscope. Score the degree of black pigment dispersal in the majority of the chromatophores on the legs of each crab (use Fig. 10-11). Record the score in Table 10-1 as the "Initial Chromatophore Stage."

Inject 0.025ml (25 μ l) of the extract into one of the crabs. Inject 0.05ml (50 μ l) of the extract into a second crab and 0.075ml (75 μ l) of the extract into a third crab. Inject the crabs at the base of one of the walking legs where the exoskeleton is thin and flexible. Three control crabs should be injected with the same volumes of 0.85% NaCl and observed along with the 3 experimental crabs.

Immediately observe and stage the chromatophores of all crabs. Continue to stage the chromatophores

every two minutes for twenty minutes. Record your observations in Table 10-1 and graph your results for each crab on Figure 10-12. Calculate the concentration of your extract in "eyestalk equivalents" per ml of extract then calculate the amount injected (dosage) as "eyestalk equivalents."

What is the general question being addressed by this experiment?

What is the hypothesis being tested?

Identify the independent variable in this experiment.

Identify the dependent variable(s) in this experiment.

What were the levels of treatment?

What served as the control treatment?

Which variables were regarded as controlled variables?

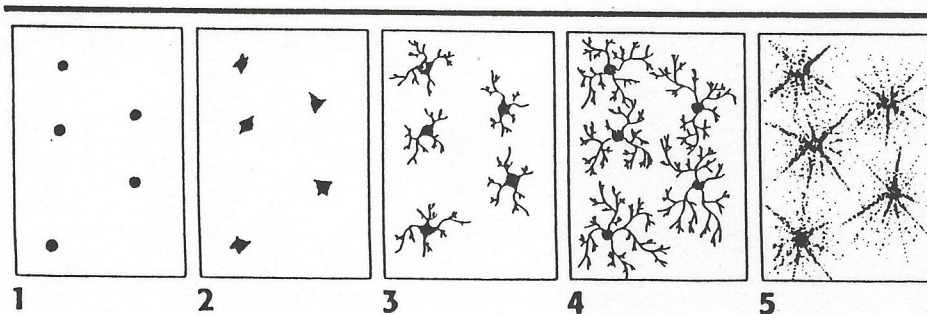


FIGURE 10-11.

Crab chromatophores—stages in pigment expansion.

Embryonic Development In Echinoderms

The echinoids have been useful in the study of animal development (also called embryology). In Chapter 4 you read how gametes are produced by meiosis. Today you will see what happens when the two haploid gametes (egg and sperm) come together. If conditions are favorable, fertilization will occur. Fertilization involves, (1) contact between sperm and egg, (2) entry of the sperm into the egg, (3) fusion of the egg and sperm pronuclei which restores the diploid condition and results in the formation of a zygote, (4) activation of certain synthetic mechanisms, and finally, (5) successive divisions of the new cell (by means of mitosis) to form the multicellular embryo.

GENERAL PRECAUTIONS FOR EMBRYOLOGICAL LABS

1. Cleanliness. Use only dishes, pipettes, etc., that are biologically clean. Detergents are good fertilization inhibitors, so rinse dishes thoroughly with distilled water and lay upside down on paper towels to dry. Do not cross-contaminate cultures. Never use a pipette for more than one vial.

2. Avoid Overinsemination. Very few sperm are necessary for successful fertilization. If the egg suspension becomes noticeably milkier after mixing, you have added too many sperm. Either use a toothpick to introduce sperm, or measure carefully.

3. Keep Temperatures Low. Remember, marine animals are accustomed to temperatures of 5–15°C. Never leave stock cultures above 20°C! Also, slides heat up quickly on the microscope stage, so do not expect to follow normal development for any length of time on a single slide. Evaporation will prohibit this as well.

4. Avoid Overcrowding. Eggs spawned under natural conditions probably never have to face the overcrowded conditions of a petri dish. Avoid this by placing very few eggs in a single culture. A continuous layer on the bottom indicates you have way too many.

The instructor will demonstrate how gametes are obtained. The live sea urchin (*Arbacia punctulata*) will be placed oral side down in a shallow dish of sea water. Inject approximately 2ml of 4% KCl into the aboral side of the echinoid to induce release of gametes via the genital pores on the aboral side.

1. Identify The Gametes. The sex of the sea urchin can be determined by the color of the gametes (white = sperm, red = eggs).

- a. If your sea urchin is a male, place it aboral side down over a dry petri dish and allow the sperm to collect on the bottom of the dish.
- b. If your sea urchin is a female, place it aboral side down in a beaker or graduated cylinder containing sea water and allow the eggs to settle to the bottom. Wash the eggs by decanting the sea water and replacing it with fresh sea water (inverting the beaker or cylinder and allowing the eggs to settle on the bottom once more). Decant again and repeat washing.

2. Fertilization. With a clean pipette, transfer a few eggs into a 60mm petri dish 2/3 full of sea water. The eggs should be sparsely distributed over the bottom. Fertilize by taking a small amount of dry sperm onto the end of a toothpick and swirling it gently through the upper level of the sea water, trying not to disturb the eggs.

3. Observing Development. Immediately prepare a microscope slide with vaseline on three sides to support the coverslip. Gently draw up some of the eggs into a clean pipette. Hold the pipette vertically and let the eggs settle toward the tip. Put one or two small drops on the microscope slide and cover with a cover slip. Note: The actual fertilization may occur too rapidly for you to observe. You can recognize eggs that have already been fertilized by the presence of a thickened fertilization membrane surrounding the fertilized egg. You may also see many unsuccessful sperm attached to the sticky fertilization membrane trying vainly to deposit their payload in the egg. The function of the fertilization membrane is to protect the egg against the entrance of more sperm. (Why is this important?)

4. Failed Attempt? If fertilization has not occurred within 10 minutes, consider the attempt a dud and try again using fresh sperm and eggs. If fertilization by the toothpick method fails twice, put a drop of dry sperm into about 10ml of sea water. Then add a drop or two of this sperm dilution to the eggs and mix gently. After two minutes (yes, you should time it), add another drop of the sperm dilution. Check for fertilization.

The process by which the fertilized egg undergoes mitotic division and develops into the embryo is called cleavage. However, during early development, other important events occur simultaneously. During mitosis, every daughter cell gets an exact set of the parental chromosomes. We know that every cell in our bodies has every genetic component of every other cell, yet a

blood cell is very different in its morphology and physiology from a nerve cell. These differences are the result of a process called differentiation which starts very early and continues throughout the development of the embryo. During cleavage, very complex differentiation events are occurring in the nucleus and in the cytoplasm of each new cell. These events are impossible to see, but you must keep in mind that they are occurring as you watch the more readily observed events of cleavage.

5. Observation Of Cleavage. Cleavage in the fertilized egg will depend on such environmental conditions as temperature and pH. A general outline of development for the sea urchin *Arbacia punctulata* is:

- | | |
|--|---------------|
| 1. Fertilization and formation of membrane | 1–5 mins. |
| 2. First cleavage | 50–70 mins. |
| 3. Second cleavage | 78–107 mins. |
| 4. Third cleavage | 103–145 mins. |
| 5. Blastula formation | 6 hours |
| 6. Hatching of blastula | 8 hours |
| 7. Gastrula formation | 12–20 hours |
| 8. Pluteus larva formation | 24–48 hours |

Observation Of Developmental Stages In The Sea Star And Sea Urchin

While you are waiting for your fertilized sea urchin eggs to divide, you should obtain a prepared slide of developing sea star eggs (*Asterias sp.*). The development of the sea star egg is very similar to the development of the sea urchin egg. The drawing in Figure 11-8 will help you identify the various stages.

Thirty minutes after fertilization, examine another wet mount. Can you detect any changes in the cytoplasm of the egg? At regular intervals throughout the remainder of the lab period, you should make wet mounts to determine whether any cleavages have taken place. If you are very careful (and very lucky) you may actually see a cleavage event in progress. Be certain to keep accurate records of both time intervals and appearance of the embryo in Table 11-1.

Your instructor will have cultures of fertilized eggs in various stages of development. Make wet mounts of some of these cultures—carefully noting the time of fertilization and calculating the age (in days and hours) of the culture at the time you examine it. Figure 11-8 gives diagrammatic representations of the various stages in the sea urchin (and sea star) development. On the data sheet provided, record the time when each of the stages was first seen. Combine data from other members of the class. Using these data you should be

able to devise a development schedule for the sea urchin with which you have been working under the conditions in your lab.

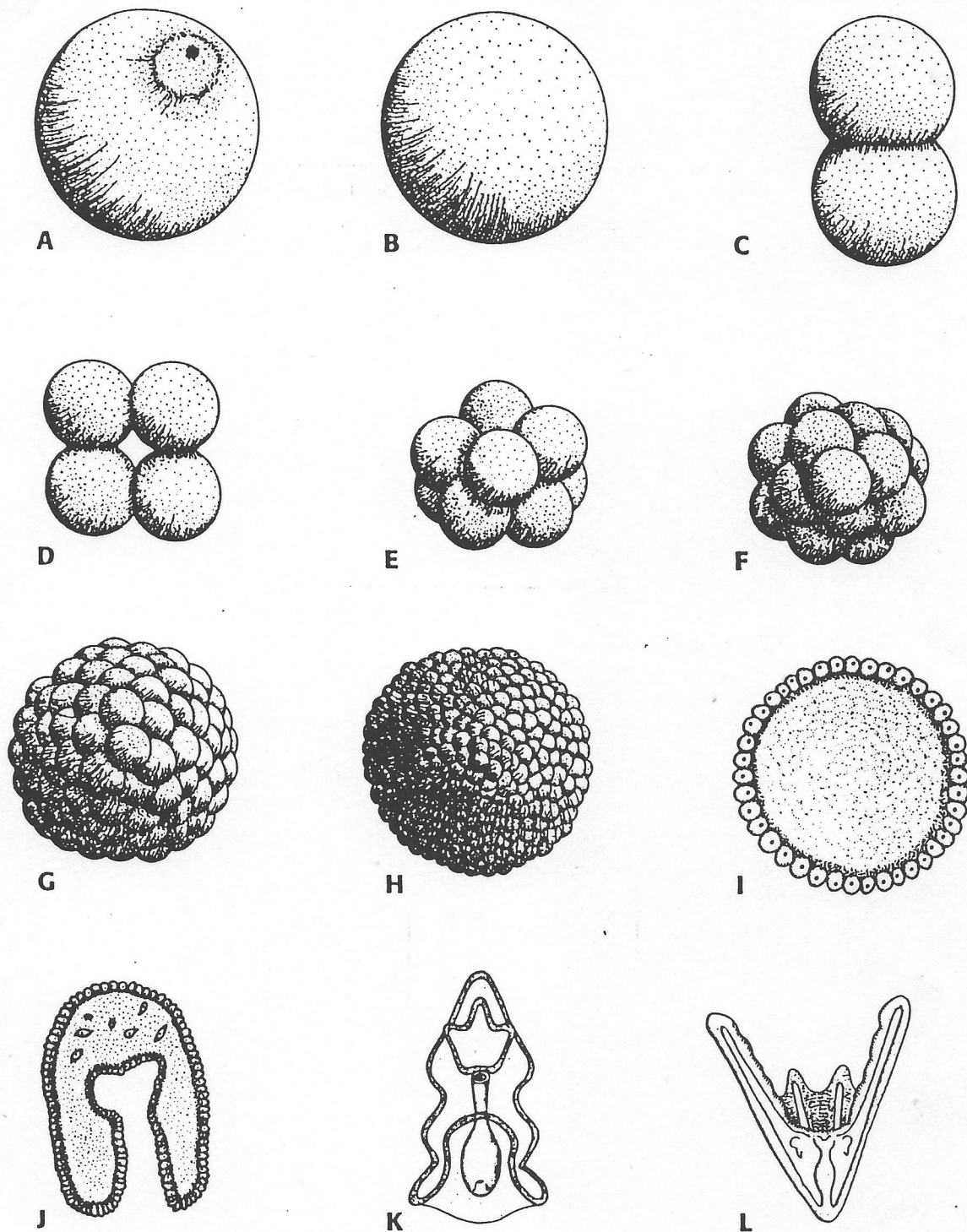
In the sea urchin, cleavage is almost identical to that of the sea star until the fourth division, at which point the developing embryo is called a morula (Fig. 11-8F). At this point, the sea urchin eggs begin to divide unevenly. See if you can find some fourth division embryos in the 3–5 hour samples that your instructor has for you. Can you see the large and small cells?

At a certain point, embryonic cell division results in the formation of a fluid-filled central cavity (Fig. 11-8G, H and I). This cavity (called the blastocoel) increases in size as the embryo continues to grow. The embryo at this point is called a blastula. The blastula is still inside the fertilization membrane. The blastula hatches out of the membrane after about 8 hours. Blastula cells are ciliated and the blastula is actually capable of moving through the water.

The free-swimming blastula continues to divide and differentiate. Approximately 12–20 hours after fertilization, the cells at one end of the hollow ball of cells begin to pulsate. This pulsation results in an invagination of cells into the hollow blastocoel. The invaginated cells extrude cytoplasmic extensions that attach to the inner walls of the ball. These cytoplasmic strands begin to contract and pull the invagination further into the blastocoel. This process of invagination of the blastula is called gastrulation. The embryo is now called a gastrula (Fig. 11-8J). The pocket that is made by the invagination is the beginning of the digestive tract. The invagination continues until the pocket meets the other side of the ball. At the point where the developing gut makes contact with the inner wall, the new mouth will form. The anus will form from the original point of invagination. Animals that develop in this manner are called deuterostomes (second-mouth). The original point of invagination becomes the anus and the point of contact with the inner wall becomes the mouth. In protostomes (first-mouth)—the original point of gut invagination becomes the mouth.

As the gut invaginates, smaller pockets of tissue begin to bud off of the forming gut. The mesoderm of the sea urchin will form from these pockets. The endoderm arises from the lining of the gut and ectoderm develops from the outside layer of the gastrula.

As the sea urchin embryo develops, the gastrula begins to change into a larva. Rod-like extensions appear around the developing anus. By twenty-four hours after fertilization, this pluteus larva is well formed (see Fig. 11-8L). This bilaterally symmetrical larval form will swim around in the ocean (as a component of the plankton) for a few months and finally it will metamorphose into the less mobile, radially symmetrical adult urchin (Fig. 11-6).

**FIGURE 11-8.**

A–K Sea Star development. A. Unfertilized egg. B. Fertilized egg. C. First cleavage (2-cell stage). D. Second cleavage. E. Third cleavage. F. Morula. G. and H. Blastula (surface view). I. Blastula (cross sectional view). J. Gastrula (longitudinal section). K. Bipinnaria larva of sea star. L. Pluteus larva of sea urchin.



TABLE 11-1.
Data Sheet for Sea Urchin Development

Event	Time Elapsed Since Fertilization
1. Membrane Formation	
2. First Cleavage (2-cell stage)	
3. Second Cleavage (4-cell stage)	
4. Third Cleavage (8-cell stage)	
5. Morula Stage (Late cleavage)	
6. Early Blastula	
7. Late Blastula	
8. Early Gastrula	
9. Late Gastrula	
10. Larva Formation	

Reassociation Of Sponge Cells

A result of the sponges' remarkable abilities to regenerate is the equally remarkable ability to reassociate. If the cellular connections that hold the sponge cells together are disrupted, the sponge will fall apart into individual cells. However, these cells can reassemble very quickly and form a new sponge.

In this experiment we will observe this reassociation phenomenon and we will test the specificity of this process. Your laboratory instructor will prepare a suspension of dissociated cells of two species of sponges, *Microciona prolifera* and *Haliclona occulata*. These cleaned sponges have been soaking in Ca^{2+} - Mg^{2+} -free sea water overnight. (Ca^{2+} and Mg^{2+} are necessary for intercellular adhesion.)

A small amount (0.5g) of each sponge is pressed through number 21 standard-quality silk bolting cloth directly into two beakers, each containing 25ml of normal sea water. The species *Microciona prolifera* is bright

red-orange and the species *Haliclona occulata* is purple. Therefore, the two suspensions of dissociated cells will appear pink and blue.

Using a pipette, remove a sample from each beaker. Place a drop on a slide, cover it and examine it with a microscope. Can you distinguish the individual cells? Record the size of these individual cells. (Use either an optic micrometer or express the size as a fraction of the diameter of the microscopic field, e.g., one cell spans approximately 1/20 of the field at 40X magnification.)

Remove 1.0ml from each beaker and mix the two samples in a 25ml Erlenmeyer flask. Place the flask on a gyratory shaker (3/4 inch diameter rotation) at 80rpm and 25°C. After 30, 60, and 90 minutes, remove a small portion of the suspension. Make a wet mount. Can you determine any differences in the size of aggregates? Are the aggregates formed from both red and purple cells or is there a segregation of cell types? Record your observations in Table 4-1. Explain your results.

Name _____

Section _____



TABLE 4-1.

Reassociation of Sponge Cells

	Time			
	30 Mins	60 Mins	90 Mins	120 Mins
Approximate Size of Aggregates				
Drawings or Observations of Aggregates				

REVIEW QUESTIONS.....



1. How many layers compose a sponge body? Are these true tissues?
2. What are the functions of water flowing through the sponge?
3. Draw and label a choanocyte. What is the function of this cell type?
4. Describe 3 sponge body forms and the selective advantage of the more complex leucon form.
5. How are larvae and gemmule formation adaptive to various environmental conditions?
6. Sponges possess spicules, which can function in:
 - a. protection
 - b. reproduction
 - c. circulation
 - d. digestion
 - e. elimination of wastes
7. Which of the following statements IS TRUE of poriferans?
 - a. they are motile
 - b. they are radially symmetrical
 - c. they stab their prey with spicules
 - d. they have no nervous system
 - e. they have two distinct tissue layers
8. Describe the physical and functional differences between mitosis and meiosis.